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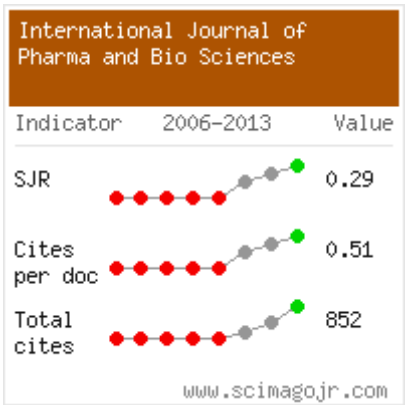

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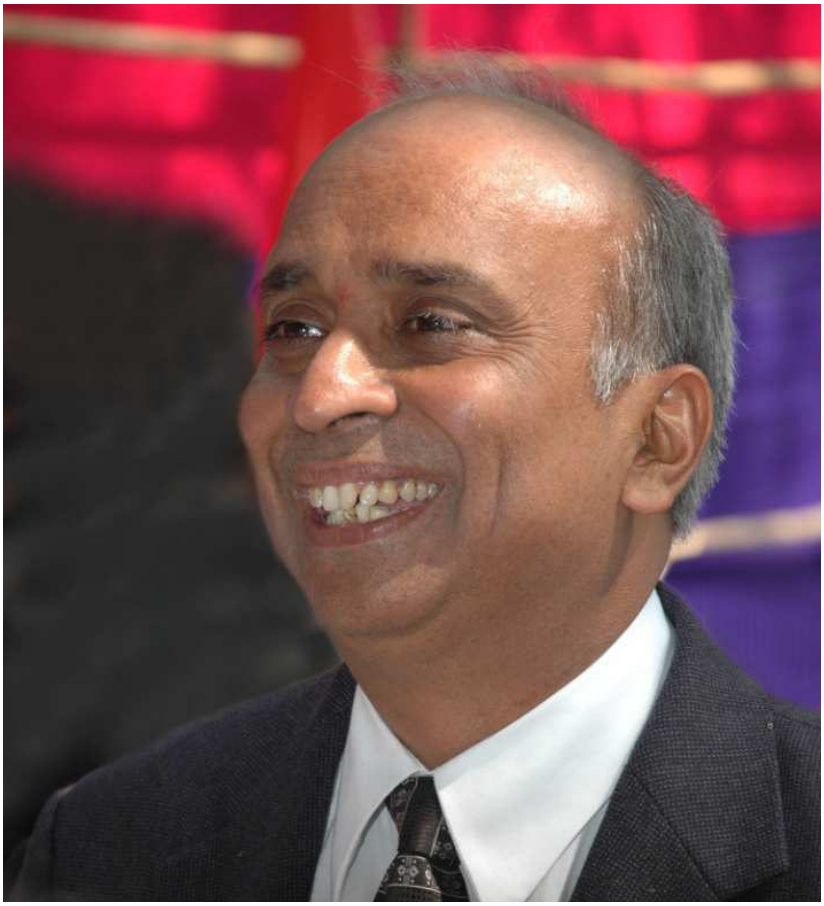
Professor Baru Vijaya Kumar

A Tribute



This special issue of IJPBS is dedicated to Prof Baru Vijaya Kumar as he celebrates his 58th birthday and moves on from the current phase of his career as a great and successful teacher and active researcher. On this memorable occasion, his colleagues, students and friends, wish him a happy, healthy and pleasant life ahead.

As a tribute to Dr. Baru, we present a brief summary of his scientific career and achievements over the past 35 years.

Dr Baru completed his Masters in Chemistry with specialization in Organic Chemistry in 1978 and joined CKM College as a faculty member in September 1978. He started his work on his Doctorate (PhD) in Organic /Medicinal Chemistry under the guidance of Prof. V. Malla Reddy in 1980. He was awarded PhD degree for the thesis entitled ***"Mannich and Michael Reactions on Benzimidazoles: Synthesis and Biological Activities of some 4(or7)–Nitrobenzimidazoles"*** in 1984 by the Kakatiya University, Warangal.

His PhD work paved a well defined path for the future research scholars in the field of Synthetic Organic Chemistry at Kakatiya University, Warangal with synthesis of several new and pharmacologically active scaffolds such as benzimidazoles-2-sulphides, sulphones S-(benzimidazol-2-yl)methyl-N-substituted dithiocarbamates, N1-substituted-N4-(benzimidazol-2-yl)methyl sulphanilamides, 1-(β-cyanoethyl)benzimidazoles, 1-(N-substituted aminomethyl)-6-nitrobenzimidazolesi-(N-alkyl/arylamidomethyl)-6- nitrobenzimidazoles .

The research work of Dr. Baru during his Ph.D program resulted in TEN research articles in reputed journals within a short span of 3 years - a proof of high quality research. Interestingly, this remarkable research work was carried out while continuing his teaching assignment at CKM College. After a successful stint of 12 years in teaching Chemistry to students at the undergraduate level, Dr. Baru also began imparting industrially and socially relevant higher education to students in the field of Medicinal Chemistry.

With his very strong desire to stay up-to-date with recent developments in Medicinal Chemistry and with active support from his department's colleagues, he designed a curriculum for Post Graduation course in Medicinal Chemistry. The Kakatiya University authorities approved the M.Sc (Medicinal Chemistry) curriculum without a single amendment, thus laying the foundation for a very ambitious post graduation program in CKM College.

As they say, the rest is history, with the students moulded by this department bagging gold medals at the university and securing positions of high responsibility in reputed academic/research institutes and pharmaceutical organizations in India and abroad. In recognition of the outstanding relevance of this course, it has been adopted by various universities/colleges in Andhra Pradesh.

Dr. Baru's current research group consists of seven Ph.D students and two research associates and assistants. Kakatiya University has awarded PhD degrees to two of his students.

Dr. Baru is a Fellow of Indian Chemical Society, Life member, Indian Pharmaceutical Association, Member, Indian Council of Chemists, Member, Indian Society of Chemists and Biologists, Member, Chemical Research Society of India, Member, American Chemical Society Division of Organic Chemistry, Member, Click Chemistry, Member, Medicinal Chemistry & Drug Discovery, Member, Process Chemistry Professionals, Member, Research & Development in India, Member, Scientific Journal Club.

On 1-7-2013, he was elevated as the Principal of his alma mater, CKM Arts & Science College affiliated to Kakatiya University, Warangal.

As a tribute to his long and successful career and as a token of gratitude to this inspirational person, we, the alumni of CKM College, in collaboration with the IJPBS (International Journal of Pharma and Bio Sciences), are bringing out this special issue.

I would like to express my heartfelt thanks to all the alumni of CKM College for contributing their articles; special thanks to my scientific review committee comprising of Dr. Kotesh Kumar, Dr. Jalapathi, Dr. Rajamohan Reddy, Dr. Venugopal, Dr. Varaprasad and Dr. Sunil Kumar for reviewing all the articles published in this issue. I sincerely thank the IJPBS team for collaborating with us in bringing out this special issue.

Dr. Kalyan Chakravarthy Akula

Guest Editor

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Mass Spectral Fragmentation Pattern Studies of N,N'-Linked Bis Azaheterocycles as a Source of Nitrogen Centered Free Radicals

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ABSTRACT

EI induced mass spectral fragmentation pattern of 2,2-dialkyl-3,3'-bisquinazolin-4,4'-diones (1**a-f**), 2,2-diaryl-tetrahydro-3,3'-bisquinazolin-4,4'-diones (**2a-f**) and 2,2-diaryl-3,3'-bisquinazolin-4,4'-diones (**3a-e**) is studied and the results are presented. Electron impact studies on 2,2-dialkyl-bisquinazolinones 1**a-f** and tetrahydro bisquinazolinones 2**a-f** indicate the N,N-bond susceptibility in their molecules by showing the prominent $M^{+\bullet}/2$ ion peak in their mass spectra. The stability nature of highly aromatized bisquinazolinones **3a-e** is also noticed in their mass spectra.

KEY WORDS

Bis quinazolinones, Nitrogen free radicals, Fragmentation pattern, N-N bond cleavage.

INTRODUCTION

N,N'-Linked bis azaheterocycles gained significant importance in view of their pharmacological activity as potential anti-convulsant[1], antidepressant[2], anti-inflammatory[3], antimicrobial[4], antifiliarial agents[5], memory enhancers[6] and anti-parkinson agents[7]. Quinazolin-4(3H)-one based bisazaheterocycles also exhibit prominent biological activities[8] as antifungal[9], antimicrobial[10] and anticancer agents[11]. 3-Quinazolinonyl thiazolidinone and 1-quinazolinonyl azetedinones were studied for their anti-parkinson activity and 3-triazinyl-4(3H)-quinazolinone derivatives showed promising anti-convulsant activity. 3-4'-Quinazolinylquinazolin-4-one exhibit anti-inflammatory properties[12]. The bacteriostatic and bactereiocidal activities of tetrahydro-3,3'-

bisquinazolin-4,4'-diones are assessed against a variety of pathogenic microorganisms[13]. 6,6'-Bisquiazolin-4,4'-dione polymers possess excellent dielectric and mechanical properties.[14]

N,N'-Linked bis azaheterocycles are also excellent precursors of nitrogen centered free radicals, which have a significant role in biological processes[15]. For example, the imidazol-1-yl radical was proposed as the key intermediate in the process of oxidative phosphorylation. Many N-centered free radicals are source for the synthesis of novel heterocyclic compounds[16]. Generally, thermolysis or photolysis of N,N'-Linked bis azaheterocycles generate nitrogen centered free radicals which undergo various types of reactions such as dimerisation, rearrangement, addition reactions to provide novel azaheterocycles[17].

In view of these observations, N,N'-linked bis azaheterocycles, particularly 3,3'-bisquinazolin-4,4'-dione derivatives were prepared and studied. As part of our scheme, we reported the synthesis and characterization of 2,2-dialkyl-3,3'-bisquinazolin-4,4'-diones (**1**), 2,2-diaryl-tetrahydro-3,3'-bisquinazolin-4,4'-diones (**2**) and 2,2-diaryl-3,3'-bisquinazolin-4,4'-diones (**3**)[18]. Here, we report the EI Induced fragmentation of the N,N'-linked bisazaheterocycles **1**, **2** and **3**. Electron impact studies on the monomer, 2,3-dihydro-4(3H)-quinazolinones was reported in literature which indicates the loss of CO, C-2 and N-3 substituents as the main fragmentation path, besides skeletal fission[19]. However, the mass spectral fragmentation pattern of tetrahydro and fully aromatized N,N'-bisquinazolinones are not reported in literature. Here, we describe some characteristic aspects of electron impact
fragmentation pattern of these 3,3'-bisquinazolin-4,4'-diones, which are potential source for cyclic nitrogen centered free radicals.

MATERIALS AND METHODS

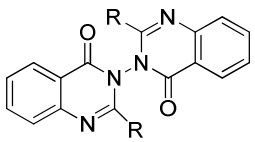
2,2-Dialkyl-3,3'-bisquinazolin-4,4'-diones (**1a-f**), 2,2-diaryl-tetrahydro-3,3'-bisquinazolin-4,4'-diones (**2a-f**) and 2,2-diaryl-3,3'-bisquinazolin-4,4'-diones (**3a-e**) were prepared according to the procedures reported from our laboratory[18]. Mass spectra were recorded on Perkin-Elmer Hitachi RMU-62 and VG-Micromass 7070Hz instrument of direct inlet probe.

RESULTS AND DISCUSSION

1. Mass spectral fragmentation pattern studies of 2,2-dialkyl-3,3'-bisquinazolin-4,4'-diones (1a-f):

The mass spectral fragmentation of 2,2-dialkyl-3,3'-bisquinazolin-4,4'-diones (**1a-f**), (Fig. 1, Fig. 2, mass spectrum of **1b**) indicate that all these compounds exhibit molecular ions $M^{+\bullet}$ of moderate intensity, whose abundance markedly decreases with increase in the bulkiness of the substituent at C-2. The compounds have shown three distinct fragmentation patterns under electron impact - (i) Loss of one C-2 substituent is very prominent in **1b, 1c** and **1d** and the resulting ion (**b**) is the base peak. When the substituent is H, and as the chain length increased, the intensity of ion **b** is decreased. The cleavage of N-N bond results in 2-alkyl-4(3H)-quinazolinonyl cation (**c**) appearing at half the molecular weight value. This process is of lesser significance when the C-2 substituent is either CH_3 or C_2H_5. However, in the higher homologues, this is the principle pathway and the resulting ion (**c**) is the base peak. In **1a**, the base peak is due to N-N bond cleavage followed by the loss of H resulting in ion radical **d**. When the 2-alkyl substituent is either n-propyl, n-butyl or n-pentyl, hydrogen migration leading to alkyl chain fragmentation is noticed. (Scheme 1, Table 1).

Figure 1



1a-f

	a	**b**	**c**	**d**	**e**	**f**
R	H	CH_3	C_2H_5	n-C_3H_7	n-C_4H_9	n-C_5H_{11}

Table 1 Partial mass spectral fragmentation pattern of 1a-f.

Entry No.	m/z (relative intensity, %)								
	a	b	c	d	e	f	g	h	i
1a	290 (65)	289 (18)	145 (45)	144 (96)	-	-	-	-	-
1b	318 (23)	303 (100)	159 (2)	144 (6)	-	-	-	-	-
1c	346 (6)	317 (100)	173 (18)	144 (1)	-	-	-	-	-
1d	374 (24)	331 (100)	187 (100)	144 (32)	-	186 (92)	185 (81)	173 (32)	160 (80)
1e	402 (11)	345 (26)	201 (100)	144 (12)	199 (57)	186 (12)	185 (42)	173 (8)	160 (53)
1f	430 (10)	359 (28)	215 (85)	144 (22)	199 (30)	186 (30)	185 (48)	173 (53)	160 (100)

Scheme 1 Mass spectral fragmentation pattern of 2,2'-dialkyl-3,3'-bisquinazolin-4,4'-diones (**1a-f**)

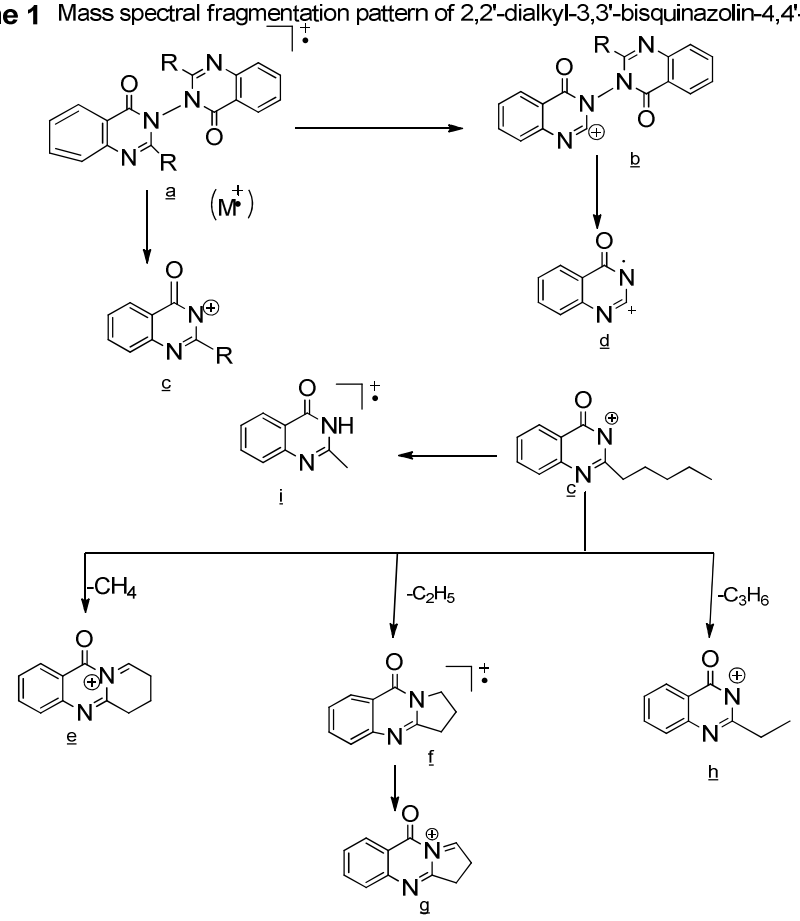


Figure 2

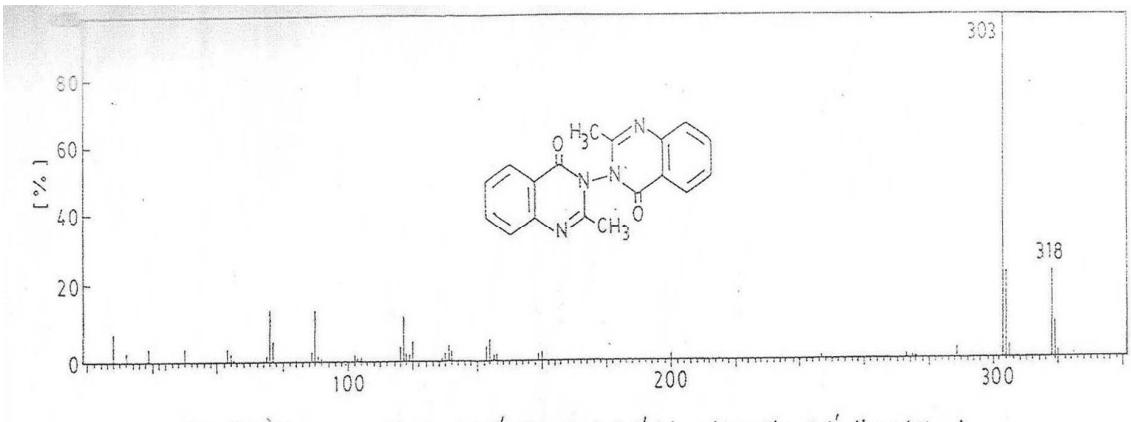
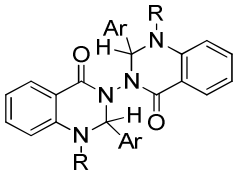


Fig. 2: Mass spectrum of 2,2'-dimethyl-3,3'-bisquinazolin-4,4'-dione (1b)

2. Mass spectral fragmentation pattern studies of 2,2'-diaryl-tetrahydro-3,3'-bisquinazolin-4,4' -diones (2a-f):

The mass spectral fragmentation pattern of 2,2'-diaryl-tetrahydro-3,3'-bisquinazolin-4,4'-diones (**2a-f**, Fig. 3), (Fig. 4, mass spectrum of **2a**; Fig. 5, mass spectrum of **2f**) revealed that the molecular ion is unstable and not recorded at 70ev. Instead, prominent ions due to 2-aryl-2,3-dihydro-4(3H)-quinazolinonyl cations are the end peaks, suggesting that the molecular ion readily undergoes N-N bond cleavage under electron impact. This is also proved by the thermolysis experiment of **2c**, **2f**, which results in the formation of the monomers[20]. The fragmented ion **e**, due to retro Diels-Alder fragmentation of ion **b**, is noticed in all the compounds (Scheme 2, Table 2).

Figure 3



2a-f

	a	b	c	d	e	f
R	H	H	CH_3	CH_3	CH_3	CH_3
Ar	2-$H_3COC_6H_4$	2-$O_2NC_6H_4$	C_6H_5	2-$H_3COC_6H_4$	4-$H_3CC_6H_4$	4-$H_3COC_6H_4$

Figure 4

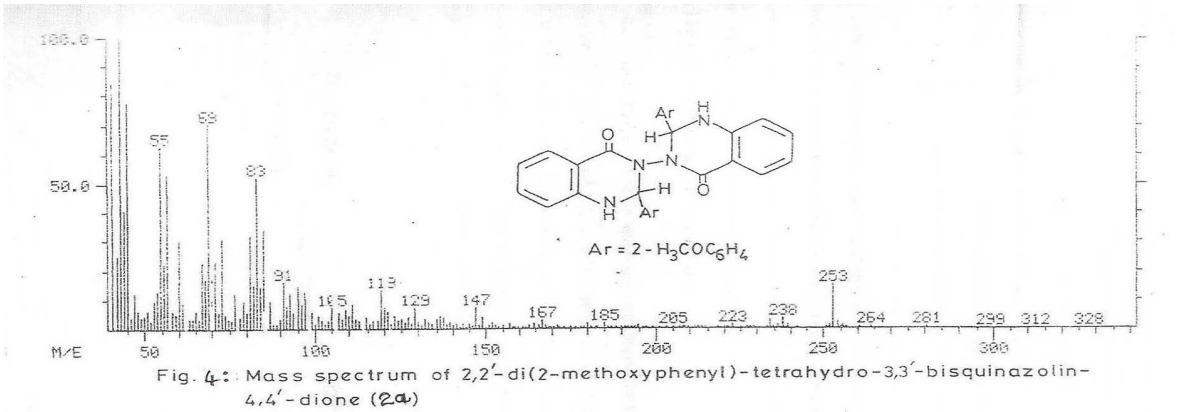


Fig. 4: Mass spectrum of 2,2′-di(2-methoxyphenyl)-tetrahydro-3,3′-bisquinazolin-4,4′-dione (2a)

Figure 5

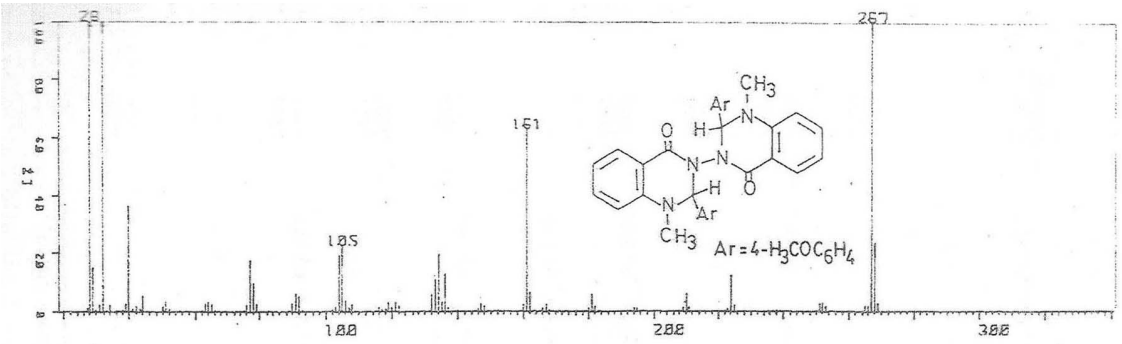


Fig. 5: Mass spectrum of 2,2′-di(4-methoxyphenyl)-1,1′-dimethyl-tetrahydro-3,3′-bisquinazolin-4,4′-dione (2f)

SCHEME 2

Mass spectral fragmentation pattern of 2,2'-diaryl-tetrahydro-3,3'-bisquinazoline-4,4'-diones (**2a-f**)

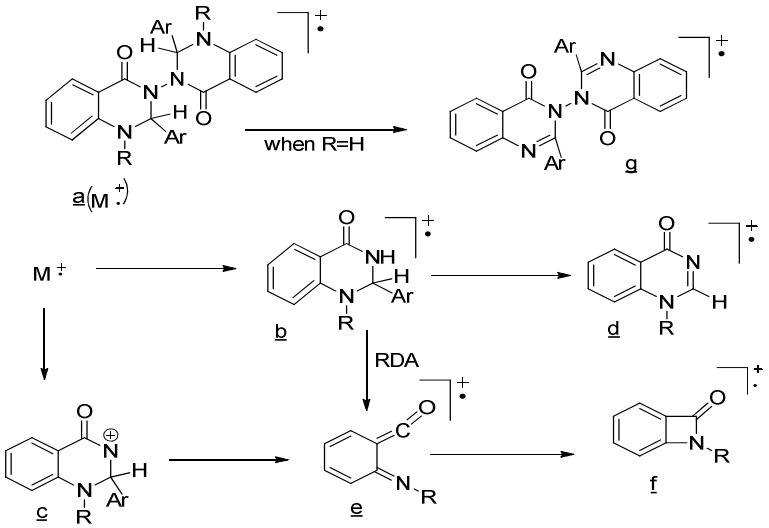


Table 2: Partial mass spectral fragmentation pattern of 2a-f

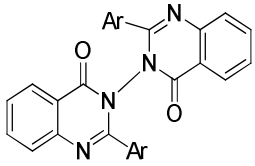
m/z (relative intensity, %)						
Entry No.	a	b $M^{\bullet +}/2 + H$	c $M^{\bullet +}$	d b-Ar	e c-ArCN	f ArCONR
2a	-	254 (2)	253 (14)	147 (8)	120 (8)	119 (13)

2b	-	268 (65)	267 (20)	147 (20)	120 (100)	119 (72)
2c	-	268 (22)	267 (95)	161 (100)	134 (30)	133 (6)
2d	-	238 (22)	237 (100)	161 (85)	134 (8)	133 (6)
2e	-	252 (22)	251 (100)	161 (84)	134 (8)	133 (4)
2f	-	268 (22)	267 (100)	161 (62)	134 (19)	133 (12)

3. Mass spectral fragmentation pattern studies of 2,2'-diaryl-3,3'-bisquinazolin-4,4'-diones (3a-e):

The mass spectra of **3a-e** (Fig. 6, Scheme 3, Table 3) (Fig. 7, Mass spectrum of **3d**) exhibit molecular ion peak of moderate intensity unlike their precursors, the tetrahydro derivatives **2**. The reason being, **3a-e** are the heteroaromatic dehydrogenated compounds which are more stable under electron impact. $M^{+\bullet}/2$ is of very low intensity revealed that N-N bond cleavage is difficult in these compounds. In the spectrum below the cation at $M^{+\bullet}/2$ is similar to that of 2-aryl-4(3H)-quinazolinones[21].

Figure 6



3a-e

	a	b	c	d	e
Ar	2-$O_2NC_6H_4$	2-ClC_6H_4	C_6H_5	4-$H_3CC_6H_4$	4-$H_3COC_6H_4$

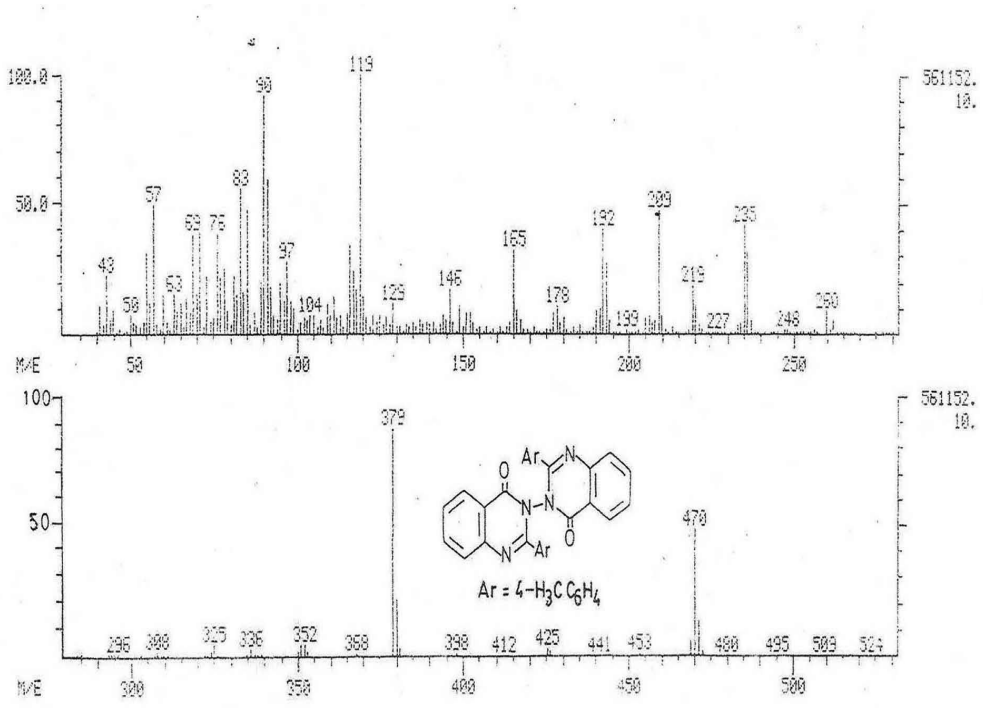


Fig.7: Mass spectrum of 2,2′-di(4-methylphenyl)-3,3′-bisquinazolin-4,4′-dione(3d)

SCHEME 3

Mass spectral fragmentation pattern of 2,2'-diaryl-3,3'-bisquinazolin-4,4'-diones (**3a-e**)

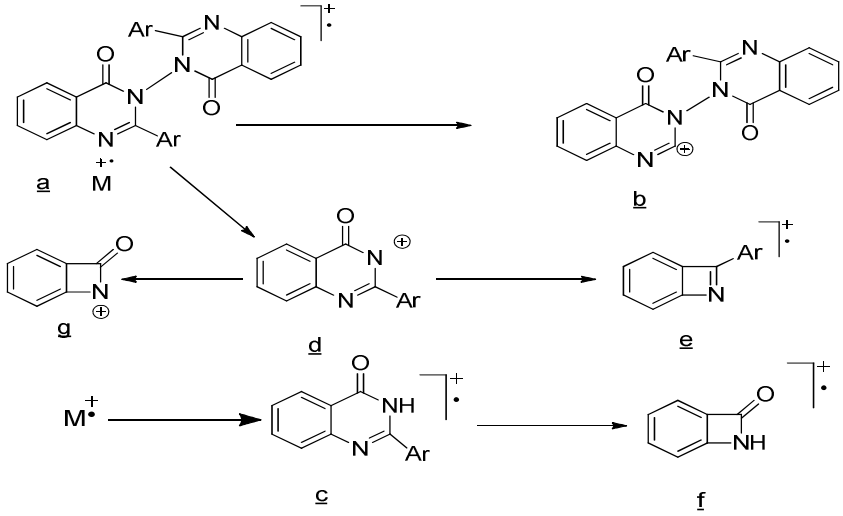


Table 3: Partial mass spectral fragmentation pattern of 3a-e

Entry No.	m/z (relative intensity, %)					
	a	b	c	d	e	f
	$M^{+\bullet}$	$M^{+\bullet}$-Ar	$M^{+\bullet}/2 + H$	$M^{+\bullet}/2$	d-NCO	C_5H_7NO
3a	532 (28)	410 (88)	267 (5)	266 (2)	224 (1)	119 (16)
3b	510 (15)	399 (80)	256 (17)	255 (16)	213 (25)	119 (26)
3c	442 (45)	365 (100)	222 (6)	221 (12)	179 (20)	116 (10)
3d	470 (49)	379 (86)	236 (31)	235 (45)	193 (23)	119 (100)
3e	502 (16)	395 (23)	252 (11)	251 (10)	209 (10)	119 (12)

CONCLUSION

EI induced mass spectral fragmentation pattern of 2,2-dialkyl-3,3'-bisquinazolin-4,4'-diones (**1a-f**), 2,2-diaryl-tetrahydro-3,3'-bisquinazolin-4,4'-diones (**2a-f**) and 2,2-diaryl-3,3'-bisquinazolin-4,4'-diones (**3a-e**) is studied and the results are presented. The fragmentation pattern pathways indicate that in 2,2-dialkyl bisquinazolinones **1a-f** and tetrahydro-bisquinazolinones **2a-f** N,N-bond is more susceptible for cleavage, providing the potential source for nitrogen centered free radicals. But the 2,2-diaryl bisquinazolinones **3a-e** are more stable and N,N-bond cleavage is not noticed in their mass spectra.

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SYNTHESIS AND CHARACTERISATION OF NEW 9-NITRO-10H BENZO-[B]-PYRIDO-[2,3-E][1,4]OXAZINE-7-SULFONIC ACID AMIDE AND DERIVATIVES

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ABSTRACT

A series of new 9-nitro-10*H*-benzo-[b]-pyrido-[2,3-e][1,4]oxazine-7-sulfonic acid amide and its derivatives **(6a-6r)** were synthesized from potassium salt of 9-nitro-10*H*-benzo-[b]-pyrido-[2,3-e][1,4]-oxazine-7-sulfonate (**4**) and thionyl chloride followed by reaction with various aliphatic/aromatic/hetero aryl amines. All the synthesized compounds were characterized by IR, ^{1}H NMR and Mass spectral data together with elemental analysis.

KEYWORDS

Potassium 9-nitro-10*H*-benzo-[b]-pyrido-[2,3-e][1,4]-oxazine-7-sulfonate, thionyl chloride, triethyl amine, sulphonamide.

INTRODUCTION

Heterocyclic skeleton benzo-[1,4]-oxazine has attracted the attention of scientists design molecules of therapeutic importance[1-2]. They posses pharmacological activities such as anti-ulcer[3], anti-hypertension[4], anti-fungal[5], anti-cancer[6], anti-thrombotic activities[7]. In addition these 1,4-oxazines act as 5- HT_6 receptor agonists[8], bladder selective potassium channel openers[9], dual selective ceratonine reuptake inhibitors (SSRIs) and 5- HT_{1a} receptor[10], dopamine agonist[11] and inhibitors of IP3 kinase[12]. Thier block TXA_2 receptor and PGI_2 receptor[13].Some 1,4-oxazines exhibit photochromic activity. Benzo fused and pyrido fused 1,4-oxazines have recently gained recognition owing to their biological importance[14]. They were utilized as suitable skeletons for the

design of biologically active compounds, ranging from anti-inflammatory, analgesics, bacteriostatic, fungistatic and MAO inhibitors[15]. It is reported that pyrido fused 1,4-oxazines are generally prepared from their 1,4-oxazinones as the synthetic routes to get 1,4-benzoxazines are not suitable for pyridine derivatives[16]. Benzo-pyrido fused 1,4-oxazines are reported by several workers[17-19], applying smile's rearrangement for pyrido[2,3-b][1,4]-oxazinones. These synthetic protocols via smile's rearrangement acquire 2-10 hours and the final products are without the presence of electron withdrawing groups in their skeletons. Fostamatinib, a molecule with pyridoxinone system is investigated for immune, thrombocytopenic purpura, a blood disorder in which immune system attacks and destroys plateletts in blood resulting in an abnormally low platelet count. Recently we have reported benzo-[1,4]-oxazine sulphonamides as potential inhibitors of PDE4[20] and insulin secretagogues.[21] We now report a new synthetic protocol with readily available synthetic starting materials prepared a versatile benzo-[b]-pyrido-[2,3-e][1,4]-oxazine system for the first time. It possesses electron withdrawing substituents such as nitro and sulphonic acids at 9 and 7 positions. These groups can be subjected to several functional group modifications resulting in new heterocyclic systems with potential pharmacophores

MATERIALS AND METHODS

All reagents used were commercial grade; melting points were determined in open capillaries and are uncorrected. ^{1}H NMR spectra were obtained on a Varian 400 MHz instrument with TMS as internal standard and chemical shifts are expressed δ ppm and mass spectrum was recorded on a Hewlett Packard mass spectrometer operating at 70ev. The purity of the compounds was checked by TLC and with E-Merck pre-coated silica gel plates (60F-254) with iodine as a developing agent. The elemental analysis of compounds was performed, and the analytical data obtained was found to be in good agreement with the calculated values.

EXPERIMENTAL DETAILS

Synthesis of potassium-4-chloro-3,5-dinitrobenzenesulfonate (2):

Chlorobenzene (50 ml) was added to a mixture of fuming sulfuric acid (260 ml) and sulfuric acid (60 ml) at 70°C. The mixture was stirred at same temperature for 1 h. Potassium nitrate (50 g) was added portion wise to the reaction mixture for about 15 min, and then fuming sulfuric acid (130 ml) was added followed by two more lots of potassium nitrate (50 g + 50 g) portion wise. The reaction mass was stirred at 130°C for 1 h, cooled to room temperature, poured in excess crushed ice and allowed to stand for 8 h. The solid was filtered and washed with cold water (3 x 200 ml), dried and washed three times with hot toluene (3x200 ml) and dried, yield 56% (80 g) of pure product; mp 295–298°C.

Synthesis of potassium;9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonate (4):

To a solution of sodium hydroxide (6.25 g, 0.156 mol) in methanol (400 ml) was added 2-Amino-pyridin-3-ol (3) (17.18 g, 0.156 mol) and potassium-4-chloro-3, 5-dinitrobenzene sulfonate (50 g, 0.156 mol). The reaction mixture was refluxed for 3 h. Then a solution of sodium hydroxide (3.5 g) in water (10 ml) was added to the reaction mass and refluxed for three more hours. Completion of the reaction was monitored by TLC. The reaction mass was cooled to room temperature and the solid was filtered and washed twice with methanol (2x 200 ml) and dried, yield 84.85% (46 g); mp >300°C; IR (KBr, cm^{-1}) λ_{max}: 3364 (NH),1190 (=C-O); MS (EI): m/z (M-K) 308.0; ^{1}H NMR (400 MHz, DMSO-d6) δ(ppm) 9.15 (s, 1H, NH), 7.78 (s, 1H), 7.73 (d, J = 16, 1H,), 7.16 (d, J = 12, 1H), 6.86 (t, J = 18,1H), ; Analysis (% Cal/fou) for $C_{11}H_6KN_3O_6S$, C: 35.04/35.02, H: 1.74 / 1.78, N: 12.10/ 12.08.

Synthesis of 9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonyl chloride (5):
Potassium; 9-nitro-10*H*-benzo[b]-pyrido-[2, 3-e] [1, 4]-oxazine-7- sulfonate (10 g) in $SOCl_2$ (80 ml) was refluxed for 3 h. Completion of the reaction was monitored by TLC. The excess of $SOCl_2$ was removed by distillation, the crude yellow coloured solid was poured in crushed ice and filtered, washed with water, cold methanol and dried, and yield 8 g (84.4%); mp 208°C (decomposition) and the product was immediately used for the next step.

Synthesis of compounds 6a-6r:
General procedure:
To a solution of substituted amine (1.0 eq) in tetrahydrofuran (50 ml) was added 1-nitro-10H-phenoxazine-3-sulfonylchloride (500 mg) at 0°C and the resulting reaction mixture was stirred for 1h at same temperature. Completion of the reaction was monitored by TLC. The excess of solvent was removed by distillation under reduced pressure and the crude solid was diluted with water and extracted with ethyl acetate (100 ml). The organic layer was collected, washed with water (50 ml), dilute HCl (50 ml), water (50 ml) and saturated sodium chloride solution (50 ml) and dried over anhydrous sodium sulphate. The organic layer was filtered and concentrated under reduced pressure.

1. Synthesis of 9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonic acid amide (6a):
Yield: 63.82% (300 mg); mp 240–242°C; MS (EI): m/z (m+1), 309; ^{1}H NMR (400 MHz, DMSO-d6), δ(ppm) 9.34 (s, 1H, NH), 7.96 (d, *J*= 16, 1H), 7.76 (d, *J*= 12.2, 1H), 7.54 (s, 2H,NH_2), 7.26 (d, *J*= 12, 1H), 7.20 (d, *J*= 16, 1H), 6.92 (m, 1H); IR (KBr, cm^{-1}) λ_{max}: 3353 (NH), 3330 (NH_2), 1153, 743; Analysis (% Cal/fou) for $C_{11}H_8N_4O_5S$, C: 42.86/43.03, H: 2.62/2.74, N: 18.17/ 18.28.
2. Synthesis of 9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonic acid phenyl- amide (6b):
Yield: 64.84% (380 mg); mp 248–250°C; MS (EI): m/z (m+1), 384.9; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 9.36 (s, 1H, NH), 7.84 (s, 1H, NH), 7.78 (d, *J*= 8, 1H), 7.32 (t, *J*= 12, 2H), 7.19-7.02 (m, 5H), 6.85 (d, *J* = 16, 1H); IR (KBr, cm^{-1}) λ_{max}: 3360 (NH), 3053 (NH), 1144, 763; Analysis (% Cal/fou) for $C_{17}H_{12}N_4O_5S$, C: 53.12 /52.89, H: 3.15 /3.20, N: 14.58 / 15.25.
3. Synthesis of 9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonicacid(4-methoxy-phenyl)-amide (6c):
Yield: 80% (400 mg); mp 255–258°C; MS (EI): m/z (M-1) 413; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 10.02 (s, 1H, NH), 9.35 (s, 1H, NH), 8.0 (s, 1H), 7.78 (d, *J*= 12, 1H), 7.19 (d, *J*= 8, 1H), 7.15-6.9 (m, 3H), 6.88-6.82 (m, 4H), 3.7 (s, 3H); IR (KBr, cm^{-1}) λ_{max}: 3352 NH), 3053 (NH), 1152, 793; Analysis (% Cal/fou) for $C_{18}H_{14}N_4O_6S$, C: 52.17/52.69, H: 3.41/3.49, N: 13.52/ 14.05.
4. Synthesis of 9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonicacid(4-chloro-phenyl)-amide (6d):
Yield: 50% (320 mg); mp 286–288°C; MS (EI): m/z (M+1) 418.91 ; ^{1}H NMR (400 MHz, DMSO-d6), δ(ppm) 10.54 (s, 1H, NH), 9.35 (s, 1H, NH), 7.88 (s, 1H), 7.75 (d, *J*= 16, 1H), 7.36 (d, *J*= 18, 2H), 7.2-7.1 (m, 4H), 6.9 (t, *J*= 24, 3H); IR (KBr, cm^{-1}) λ_{max}: 3364 (NH), 3095 (NH), 1452, 746; Analysis (% Cal/fou) for $C_{17}H_{11}ClN_4O_5S$, C: 48.75/49.5, H: 2.65/2.89, N: 13.38/ 13.86.
5. Synthesis of 9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonic acid cyclohexyl amide (6e):
Yield: 76.27% (450 mg); mp 232–234°C; MS (EI): m/z (M+1) 391; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 9.35 (s, 1H, NH), 7.92 (s, 1H), 7.77 (d, *J*= 16, 2H), 7.22 (t, *J*= 24, 2H), 6.92 (t, *J*= 12, 1H), 2.96 (s,

1H), 1.69-1.55 (m, 4H), 1.45 (d, *J*= 28, 1H, NH), 1.20-1.14 (m, 5H); IR (KBr, cm^{-1}) λ_{max}: 3338 (NH), 3106 (NH), 1454, 799; Analysis (% Cal/fou) for $C_{17}H_{18}N_4O_5S$, C: 52.30/52.50, H: 4.65/4.79, N: 14.35/14.89.

6. Synthesis of 9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonicacid(2-hydroxy-ethyl)amide (6f):

Yield: 70.89% (380 mg); mp 262–264°C; MS (EI): m/z (M-1) 351; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 9.36 (s, 1H, NH), 7.85 (s, 1H), 7.74 (d, *J*= 16, 2H), 7.21 (s, 1H), 7.19 (d, *J*= 8, 1H), 6.88-6.82 (m, 1H), 3.58 (t, 1H, OH), 3.39-3.36 (q, 2H), 2.84-2.78 (q, 2H); IR (KBr, cm^{-1}) λ_{max}: 3345 (NH), 3061 (NH), 1157, 746; Analysis (% Cal/fou) for $C_{13}H_{12}N_4O6S$, C: 44.32/45.19, H: 3.43/3.85, N: 15.90/15.30.

7. Synthesis of 4-(9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonylamino)benz-enesulfonic acid (6g):

Yield: 52.70% (370 mg); mp 278–280°C; MS (EI): m/z (M-1) 463; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 9.42 (s, 1H, SO_3H), 9.15 (s, 1H, NH), 7.78 (s, 1H), 7.76 (d, *J*= 18, 1H), 7.32(d, *J*= 24, 2H), 7.18 (d, *J*= 12, 1H), 7.06 (s, 1H), 6.84 (t, *J*= 8,1H), 6.52 (d, , *J*= 24, 1H), 5.88 (bs, 1H); IR (KBr, cm^{-1}) λ_{max}: 3424 (NH), 3338 (NH), 1599, 695; Analysis (% Cal/fou) for $C_{17}H_{12}N_4O_6S$, C: 43.96/44.85, H: 2.60/2.82, N: 12.06/12.69.

8. Synthesis of 9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonic acid (2-hydroxy phenyl)-amide (6h):

Yield: 95.8% (580 mg); mp 260–262°C; MS (EI): m/z (M-1) 398.65; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 9.7 (bs, 2H, NH), 7.92 (s, 1H), 7.79 (d, *J*= 8, 1H), 7.38 (s, 1H), 7.20(d, *J*= 12, 1H), 7.05 (d, *J*= 16, 1H), 7.04-6.86 (m, 2H), 6.72 (d, *J*= 16, 1H), 6.54 (t, *J*= 18, 1H), 5.18 (s, 1H, OH); IR (KBr, cm^{-1}) λ_{max}: 3454 (NH), 3350 (NH), 1453, 799; Analysis (% Cal/fou) for $C_{17}H_{12}N_4O_8S_2$, C: 43.96/44.85, H: 2.60/2.82, N: 12.06/12.69.

9. Synthesis of 9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonicacid(2-amino-phenyl)amide (6i):

Yield: 65.68% (400 mg); mp 252–253°C; MS (EI): m/z (M-1) 398; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 9.42 (s, 1H, NH), 9.32 (s, 1H, NH), 7.78 (s, 2H, NH_2), 7.18 (d, *J*= 28, 2H), 7.05 (s, 1H), 6.88-6.82 (m, 3H), 6.82 (d, *J*= 16, 1H), 6.62 (d, *J*= 10, 1H), 6.46 (t, *J*= 24, 1H); IR (KBr, cm^{-1}) λ_{max}: 3432 (NH), 3357 (NH), 1474, 759; Analysis (% Cal/fou) for $C_{17}H_{13}N_5O_5S$, C: 51.12/51.04, H: 3.28/3.54, N: 17.54/16.89.

10. Synthesis of 9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonicacid(3-hydroxy-pyridin-2-yl)amide (6j):

Yield: 89.8% (550 mg); mp 272–274°C; MS (EI): m/z (M+1) 401.72; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 9.52 (bs, 2H, NH), 7.92-7.78 (m, 3H), 7.44 (t, *J*= 24, 2H), 7.18 (d, *J*= 16, 1H), 6.92 (t, *J*= 18, 1H), 6.58-6.54 (m, 1H), 6.18 (s, 1H, OH); IR (KBr, cm^{-1}) λ_{max}: 3474 (NH), 3315 (NH), 1451, 817; Analysis (% Cal/fou) for $C_{17}H_{13}N_5O_5S$, C: 51.12/51.04, H: 3.28/3.54, N: 17.54/16.89.

11. Synthesis of 9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonic acid pyridin-2-yl amide (6k):

Yield: 42.73% (250 mg); mp 284–286°C; MS (EI): m/z (M-100) 384; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 9.42 (s, 1H, NH), 9.32 (s, 1H, NH),8.26 (s, 1H), 8.18 (d, *J*= 12, 1H), 8.04 (d, *J*= 12, 1H), 7.89 (s, 1H), 7.53-7.42 (m, 2H), 7.12-6.92 (m, 3H); IR (KBr, cm^{-1}) λ_{max}: 3367 (NH), 3088 (NH), 1531, 773; Analysis (% Cal/fou) for $C_{16}H_{11}N_5O_5S$, C: 49.87/49.53, H: 2.88/3.24, N: 18.17/18.56.

12. Synthesis of 9-nitro-10H-benzo-[b]-pyrido-[2, 3-e]-[1, 4]-oxazine-7-sulfonic acid hydrazide (6l):

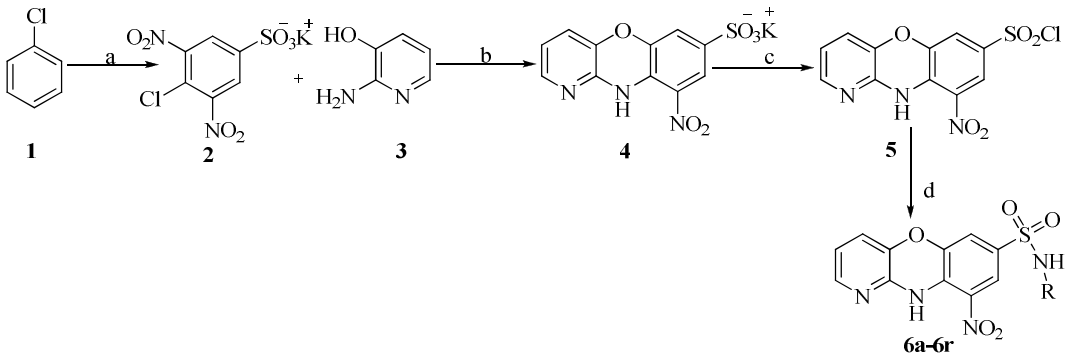
Yield: 81% (400 mg); mp 242–243°C; MS (EI): m/z (M-1) 322; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 9.36 (s, 1H, NH), 9.28 (s, 1H, NH), 7.96 (d, *J*= 14, 1H), 7.82 (d, *J*= 12.2, 1H), 7.454 (s, 2H,NH_2), 7.28 (d, *J*= 14, 1H), 7.26 (d, *J*= 18, 1H), 6.92-6.90 (m, 1H); IR (KBr, cm^{-1}) λ_{max}: 3370 (NH), 3272 (NH), 745; Analysis (% Cal/fou) for $C_{11}H_9N_5O_5S$, C: 40.87/40.53, H: 2.81/2.76, N: 21.66/20.96.

13. Synthesis of 9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonicacid-N'-phenyl-hydrazide (6m):
Yield: 32.8% (200 mg); mp 268–270°C; MS (EI): m/z (M-1) 398; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 9.28 (s, 1H, NH), 8.64 (s, 1H, NH), 8.52 (s, 1H, NH), 8.14 (s, 1H),7.76 (d, *J*= 9, 1H), 7.64 (s, 1H),7.42-7.31 (m, 3H), 7.24-7.13 (m, 4H); IR (KBr, cm^{-1}) λ_{max}: 3367 (NH), 3088 (NH), 1531, 773; Analysis (% Cal/fou) for $C_{17}H_{13}N_5O_5S$, C: 51.12/51.89, H: 3.28/3.76, N: 17.54/18.63.
14. Synthesis of 9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonic acid methyl amide (6n):
Yield: 20.3% (100 mg); mp 232–234°C; MS (EI): m/z (M+1) 323; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 9.46 (s, 1H, NH), 7.86 (d, *J*= 14, 1H), 7.74 (d, *J*= 12.4, 1H), 7.54 (s, 1H, NH), 7.28 (d, *J*= 18, 1H), 7.22 (d, *J*= 14.4, 1H), 6.94 (s, 1H), 2.86 (s, 3H, CH_3); IR (KBr, cm^{-1}) λ_{max}: 3371 (NH), 3091 (NH), 796; Analysis (% Cal/fou) for $C_{12}H_{10}N_4O_5S$, C: 44.72/44.85, H: 3.13/3.56, N: 17.38/17.83.
15. Synthesis of 9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonic acid diethyl amide (6o):
Yield: 63.06% (350 mg); mp 246–248°C; MS (EI): m/z (M+1) 365; ^{1}H NMR (400 MHz, DMSO-d6), δ(ppm) 9.28 (s, 1H, NH), 7.84 (s, 1H), 7.68 (d, *J*= 8, 1H), 7.22 (s, 1H), 7.18 (d, *J*= 16, 2H), 6.86 (t, *J*= 14, 1H), 3.26 (q, *J*= 14, 4H, CH_2), 0.94 (t, *J*= 24, 6H, CH_3); IR (KBr, cm^{-1}) λ_{max}: 3368 (NH), 3081 (NH), 1275, 702; Analysis (% Cal/fou) for $C_{15}H_{16}N_4O_5S$, C: 49.44/49.85, H: 4.43/4.56, N: 15.38/15.83.
16. Synthesis of 9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonic acid biphenyl amide (6p):
Yield: 44.44% (320 mg); mp 288–290°C; MS (EI): m/z (M+1) 460.99; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 9.34 (s, 1H, NH), 7.76 (d, *J* = 24, 1H), 7.64 (s, 1H), 7.42-7.38 (m, 5H), 7.22-7.02 (m, 5H), 6.88 (d, *J* = 16, 3H); IR (KBr, cm^{-1}) λ_{max}: 3366(NH), 3055, 1151, 744; Analysis (% Cal/fou) for $C_{23}H_{16}N_4O_5S$, C: 59.99/60.89, H: 3.50/4.20, N: 12.17/13.25.
17. Synthesis of 7-(morpholine-4-sulfonyl)-9-nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine (6q):
Yield: 43.32% (250 mg); mp 268–270°C; MS (EI): m/z (M-1) 377; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 9.42 (s, 1H, NH), 7.82 (d, *J*= 16, 2H), 7.22 (d, *J*= 12 Hz, 2H), 6.92 (t, *J*= 14, 1H), 3.64 (t, *J*= 20, 4H, N-CH_2), 2.96 (t, *J*= 22, 4H, O-CH_2); IR (KBr, cm^{-1}) λ_{max}: 3368 (NH), 3081 (NH), 702; Analysis (% Cal/fou) for $C_{15}H_{14}N_4O_6S$, C: 47.62/48.05, H: 3.73/4.35, N: 14.81/14.80.
18. Synthesis of 9-Nitro-10H-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonicacid(2-amino-5-nitro-phenyl)amide (6r):
Yield: 29.85% (200 mg); mp 282–283°C; MS (EI): m/z (M-1) 443; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 9.23 (s, 1H, NH), 8.68 (s, 1H, NH), 8.22 (s, 1H), 8.14 (d, *J*= 9.5 Hz, 1H), 8.02 (s, 1H), 7.86 (d, *J*= 9, 1H), 7.64 (s, 1H), 7.34-7.26 (m, 3H), 7.14-7.04 (m, 2H); IR (KBr, cm^{-1}) λ_{max}: 3399 (NH), 3088 (NH), 3329 (NH_2), 1159, 746; Analysis (% Cal/fou) for $C_{17}H_{12}N_6O_7S$, C: 45.95/45.05, H: 2.72/3.25, N: 18.91/18.80.

RESULTS AND DISCUSSION

Potassium-4-chloro-3,5-dinitrobenzenesulfonate (Scheme-1, 2) was prepared by treating chlorobenzene with a mixture of fuming sulfuric acid followed by nitration with potassium nitrate. It was converted to potassium; 9-nitro-10*H*-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonate (Scheme-1, 4) on reaction with 2-Amino-pyridin-3-ol (**3**). The potassium salt of pyridoxazine **4** on reaction with thionyl chloride gave 9-nitro-10*H*-benzo-[b]-pyrido-[2,3-e]-[1,4]-oxazine-7-sulfonyl chloride (Scheme-1, **5**). The acid chloride **5** on treatment with different aliphatic/aromatic/heteroaromatic amines in presence of triethylamine/pyridine in tetrahydrofuran/ dichloromethane yielded the title compounds **(**Scheme-1, **6a-6r).**

Scheme-1



R= a) H, b) phenyl, c) 4-methoxy phenyl, d) 4-chloro phenyl, e) cyclohexyl, f) 2-hydroxy ethyl, g) 4-sulfonic acid phenyl, h) 2-hydroxy phenyl, i) 2-amino phenyl, j) 3-hydroxy-2-pyridyl, k) 2-piridyl, l) NH2, m) NH-phenyl, n) methyl, o) diethyl, p) biphenyl, q)tetrahydro pyron, r) 2-amino-5-nitro phenyl

Reagents and conditions:

(a) Oleum, (70°C for 1h), Potassium nitrate (130°C). (b) NaOH, Methanol, Reflux/6h. (c) $SOCl_2$ Reflux/4h. (d) substituted amine, THF, 0°C/1h.

CONCLUSION

In the present study, 9-nitro-10*H*-benzo-[b]-pyrido-[2,3-e][1,4]oxazine-7-sulfonic acid amide and derivatives **(6a-6r)** were synthesized and characterized by IR, ^{1}H NMR and Mass spectral data together with elemental analysis.

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ANTIOXIDANT ACTIVITIES OF SOME N-MANNICH BASES OF SUBSTITUTED 2-MERCAPTO-1H-BENZIMIDAZOLES

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ABSTRACT

The antioxidants reduce the risk for chronic diseases including cancer and heart disease and play an important role as a health protecting factor. In view of this, a series of twenty four new [1-(*N,N*-disubstituted)aminomethyl-2-(2,4-dinitrophenyl)sulphanyl-6-substituted-1*H*-benzimidazoles (**16a-19f**) were synthesized by the Mannich reaction of 2-[(2,4-dinitrophenyl)sulphanyl]-5(or 6)-substituted-1*H*-benzimidazoles (**10-13**) with appropriate secondary amine and paraformaldehyde in presence of concentrated hydrochloric acid in ethanol. The synthesized compounds were characterized by elemental analysis, IR, ^{1}H NMR, and Mass spectral data. These were evaluated for antioxidant activity by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging method. Considerable number of *N*-Mannich bases have shown significant activity at different concentrations compared to standard.

KEYWORDS

2-Mercaptobenzimidazole, Mannich reaction, Antioxidant activity, DPPH, IC_{50}

INTRODUCTION

The antioxidants are chemical substances that avert oxidation of other substances by donation of an electron to the free radical and convert it to a harmless molecule. Moreover, they protect body from damage caused by oxidation, a process that causes damage in our tissues by action of free radicals. The nutrient antioxidants such as β-carotene, vitamin-C, vitamin-E, and selenium have been found to act as antioxidants. Benzimidazole nucleus a constituent of vitamin-B_{12}[1], exhibits a wide range of biological activities[2]. Furthermore, benzimidazole derivatives are structural isosters of naturally occurring nucleotides, which allow them to interact easily with the biopolymers of the living system. 2-Mercaptobenzimidazole and its derivatives are known to possess various biological activities such as antibacterial[3], antifungal[4], antiviral[5,6], anti-ulcerative[7], antitumor[8], antioxidant[9], absorbents[10], and are also important industrial inhibitors[11,12]. The benzimidazole-2-sulphides exhibit the antimicrobial, analgesic, anti-inflammatory and antioxidant activities[13-17]. Introduction phenyl ring with nitro substituent was shown to increase the antibacterial and antifungal

activity of heterocyclic systems[18]. Larry K. Keefer et al. found that the presence of 2,4-dinitrophenyl group enhances the antitumor activity both *in vitro* and *in vivo*[19]. Introduction of dialkylaminomethyl side chain on benzimidazole nucleus was found to enhance the antibacterial, antifungal, analgesic and anti-inflammatory activities[20-27]. It is therefore planned to introduce both the 2,4-dinitrophenyl and the dialkylaminomethyl side chain into mercaptobenzimidazole skeleton and screen the compounds for antimicrobial activities. We now report for the first time, the antioxidant activity of 1-(*N,N*-disubstituted)aminomethyl-2-(2,4-dinitrophenyl)sulphanyl-6-substituted-1*H*-benzimidazoles (Figure 1) in continuation of our work on Mannich reactions of substituted benzimidazoles[20-27].

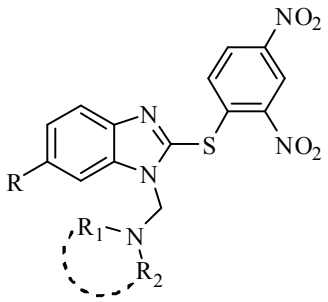


Figure 1: ***General structure of Mannich bases (19a-19f)***

MATERIAL AND METHODS

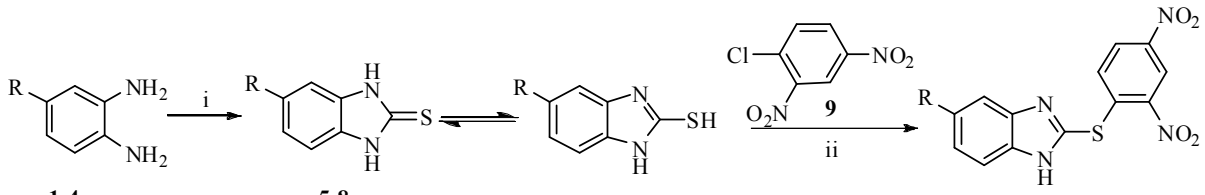
INSTRUMENTATION

Melting points of the synthesized Mannich bases were determined with an electro thermal melting point apparatus (Seatal Scientific Ltd.) and are uncorrected. Q Pro-M microwave sample preparation system was used for microwave assisted reactions. A 2450 MHz frequency Microwave oven was used that has an output range of 100-500 W with fiber optic sensor to control the temperature. The progress of the reactions was monitored by thin layer chromatography (TLC) using UV light or iodine vapor as visualizing agent. Mannich bases were purified by column chromatography using suitable solvents mixture as eluant. IR spectra (υ in cm^{-1}) were recorded on FT-IR spectrometer using KBr pellets, ^1H NMR (δ in ppm) spectra were recorded on 200 MHz / 400 MHz instrument using $CDCl_3$ or DMSO-d_6 as the solvent, and mass spectra were acquired on a Jeol TMS D-300 spectrometer operating at 75 eV. The elemental analysis of compounds was performed, and the analytical data obtained was found to be in good agreement with the calculated values.

SYNTHESIS

1H-Benzimidazole-2-thiols were prepared by refluxing respective o-phenylenediamine (**1-4**) with carbon disulphide in ethanol-water solution of sodium hydroxide[28]. The reaction between 2,4-dinitrochlorobenzene (**9**) and 5-(un)substituted-1*H*-benzimidazole-2-thiols (**5-8)** in presence of a base by conventional method and microwave induced method afforded 2-[(2,4-dinitrophenyl)sulphanyl]-5-(un)substituted-1*H*-benzimidazoles[26] (**10-13,** Scheme-1).

Scheme 1



1-4 **5-8** **10-13**

1: R = H
2: R = CH_3
3: R = OCH_3
4: R = NO_2

5: R = H
6: R = CH_3
7: R = OCH_3
8: R = NO_2

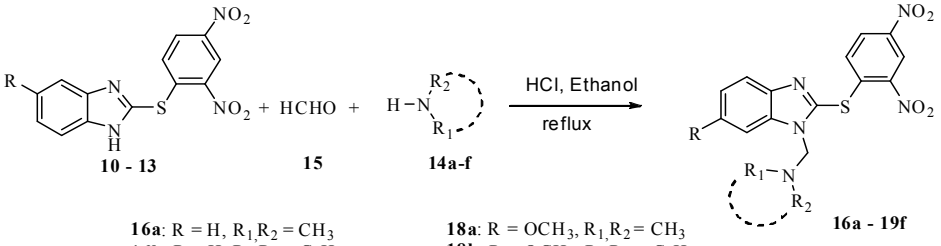
10: R = H
11: R = CH_3
12: R = OCH_3
13: R = NO_2

Reagents and conditions

(i) CS_2, NaOH, aq.ethanol, reflux (ii) a) conventional method: Ethanol, reflux, 3 h. b) microwave method: DMF, 2-5 min.

Mannich bases **16a-19f** were prepared by the reaction of **10-13** with cyclic/acyclic secondary amines **14a-14f** and paraformaldehyde (**15)** in presence of concentrated hydrochloric acid in alcohol[26] [**Scheme-2**]. The synthesized compounds were characterized by elemental analysis, IR, ^{1}H NMR, and Mass spectral data.

Scheme 2



10 - 13 **15** **14a-f** **16a - 19f**

16a: R = H, R_1R_2 = CH_3
16b: R = H, R_1R_2 = C_2H_5
16c: R = H, R_1R_2 = C_2H_4OH
16d: R = H, R_1R_2 = Morpholino
16e: R = H, R_1R_2 = Pyrrolidino
16f: R = H, R_1R_2 = Piperidino
17a: R = CH_3, R_1R_2 = CH_3
17b: R = CH_3, R_1R_2 = C_2H_5
17c: R = CH_3, R_1R_2 = C_2H_4OH
17d: R = CH_3, R_1R_2 = Morpholino
17e: R = CH_3, R_1R_2 = Pyrrolidino
17f: R = CH_3, R_1R_2 = Piperidino

18a: R = OCH_3, R_1R_2 = CH_3
18b: R = OCH_3, R_1R_2 = C_2H_5
18c: R = OCH_3, R_1R_2 = C_2H_4OH
18d: R = OCH_3, R_1R_2 = Morpholino
18e: R = OCH_3, R_1R_2 = Pyrrolidino
18f: R = OCH_3, R_1R_2 = Piperidino
19a: R = NO_2, R_1R_2 = CH_3
19b: R = NO_2, R_1R_2 = C_2H_5
19c: R = NO_2, R_1R_2 = C_2H_4OH
19d: R = NO_2, R_1R_2 = Morpholino
19e: R = NO_2, R_1R_2 = Pyrrolidino
19f: R = NO_2, R_1R_2 = Piperidino

ESTIMATION OF TOTAL ANTIOXIDANT ACTIVITY

The free radical scavenging activity of Mannich bases was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method of Blois, a method based on the reduction of a methanolic solution of the coloured DPPH radical.[29] 0.2 mM solution of DPPH and compounds solution were prepared in methanol. To estimate antioxidant activity, 0.1 ml solution of different compounds (i.e. 200, 400, 600 µ gm) were added to 2.5 ml of methanol and 0.5 ml of 0.2 mM of DPPH solution. The solution was mixed thoroughly and absorbance was noted at 517 nm against blank using spectrophotometer method. All the tests were performed in triplicate and the results averaged. Ascorbic acid was used as a reference standard and the antioxidant activity was expressed in terms of % inhibition of 0.2mM DPPH. The standard graph was plotted using different concentrations of

ascorbic acid and the antioxidant values were expressed in terms of nM of ascorbic acid. The percentage reduction in absorbance was calculated from the initial and final absorbance of each solution.[30] The percentage inhibition was calculated by comparing the absorbance values of control and samples. Percentage scavenging of DPPH radical was calculated using the formula,

$$\% \text{ Scavenging of DPPH} = \frac{[\text{Absorbance of Control- Absorbance of Test}]}{[\text{Absorbance of Control}]} \times 100$$

IC_{50} value is a measure of the effectiveness of a compound in inhibiting biological or biochemical function and represents the concentration of drug that is required for 50% inhibition in vitro. IC_{50} values of compounds under investigation were determined by constructing dose response curve and are presented (Table 1) along with standard antioxidant ascorbic acid.

RESULTS AND DISCUSION

A series of new [1-(*N,N*-disubstituted)aminomethyl-2-(2,4-dinitrophenyl)sulphanyl-6-substituted-1*H*-benzimidazoles (**16a-19f**) were synthesized by the Mannich reaction of 2-[(2,4-dinitrophenyl)sulphanyl]-5(or 6)-substituted-1*H*-benzimidazoles (**10-13)** with appropriate secondary amine and paraformaldehyde in presence of concentrated hydrochloric acid in ethanol.

In vitro antioxidant activity of test compounds was assessed by measuring DPPH scavenging activity. The electron donating ability of the test compounds was measured spectrophotometrically and compounds have shown moderate to strong antioxidant activities (Table 1). The IC_{50} value of ascorbic acid was 116.6 and values of IC_{50} were found to be inversely proportional to antioxidant activity. Most of the test compounds of synthesized have shown moderate antioxidant activity which is expressed in terms of percentage inhibition of DPPH. The morpholino substituted **(16d)** and piperidino substituted **(16f)** Mannich bases of 2-[(2,4-dinitrophenyl)sulphanyl]-1*H*-benzimidazole were exhibited excellent antioxidant activity and its activity is more pronounced than the standard drug ascorbic acid. Out of all the test compounds **18a** has shown minimum antioxidant activity where as compounds **16c, 17c, 17f, 18c, 18e, 18f, 19a** and **19c** were good active remaining test compounds moderate to less active.

Table 1
Antioxidant activities of Mannich bases

S. No.	Compounds	% inhibition of DPPH			IC_{50}
		200(µg/ml)	400(µg/ml)	600(µg/ml)	
1	16a	42.1	60.7	65.7	237.5
2	16c	52.2	65.7	89.2	192.3
3	16d	74.1	89.3	98.9	**134.9**
4	16e	35.4	53.7	64.08	371.0
5	16f	63.5	82.3	90.4	**157.8**
6	17a	38.7	52.2	65.7	383.1
7	17c	49.5	67.2	80.0	202.0
8	17d	37.6	57.2	62.3	349.6
9	17e	47.1	66.7	72.1	212.3
10	17f	51.4	77.5	88.2	194.5
11	18a	38.2	48.2	66.1	408.9
12	18c	50.7	60.7	96.0	197.2
13	18d	38.7	53.9	86.0	371.0
14	18e	49.2	57.3	96.8	203.2
15	18f	50.5	72.4	90.8	198.0
16	19a	52.2	69.1	98.2	191.5
17	19c	50.8	60.7	70.8	196.8
18	19d	48.9	52.2	64.0	204.4
19	19e	48.2	64.0	70.7	207.4
20	19f	50.3	67.2	71.5	198.8
21	Ascorbic acid	60.2	89.5	96.04	166.6

CONCLUSIONS

A series of *N*-Mannich bases were synthesized and tested for antioxidant activity against the DPPH radical was performed by the spectrophotometrically measurement of DPPH consumption in the presence of antioxidants. It is concluded that most of the Mannich bases of benzimidazoles were effective antioxidants. Among the Mannich bases **16d** and **16f** showed better antioxidant activity than the standard.

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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NEW 3,4-DIHYDRO-2H-BENZO-[1,4]-OXAZINE-7-SULFONAMIDE DERIVATIVES AS ANTI DIABETIC AGENTS

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ABSTRACT

In this study, a library of new 5-nitro-3,4-dihydro 2*H*-benzo [1,4] oxazine-7- sulfonamides (**5a-5t**) were synthesized and evaluated for their *in vivo* anti diabetic activity. All the compounds were prepared in a multistep process involving the initial preparation of potassium; 5-nitro-3,4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonate (4a). **4a** was converted to 5-nitro-3,4-dihydro-2*H*-benzo-[1,4]-oxazine-7- sulfonamides (**5a-5t**). The structures of the compounds were established on the basis of spectral (IR, ^{1}HNMR, ^{13}CNMR, and Mass) data. All the synthesized compounds were subjected to oral glucose tolerance test (OGTT) to gain preliminary information regarding the anti-diabetic activity. They were evaluated for anti-diabetic activity using glibenclamide as the standard. Among the test compounds **4a, 5m** were significant in their anti-diabetic activity in comparison with standard. All the remaining were poor anti-diabetic in nature.

KEYWORDS

3, 4-Dihydro 2*H*-benzo [1,4] oxazine, Sulfonamide, Anti-diabetic activity.

INTRODUCTION

Benzoxazine and its derivatives are used in organic synthesis for building natural compounds and to design synthetic compounds which have been used as a suitable skeleton for the synthesis of biologically active compounds. Benzoxazines are generated by the Mannich condensation of phenol, formaldehyde and a primary amine[1]. Aromatic oxazines were first synthesized by Holly and Cope in 1944 through Mannich reaction from phenols, formaldehyde, and amines[2]. The literature survey updated information on several benzoxazine derivatives in the development phase as potential new drugs. Among different isomers of benzoxazines, 1,4-benzoxazine derivatives exhibit wide range of biological, therapeutic properties and pharmacological activities such as central nervous system drugs[3], calcium channel antagonists[4], analgesics[5] and others[6]. 3,4-Dihydro-*2H*-1,4-benzoxazines constitute an interesting group, which are key intermediates in several synthetic pathways

for the preparation of bioactive polycyclic heterocyclic systems. The versatility of the benzoxazine skeleton, its chemical simplicity, makes these chemicals the most promising sources of bioactive compounds. 3,4-Dihydro-2*H*-benzo[1,4]oxazin-8-yloxy acetic acid derivatives are reported as dual thromboxane A_2 receptor antagonists and prostacyclin receptor agonists[7]. Carboxamide derivatives of 3,4-dihydro-2*H*-1,4-benxoxazines as potent serotonin-3-(5-HT_3) receptor antagonists was reported[8,9,10]. Recently, a series of 3,4-dihydro-2*H*-1,4-bezoxazine derivatives as a novel class of anti-thrombotic compounds with dual function of thrombin inhibitory and glycoprotein IIb/IIIa (GpIIb/IIIa) receptor antagonistic activities was reported[11]. Novel benzoxazine compounds have been synthesized by Wang et.al, which are protective in tissue culture[12]. Dong et.al group reported recently a new series of 6-amino-2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine derivatives. This series of compounds could improve the proliferation of human umbilicalvein endothelial cells without basic fibroblast growth factor and serum[13]. Recently, a short review on pharmacological profile of benzoxazines was reported[14]. Several 1,4-benzoxazines and their dihydro derivatives are frequently found in naturally occurringcompounds[15] and pharmacologically active substances including drugs[16] (e.g., Levofloxacin). The presence of sulfonic acid group at C-3 position of benzoxazine nucleus imitate like p-aminobenzoicacid pharmacophore in benzoxazine moiety. This sulfonicacid group was converted to sulfonamide group, an important pharmacophore in medicinal chemistry. Sulfonamides were amongst the oldest chemically synthesised compounds which are still widely used for the treatment of various bacterial, protozoal and fungal infections[17]. Recently, we reported the synthesis of1-aryl imidazo[4,5,1-k*l*]phenoxazine-4-sulfonamide derivatives,[18] starting from 2-aminophenol and potassium-4-chloro-3,5-dinitro benzene sulfonate. Due to the interesting pharmacological importance of 1,4-benzoxazines and our long standing interest in synthesising the novel heterocyclic systems with promising biological activity. Herein, we report a new series of sulphurnamide derivatives of 5-nitro-3,4-dihydro-2*H*-benzo[1,4]oxazine. While there are many synthetic routes for the preparation of 1,4-benzoxazines[19], incorporation of two substituents, nitro and sulfonic acid groups in to benzoxazine moiety in a single step is a unreported method. The basic synthon potassium-4-chloro-3,5-dinitrobenzene sulfonate[20] which was used to build heterocyclic nuclei in previous work has been used in the present synthesis. Keeping in view, the target compound (**4a**) was synthesised by using ethanol amine. The applications of sulphurnamides has been extended from their primary function as antimicrobial agents to targets such as anti-cancer, anti-inflammatory, anti-diabetic, anti-viral, COX-II inhibitors, loop diuretics, carbonic anhydrase inhibitors and even as anti-impotence drugs[21].

MATERIAL AND METHODS

EXPERIMENTAL DATA

All reagents used were commercial grade. Unless otherwise noted reactions were performed under nitrogen atmosphere. Progresses of reaction were monitored by TLC using silicagel plates. The spots were located by ultra violet light and iodine vapours spray. Column chromatography was performed on silica gel (60-120 mesh) using hexane, ethyl acetate, dichloromethane and methanol. Melting points are measured on a melting point apparatus and are uncorrected. Infrared spectra were obtained on a FTIR (Perkin- Elmer 383 Spectrophoto meter) using KBr discs. ^{1}H NMR spectra were recorded on 200 & 400 MHz spectrometer in DMSO-d6 solution and the chemical shifts were reported relative to internal standard TMS (δ ppm). The following abbreviations are used to describe peak patterns where appropriate: s= singlet, d= doublet, t= triplet, q= quartet, m= multiplet, b= broad. Coupling constant (*J*) are reported in Hertz (Hz). ^{13}CNMR were recorded in 100 MHZ and referred to the internal solvent. Mass Spectra were obtained on a mass spectrometer using LCMS & EI Ionization.

CHEMISTRY

1. Synthesis of potassium; 4-(2-hydroxy ethyl amino)-3,5-dinitrobenzenesulfonate (3)

To a solution of ethanol amine (**2)** (3.75g, 62.5 mmol) in alcohol (30 ml), sodium acetate (10.8g, 125 mmol) was added and refluxed for 1hour. To this clear solution, compound **1** (20 g, 62.5 mmol) was added, the resulting mixture was refluxed for 2 hours and the completion of the reaction was monitored by TLC eluted in 1:9 methanol in ethyl acetate. The reaction mass was then cooled to room temperature, separated yellow coloured solid was filtered under reduced pressure and dried, yield 16.5 gm (86.3%), mp 292-293^{0}C. ^{1}HNMR (200 MHz, DMSO- d_6): δ (ppm): 8.58 (t, *J* = 4.0 Hz, NH), 8.32 (s, 2H), 5.10 (t, *J* = 6.0 Hz, OH), 3.57 (q, *J* = 4.0 Hz, 2H), 2.97 (q, *J* = 4.0 Hz, 2H). Mass: calculated for $C_8H_9N_3O_8S$ 307, found 306(M-H$^+$).

2. Synthesis of Potassium; 5-nitro-3,4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonate (4a)

To a solution of **3** (10g, 32.5 mmol) in alcohol (30 ml), sodium acetate (10.5 g, 130 mmol) was added and refluxed for 4 hours, the completion of the reaction was monitored by TLC eluted in 2:8 methanol in ethyl acetate. The reaction mass was then cooled to room temperature, yellow coloured solid was filtered under reduced pressure. The product was washed twice with limited amount of cold methanol. mp: above 300°C, yield: 8.1g, (85%). ^{1}HNMR (400MHz , DMSO- d_6): δ (ppm): 8.35 (s, 2H), 8.29 (t, *J* = 4.8 Hz, NH), 3.88 (t, *J*=8 Hz, 2H), 3.241 (q, *J*=5.2 Hz, 2H). ^{13}CNMR (100MHz, DMSO-d_6) δ (ppm): 139.8, 136.5, 134.0, 131.6, 128.8, 122.2, 59.1, 48.3. IR (KBr, cm^{-1}) υ_{max}: 3348 (NH), 3070 (aryl CH), 2937 (aliphatic CH), 1526, 1341(NO_2), 1261, 1057 (SO_2) 1228 (aryl C-O), 925, 908, 761, 727. Mass: calculated for $C_8H_8N_2O_6S$: 260.23, found: 259.9, 260 (M$^+$), 300 (M+K).

3. General procedure for synthesis of 5-nitro-3,4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonamide derivatives (5a-5t)

To compound 4a (2g, 0.0671mol) in dimethylformamide (0.2 ml), thionyl chloride (10 ml) was added and refluxed under nitrogen atmosphere for 3hours. Progress of reaction was monitored by TLC using 30% chloroform in hexane. The excess solvent was removed from the reaction mass under reduced pressure to get residue 5-nitro-3,4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonyl chloride (4b) (3.0mmol). It was immediately used for the next step. To a solution alkyl/aryl/hertero aryl amine (3.0 mmol) in dichloromethane / tetrahydrofuran (10 ml) was added the compound (4b) and triethylamine (0.2 ml) at 0-5°C. The reaction mass was then stirred for 2 hours at room temperature and refluxed for another 1 hour. Completion of the reaction was monitored by TLC by eluting in 10 % methanol in ethyl acetate. Excess solvent was distilled off under vacuum. The product thus separated was filtered under suction and dried. Crude residue was purified by column chromatography with 1:9 methanol in chloroform as eluting system.

4. 5-Nitro-3,4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonic acid(phenyl)amide (5a)

Yield 0.72 g (72.1 %), mp 297-298°C. m/z: calculated: 335.03, Found: 335.09. ^{1}HNMR (200 MHz, DMSO-d_6): δ (ppm): 10.28 (s, SO_2NH), 8.36 (s, 2H), 8.28 (t, *J* = 6.0 Hz, NH), 7.25-7.50 (m, 5H), 3.85 (t, *J* = 4.0 Hz, 2H), 3.24 (q, *J* = 6.0 Hz). ^{13}CNMR (100 MHz, DMSO:158.1, 153.4, 151.6, 143.6, 131.1, 128.0, 125.6, 125.4, 120.9, 115.4, 115.0, 114.6, 55.5, 55.4. IR (KBr, cm^{-1}) υ_{max}: 3329, 3232, 3063, 2943, 1498, 1529, 1348, 1276, 1156, 1276, 944, 764.

5. 5-Nitro-3,4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonic acid (4-methoxy-phenyl)amide (5b)

Yield 0.93g (75.6%), mp: 234-235 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm): 10.30 (s, NH), 8.36 (s, 2H), 8.25 (t, *J* = 2.0 Hz, NH), 7.30 (d, *J* = 8.0 Hz, 2H), 7.01 (d, *J*=8.0 Hz, 2H), 3.90 (t, *J* = 4.0 Hz, 2H) , 3.76 (s, 3H), 3.23 (q, *J*=6.0 Hz). ^{13}C NMR (100M Hz, DMSOd_6) δ (ppm) 135.2, 129.0, 128.98, 128.95,

128.7, 48.4, 44.1, 34.0; IR (KBr, cm^{-1}) υ_{max}: 3419, 3308, 3096, 2841, 1571, 1523, 1343, 1276, 1201, 1239, 910, 764, 725, 680.

6. 5-Nitro-3,4-dihydro-2*H*- benzo-[1,4]-oxazine-7-sulfonic acid o tolyl amide (5c)

Yield 0.72 g (63.5 %), mp 245-246 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 10.60 (s, 1H), 8.1 (s, 2H), 8.06 (s, 1H), 7.7 (s, 1H), 7.39 (d, *J* = 8.0 Hz,1H), 7.03 (d, *J* = 8.0 Hz, 1H), 6.9 (s, 1H), 3.70 (s, 3H), 3.2 (s, 2H), 3.1 (s, 2H).

7. 5-Nitro-3,4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonic acid (2-nitro-phenyl)-amide (5d)

Yield 0.38g (50.9%), mp. 284-286°C. ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm): 158.1, 153.4, 151.6, 143.6, 133.4, 131.1, 128.0, 125.6, 124.4, 120.9, 115.0, 114.6, 55.5, 55.1. IR (KBr, cm^1) υ_{max}: 3419, 3307, 3096, 2947, 1572, 1524, 1506, 1344, 1277, 1239, 1201, 1050, 910, 873, 765, 680.

8. 5-Nitro-3,4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonic acid (2-aminoethyl)-amide (5e)

Yield 0.78 g (78.2 %), mp. 256-257°C. ^{1}HNMR (200 MHz, DMSO-d_6): δ (ppm): 8.8 (s, NH), 8.37 (s, 2H), 8.25 (t, *J* = 6 Hz, NH), 8.04 (s, NH_2), 3.86 (t, *J*= 4.0 Hz, 2H), 3.56 (t, *J* = 4.0 Hz) 3.15 (q, *J*= 6.0 Hz) 2.86 (q, *J*= 4.0 Hz, 2H). IR (KBr, cm^1) υ_{max}: 3365, 3331, 3254, 3095, 2979, 1575, 1536, 1501, 1332, 1287, 1267, 1144, 1091, 938, 758.

9. 5-Nitro-3,4-dihydro-2*H*- benzo-[1,4] oxazine-7-sulfonic acid (amino)-amide (5f)

Yield 0.5.g (55.5%), mp 214-216 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm): 8.78 (s, 3H, NH), 8.34 (s, 2H), 8.3 (t, NH, *J* = 2.0 Hz, NH), 3.84 (t, *J* = 4.0 Hz, 2H), 3.26 (q, *J*= 6.0 Hz).

10. 5-Nitro-3,4-dihydro-2*H*- benzo-[1,4]-oxazine-7-sulfonic acid cyclohexyl amide (5g)

Yield 0.86g (74.7%), mp 224-225 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm): 8.34 (s, 2H), 8.25 (t, *J* = 6 Hz, NH), 7.82 (s, NH), 3.84 (t, *J* = 4.0 Hz, 2H), 3.26 (q, *J* = 6.0 Hz), 2.93 (s, 1H), 1.90 (s, 2H), 1.76 (s, 2H), 1.58 (s, 1H), 1.25 (t, *J* = 8 Hz). IR (KBr, cm^{-1}) υ_{max}: 3308, 3226, 3076, 2940, 2864, 1524, 1341, 1279, 1241, 1180, 1276, 1040, 993, 763, 727.

11. 5-Nitro-3,4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonic acid (2-hydroxy-ethyl) - amide (5h)

Yield 0.72g (73.2%), mp 229-230 °C. m/z: calculated: 303.99, found: 305.1 ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm): 8.39 (s, 2H), 8.28 (t, *J* = 6 Hz, NH), 8.02 (s, NH), 5.08 (s. OH), 3.84 (t, *J* = 4.0 Hz, 2H), 3.58 (t, *J* = 4.0 Hz), 3.15 (q, *J* = 6.0 Hz), 2.87 (q, *J*= 4.0 Hz, 2H). IR (KBr, cm^{-1}) υ_{max}: 3550, 3332, 3288, 3100, 2979, 1575, 1533, 1501, 1323, 1286, 1147, 1049, 878, 755.

12. 5-Nitro-3, 4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonic acid isoquinolin-8-yl amide (5i)

Yield 0.5 g (55.5 %), mp 238-239 °C. m/z: calculated: 386.0, found: 386.2. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm): 9.9 (s, NH), 8.40 (s, 1H), 8.35 (s, 2H), 8.23 (t, *J* = 6.0 Hz), 8.19 (d, *J* = 4.0 Hz, 2H), 7.82 (t, *J* = 6.0 Hz, 1H), 7.23 (d, *J* = 4.0 Hz, 1H), 3.82 (t, *J* = 6.0 Hz, 2H), 3.24 (q, *J* = 4.0 Hz, 2H). IR (KBr, cm^{-1}) υ_{max}: 3508, 3196, 3049, 2979, 1524, 1506, 1276, 1239, 1201, 1049, 996, 910, 835, 765

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13. 5-Nitro-3, 4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonic acid ethyl amide (5*J*)

Yield 0.52 g (57.6%), mp 287-289 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 8.71(s, NH), 8.37 (s, 2H), 8.26 (t, *J* = 12.0 Hz, NH), 3.89 (t, *J* = 8.0 Hz, 2H), 3.25 (q, *J* =12.0 Hz, 2H), 2.86 (m, 2H), 1.20 (t, *J* = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm): 139.4, 139.3, 137.3, 137.2, 135.2, 128.7, 48.4, 44.2,

44.1, 34.0. IR (KBr, cm^{1}) υ_{max}: 3431, 3307, 3096, 2949, 2779, 1571, 1524, 1344, 1277, 1239, 1201, 1049, 910, 872, 765, 725, 680.

14. 5-Nitro-3, 4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonic acid diphenylamide (5k)
Yield 0.94g (69.1%), mp: 276-278°C.

15. 5-Nitro-3, 4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonic acid (2-hydroxy-phenyl)amide (5l)
Yield 0.73 g (61.3 %), mp: 287-289 °C. m/z: calculated: 351.3, found: 352.1. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 10.5 (s, NH), 8.1 (s, 2H), 8.06 (t, *J* = 8.0 Hz, NH), 7.7 (s, 1H), 7.4 (s, 1H), 7.04 (s, 1H), 6.7 (s, 1H), 4.5 (s, OH), 3.8 (q, *J* = 8.0 Hz, 2H), 3.57 (t, *J* = 6.0 Hz, 2H).

16. 5-Nitro-3,4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonic acid (4-chloro-phenyl)amide (5m)
Yield 0.53 g (59.3%), mp: 287-289°C.

17. 5-Nitro-3,4-dihydro-2*H*- benzo-[1,4]-oxazine-7-sulfonic acid furan-2-yl amide (5n)
Yield 0.65 g (70.3%), mp: 276-277 °C, m/z: calculated: 339.3, found: 339.1. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm): 8.61 (t, *J* = 6.0 Hz, NH), 8.45 (s, 2H), 7.70 (s, 1H), 6.53-6.59 (m, 2H), 6.18-6.20 (m, 1H), 4.14 (d, *J* = 8.0 Hz, 2H), 3.90 (t, *J* = 4.0 Hz, 2H), 3.25 (q, *J* = 6.0 Hz). IR (KBr, cm^{1}) υ_{max}: 3302, 3281, 3131, 3077, 2979, 2706, 2614, 1623, 1536, 1527, 1338, 1278, 1238, 1177, 1138, 1010, 916, 826, 752, 678.

18. 5-Nitro-3, 4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonic acid (2-amino-phenyl)amide (5o)
Yield 0.68 g (62.2 %), mp: 236-237 °C, m/z: calculated: 350.9, found: 349.9. ^{13}C NMR (100 MHz, DMSO-d_6): 158.1, 152.4, 151.6, 143.6, 133.4, 131.1, 128.0, 125.6, 123.9, 120.9, 115.0, 114.6, 55.5, 43.1. IR (KBr, cm^{1}) υ_{max}: 3365, 3331, 3254, 3095, 2979, 1575, 1536, 1501, 1332, 1287, 1267, 1144, 1091, 938, 758.

19. 7-(Morpholine-4-sulfonyl)-5-nitro-3,4-dihydro-2*H*-benzo-[1,4]-oxazine (5p)
Yield 0.42.g (65.5%), mp 218-220 °C. m/z: calculated: 329.3, found: 330.9. ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 139.3, 137.3, 137.2, 135.2, 128.8, 128.7, 48.5, 48.4, 44.1, 44.0, 41.2, 34. IR (KBr, cm^{1}) υ_{max}: 3275, 3082, 2879, 2842, 2691, 2604, 1538, 1514, 1274, 1180, 1102, 1050, 933, 909, 763, 699.

20. 5-Nitro-3,4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonic acid (4-acetyl-phenyl)amide (5q)
Yield 0.38 g (55.2 %), mp: 242-244°C. m/z: calculated: 377.3, found:378.1.

21. 4-(5-Nitro-3,4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonyl amino)benzoic acid methyl ester (5r)
Yield 0.35 g (51.6 %), mp: 267-268°C.

22. 5-Nitro-3,4-dihydro-2*H*-benzo-[1,4]-oxazine-7-sulfonic acid (2,4-dinitro-phenyl)amide (5s)
Yield 0.78 g (60.9 %), mp 294-296 °C. m/z: calculated:425.3, found: 423.9.

23. 7-(N,N-dimethylamino-4-sulfonyl)-5-Nitro-3, 4-dihydro-2*H*- benzo [1, 4] oxazine (5t)
Yield 0.42.g (65.5 %), mp 221-223°C.

PHARMACOLOGICAL EVALUATION

1. Effect on Oral Glucose Tolerance Test (OGTT)

15 test compounds are used for this test. The animals were divided into 17 groups (n =6)

Group I: Rats served as normal-control and received water.

Group II: Rats served as standard and received glibenclamide (10 mg/kg).
Group III – Group XVII: Rats were administered (10 mg/kg b.wt) orally with test compounds.

For Oral Glucose Tolerance Test, overnight fasted animals were loaded with glucose (2 g/kg i.p), 60 min after the administration of test compounds and the blood Samples were collected on 0, 30, 60, 90, 120 minutes time Interval and the blood glucose levels were determined by making use of SUGAR SCAN Glucometer (Thyrocare).

Table 1.
Oral Glucose Tolerance Test

Group	Treatment	Dose (Kg^{-1} b.wt)	Blood Glucose levels (mg/dl)				
			0 mins	15 mins	30 mins	60 mins	120 mins
I	Control (Water)	2ml	78.83±1.302	109.16±2.798	108± 1.368	94.83±0.945	94.33± 0.557
II	Standard (GBC)	10mg	81.16±1.327^{ns}	105.16±1.74^{ns}	99.33± 1.358**	84.833±1.167**	84.5± 0.922**
III	Test Compound 4a	10mg	97±0.577**	144.5±1.708**	89.33±1.406**	75.5±3.082**	73±0.856**
IV	Test Compound 5a)	10mg	85±2.03^{ns}	158.16±1.515**	85.16±1.815**	74.166±1.078**	71.66±0.881**
V	Test (Compound 5b)	10mg	82.16±0.703^{ns}	161.83± 1.302**	125.83±1.515**	112.33± 1.406**	80.83±1.352^{ns}
VI	Test (Compound 5c)	10mg	86.5± 1.72^{ns}	133.16± 1.167**	121.83± 0.94**	110.66± 1.687**	99.83±1.470**
VII	Test (Compound 5d)	10mg	81.66±0.881^{ns}	138± 1.155**	130±1.528**	110.33± 1.282**	100.33±1.476**
VIII	Test (Compound 5e)	10mg	89.16±1.167**	147.33±1.145**	127.5± 1.057**	112.83± 1.352**	101.5± 1.147**
IX	Test (Compound 5f)	10mg	75.33±1.874**	155± 1.438**	136.16±1.537**	124.33± 2.06**	112.66±1.382**
X	Test (Compound 5g)	10mg	80.83± 1.352^{ns}	155.166± 1.6**	144.83±1.621**	124.5± 1.784**	118.33±1.585**
XI	Test (Compound 5h)	10mg	71.5± 1.118**	120.83± 1.167**	102.33±1.667**	88.66± 0.88**	79.33±1.116**
XII	Test (Compound 5i)	10mg	76.66± 1.054^{ns}	154.66± 1.33**	134.5± 1.176**	124.83± 1.493**	105.5± 1.727**
XIII	Test (Compound 5j)	10mg	76.83± 1.249^{ns}	135.83± 1.647**	127.16±1.078**	114± 1.528**	102.66±1.498**
XIV	Test (Compound 5k)	10mg	81± 1.414^{ns}	157.16± 1.352**	143.5± 1.893**	128.33± 2.362**	115.33±2.275**
XV	Test (Compound 5l)	10mg	75.33± 1.476**	134.166±1.195**	127.83±0.749**	122± 0.966**	110.66±1.430**
XVI	Test (Compound 5m)	10mg	79.33± 1.892^{ns}	140.83± 1.195**	120.83±1.641**	100.33± 1.498**	85.33± 2.231**
XVII	Test (Compound 5o)	10mg	88.5± 0.99**	155.83± 2.509**	136.16±1.302**	123± 1.506**	109.166±1.302**

*Comparision: Group II v/s Group I and Group II v/s Group III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XV, XVI and XVII. Statistical significance test for comparisions were done by ANOVA, followed by Dunnet's multiple comparision test. N=6; Values are expressed in mean ± SEM; *p<0.05. **p<0.01, ***p<0.01 Compare with Diabetic rats.*

2. Effect on Diabetic rats (Alloxan induced rats)

Compound (4a), Compound (5a), Compound (5b), Compound (5h), Compound (5m) were selected for the study based on Oral Glucose Tolerence Test (Table.1).

1) Experimental Induction of Diabetes: The animals (male rats) were fasted for 24 h and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of alloxan monohydrate (130 mg/kg) in icecold 0.9% saline (NaCl) solution. The animals were given 2 ml of 5% dextrose solution using orogastric tube immediately after induction to overcome the drug induced hypoglycemia.Seventy two hours later, rats with blood glucose levels (BGLs)above 200 mg/dL were considered diabetic and selected for the experiment.
2) Grouping of animals: The rats were divided into eight groups of six (n=6) each randomly.
Group I: Rats served as normal- control and receives 5% gum acacia
Group II: Diabetic rats received 5% gum acacia served as diabetic control
GroupIII: Diabetic rats served as standard received glibenclamide (10mg/kgb.wt).
Group IV – Group VIII: Rats were administered with (test compounds, 10 mg/kg b.wt) orally.

On the final day of the study blood was collected form the retro-orbital sinus and parameters like blood glucose levels, SGPT, SGOT, Total cholesterol, triglycerides, HDL, LDL levels are estimated. Blood glucose levels are estimated by making use of GOD-POD method SGPT, SGOT by 2,4-DNPH method

Table 2

Body Weights in Diabetic Rats and in Normal Rats (Sub Acute Study)

Group	Treatment	Dose (Kg^{-1} Body Weight)	Body Weights(gms)				
			Intial Weight	1st Day	3rd Day	7th Day	14th Day
I	Control (Water)	2ml	188.33±6.009	188.33±6.009	187.5± 6.021	186.66±6.280	186.66± 6.280
II	Diabetic control+ (Alloxan)	130mg	180± 7.303^{ns}	173.33±6.667^{ns}	168.33±6.009^{ns}	157.5±6.158^{ns}	145.83±5.388*
III	Standard (GBC+Alloxan)	10mg	167± 5.737^{ns}	172.5±5.737^{ns}	178.33±5. 869^{ns}	180.833±5.540^{ns}	185±5.164*
IV	Test (Compound4a)+Alloxan)	10mg	175± 8.507^{ns}	149.166±11.649^{ns}	155.833± 12.343^{ns}	159.166±11.649^{ns}	162.5±11.087^{ns}
V	Test (Compound5a)+Alloxan)	10mg	170.833±8.207^{ns}	151.66±8.724^{ns}	150± 9.574^{ns}	155.83± .308^{ns}	160±8.165^{ns}
VI	Test (Compound5b)+Alloxan)	10mg	190.83±9.867^{ns}	175± 7.853^{ns}	169.166± 9.523^{ns}	180± 9.220^{ns}	188.33±7.032*
VII	Test (Compound5h)+Alloxan)	10mg	195.83±8.207^{ns}	169.166±7.35^{ns}	169.166± 9.435^{ns}	180± 9.661^{ns}	202.5±13.525**
VIII	Test (Compound5m)+Alloxan)	10mg	212.5±10.468*	192.5±12.093^{ns}	191.66±12.494^{ns}	196.66±12.225*	203.33±11.738**

*Comparision: Group II v/s Group I and Group II v/s Group III, IV, V, VI, VII and VIII. Statistical significance test for comparisions were done by ANOVA, followed by Dunnet's multiple comparision test. N=6; Values are expressed in mean ± SEM; *p<0.05. **p<0.01, ***p<0.01 Compare with Diabetic rats.*

Body weights.

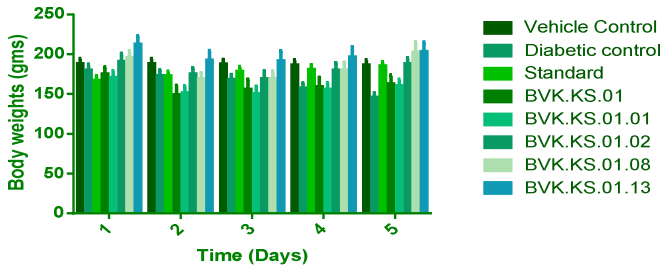

Figure 1

*Body weights in normal and diabetic rats, values are expressed in mean ± SEM, on various days of the study, N=6 in each group, *p<0.05. **p<0.01, ***p<0.01 when compared with diabetic control group dunnet's test.*
BVK.KS.O1-Compound (4a); BVK.KS.01.01-Compound (5a); BVK.KS.01.02-Compound (5b), BVK.KS.01.08-Compound (5h); BVK.KS.01.13-Compound (5m).

Table 3.

Anti diabetic effect of Test Compounds

Group	Treatment	Dose (Kg^{-1}Body Weight)	Blood Glucose Levels(mg/dl)			
			0 min	60 min	120 min	180 min
I	Control(Water)	2ml	94.33±1.994	93.66±1.174	96.33±2.216	94.5±2.217
II	Diabetic control (Alloxan)	130mg/kg	488.833±36.697**	472.66±32.543**	474.16±32.185**	482.66±35.08**
III	Standard(GBC+Alloxan)	10mg/kg	410.66±22.399^{ns}	308.83±19.272**	293.5±18.929**	157.66±6.211**
IV	(CompoundI4a+Alloxan)	10mg/kg	485.5±15.163^{ns}	460.5±15.414^{ns}	401.33±19.820^{ns}	370±19.370*
V	(Compound5a +Alloxan)	10mg/kg	503.5±50.775^{ns}	460.16±44.051^{ns}	431.5±42.171^{ns}	404.33±39.356^{ns}
VI	(Compound5b +Alloxan)	10mg/kg	528.33±23.511^{ns}	495.66±24.909^{ns}	461.5±29.616^{ns}	429.33±31.395^{ns}
VII	(Compound5h +Alloxan)	10mg/kg	507.833±45.310^{ns}	480.16±46.552^{ns}	443.166±46.789^{ns}	420.5±46.238^{ns}
VIII	(Compound5m +Alloxan)	10mg/kg	454.166±9.971^{ns}	431.5±8.86^{ns}	384.166±12.001^{ns}	358.5±8.484*

*Comparision: Group II v/s Group I and Group II v/s Group III, IV, V, VI, VII and VIII. Statistical significance test for comparisions were done by ANOVA, followed by Dunnet's multiple comparision test. N=6; Values are expressed in mean ± SEM; *p<0.05. **p<0.01, ***p<0.01 Compare with Diabetic rats.*

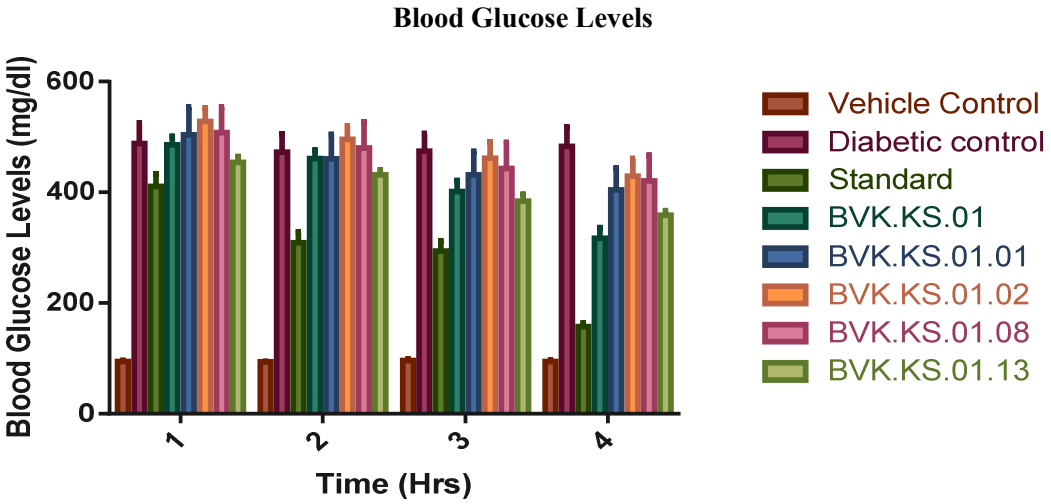

Figure 2

*Blood glucose levels in normal and diabetic rats, values are expressed in mean ± SEM, on day one, N=6 in each group, *p<0.05. **p<0.01, ***p<0.01 when compared with diabetic control group dunnet's test. BVK.KS.O1-Compound (4a); BVK.KS.01.01-Compound (5a); BVK.KS.01.02-Compound (5b), BVK.KS.01.08-Compound (5h); BVK.KS.01.13-Compound (5m).*

Table 4
Anti diabetic effect of Test compounds (sub-acute study)

Group	Treatment	Dose (Kg^{-1}Body Weight)	Blood Glucose Levels(mg/dl)			
			1st Day	3rdDay	7thDay	14thDay
I	Control(Water)	2ml	94.33±1.994	95.16±1.740	98±1.125	95.66±1.358
II	Diabetic control (Alloxan)	130mg/kg	488.83±36.697**	490.33±32.478**	480.33±38.522**	477±37.487**
III	Standard(GBC+Alloxan)	10mg/kg	410.66±22.399^{ns}	363.166±20.247*	242.5±13.119**	131.833±8.276**
IV	(Compound 4a +Alloxan)	10mg/kg	485.5±15.163^{ns}	394±24.809^{ns}	272.33±19.592**	177.66±8.988**
V	(Compound5a +Alloxan)	10mg/kg	503.5±50.775^{ns}	392.33±36.394^{ns}	320.5±29.743**	239.166±23.234**
VI	(Compound5b+Alloxan)	10mg/kg	520±27.447^{ns}	416.33±18.894^{ns}	306.83±21.932**	220±11.486**
VII	(Compound5h+Alloxan)	10mg/kg	507.833±45.310^{ns}	429.166±47.406^{ns}	360.16±44.389*	267.66±28.879**
VIII	(Compound5m+Alloxan)	10mg/kg	454.116±9.971^{ns}	387.166±10.505^{ns}	281.66±18.438**	199.33±11.108**

Comparision: Group II v/s Group I and Group II v/s Group III, IV, V, VI, VII and VIII. Statistical significance test for comparisions were done by ANOVA, followed by Dunnet's multiple comparision test. N=6; Values are expressed in mean ± SEM; *p<0.05. **p<0.01, ***p<0.01 Compare with Diabetic rats.

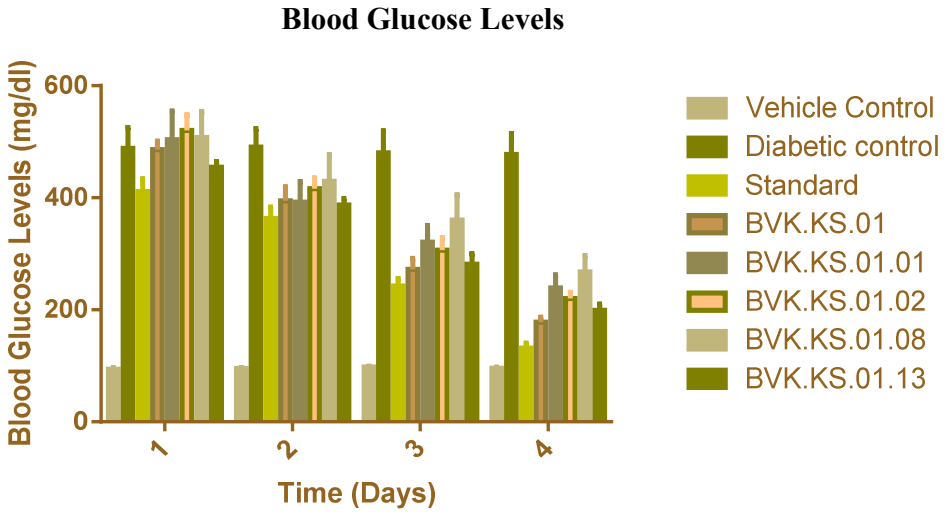

Figure 3

*Blood glucose levels in normal and diabetic rats, values are expressed in mean ± SEM, on 14th day, N=6 in each group, *p<0.05. **p<0.01, ***p<0.01 when compared with diabetic control group dunnet's test.*
BVK.KS.O1-Compound (4a); BVK.KS.01.01-Compound (5a); BVK.KS.01.02-Compound (5b), BVK.KS.01.08-Compound (5h); BVK.KS.01.13-Compound (5m).

RESULTS AND DISCUSSSION

Although, many synthetic methods have been reported, sulfonylation of amines with sulfonyl chlorides in the presence of a base is still the most commonly employed method for the synthesis of sulphurnamides[22,23]. In the present study, the key starting material (**4a**) was prepared by the condensation of ethanol amine with potassium salt of 4-chloro-3,5-dinitro benzene sulfonate (**1**). Compound (**4a**) was treated with thionyl chloride to get the corresponding sulfonylchloride(**4b**) which was subsequently treated with amine in the presence of base to afford the compound **5(a-t)**. All the synthesised compounds were characterized by IR, ^{1}HNMR, ^{13}CNMR, Mass spectral data and evaluated for anti-diabetic activity. Fifteen compounds were screened for Oral Glucose Tolerance Test for preliminary study. Among them, five compounds (**4a, 5a, 5b, 5h** and **5m**) were selected for further study based on OGTT. Among the test compounds **4a, 5m** were significant in their anti-diabetic activity in comparison with standard. All the remaining was poor anti-diabetic in nature

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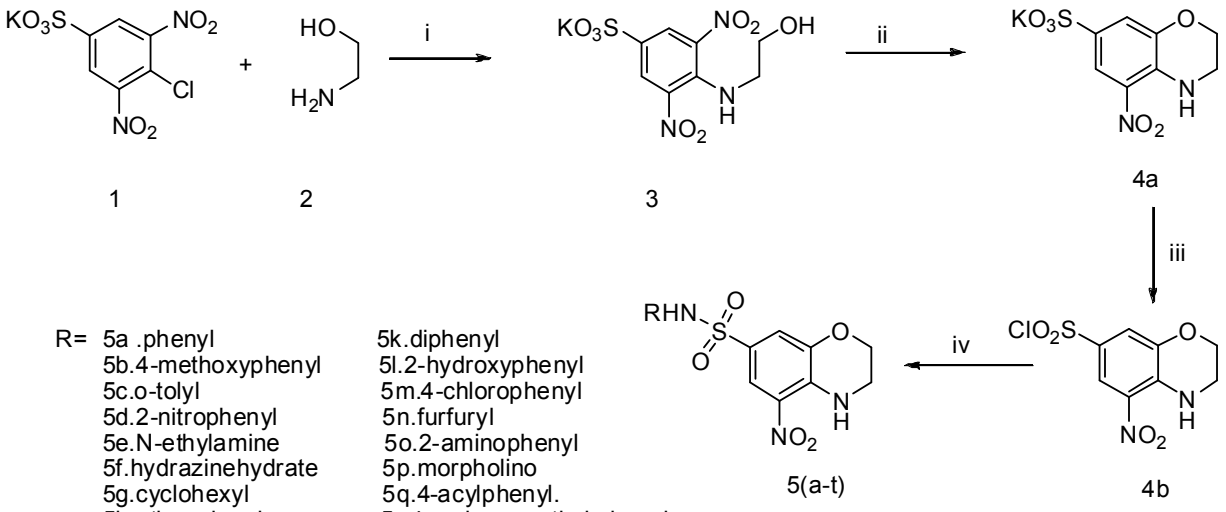
Scheme 1



R= 5a .phenyl
5b.4-methoxyphenyl
5c.o-tolyl
5d.2-nitrophenyl
5e.N-ethylamine
5f.hydrazinehydrate
5g.cyclohexyl
5h.ethanol amine
5i.8-amino isoquinoline
5j.ethylamine
5k.diphenyl
5l.2-hydroxyphenyl
5m.4-chlorophenyl
5n.furfuryl
5o.2-aminophenyl
5p.morpholino
5q.4-acylphenyl.
5r.4-carboxymethyl phenyl
5s.2, 4-dinitrophenyl
5t.N,N-dimethyl

Reagents and conditions i) NaOAc, methanol, reflux, 2h. ii) NaOAc, methanol, reflux, 4h. iii) $SoCl_2$, DMF, reflux, 3h. iv) RNH_2, THF/DCM, 0^{o}C- reflux, 1h.

CONCLUSION

From the above results, it is concluded that among the synthesized compounds **4a, 5m** exhibited significant anti-diabetic activity compared than standard.

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SYNTHETIC APPROACH FOR THE PREPARATION OF 1,3,4-OXADIAZOLE BENZAMIDES AS ANTIFUNGAL AND ANTIMICROBIAL AGENTS

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ABSTRACT

1,3,4-Oxadaizole bezamides are versatile lead molecule for designing potential bioactive agents. All the newly synthesized compounds were screened for their antibacterial and antifungal studies. Antimicrobial studies revealed that compounds **10e, 10i,** and **14b** showed significant antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa*. Compounds **10a, 10b, 14a, 14b, 14f** Showed significant antifungal activity against *C. albicans*

KEY WORDS

1,3,4-oxadiazole benzamides, 2-methyl benzamides, Antifungal activity, Antimicrobial activity.

INTRODUCTION

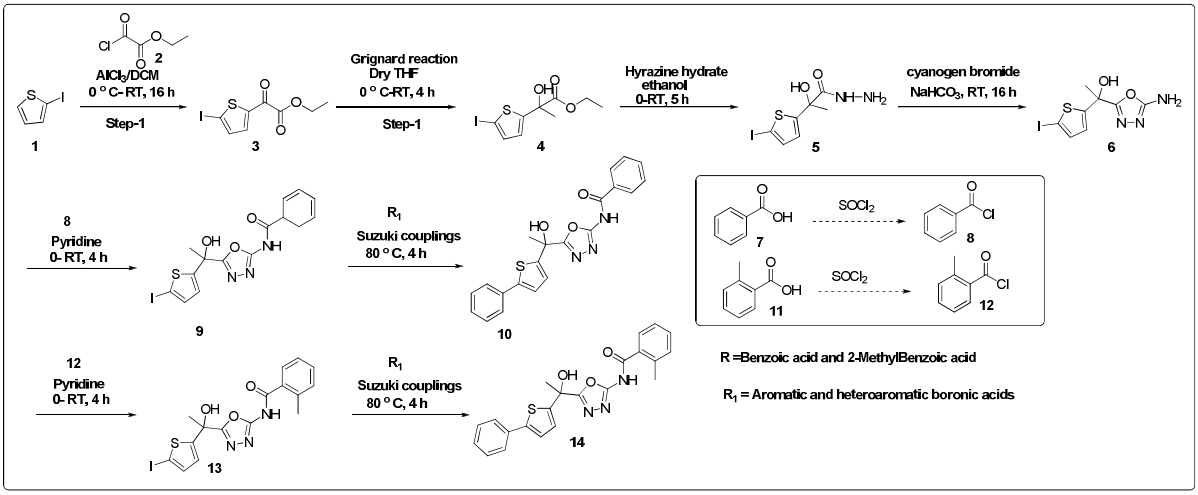
1,3,4-Oxadiazoles constitute an important family of heterocyclic compounds as they have attracted significant interest in medicinal chemistry, pesticide chemistry and polymer science. Since many of 1,3,4-oxadiazoles display a remarkable biological activity, their syntheses and transformations have been received particular interest for a long time. The 1,3,4-oxadiazoles have been found to exhibit diverse biological activities such as antimicrobial[1], anti-HIV[1], ant tubercular[2], antimalarial[3], analgesic[4], antiinflammatory[5], anticonvulsant[6], hypoglycemic[7] and other biological properties such as genotoxic[8] and lipid peroxidation inhibitory activities[9]. 5-Substituted-1,3,4-oxadiazole-2-thiones possess CNS depressant[10], pesticidal[11,12], tyrosine's inhibition[13] property. We therefore are interested in

exploring the biological activity of such molecules through structural medications. In view of these observations, we hereby report syntheses, characterization and antifungal antimicrobial activity of some disubstituted oxadiazoles.

PRESENT WORK

2-Iodo thiophene was converted to ethyl oxo 5-iodo thiophene using $AlCl_3$ and ethyl 2-chloro-2-oxo-acetate. Further this was converted to 2-Hydroxy-2-(5-iodo-thiophen-2-yl)-propionic acid ethyl ester used 2M methyl megnium chloride solution in THF. Further this ester was converted to hydrazide by using hydrazine hydrate in ethanol medium[14]. 1-(5-Amino-[1,3,4]oxadiazol-2-yl)-1-(5-iodo-thiophen-2-yl)-ethanol was prepared from the cyanogens bromide. Title compound was prepared from the substituted 5-amino 1,3,4-Oxadaizole derivatives using pyridine (as a base) and coupled with different aromatic acid chloride (Scheme: 1).

Scheme I:



EXPERIMENTAL SECTION

All reactions were carried out under an atmosphere of dry nitrogen in dried glassware. Indicated reaction temperatures refer to those of the reaction bath, while room temperature (RT) is noted as 25°C. Commercially available starting materials and reagents were used as received. Thin layer chromatography (TLC) was performed with Merck TLC plates (20 X20 cm). Visualization was accomplished by irradiation under a 254 nm UV lamp. Chromatography on silica gel was performed using forced flow (liquid) of the indicated solvent system on Biotage KP-Sil prepacked cartridges and using the Biotage SP-1 automated chromatography system. ^1H and ^{13}C NMR spectra were recorded on aGemini Varian 300 MHz spectrometer. Chemical shifts are reported in ppm, with the solvent resonance as the internal standard ($CDCl_3$ 7.26 ppm, 77.00 ppm, DMSO-d6 2.49 ppm, 39.51 ppm for ^1H, ^{13}C, respectively). Data are reported as follows: chemical shift, multiplicity (s=singlet, d=doublet, t = triplet, q = quartet, brs = broad singlet, m = multiplet), coupling constants, and number of protons. Low resolution mass spectra (electrospray ionization) were acquired on an Agilent Technologies 6130 quadrupole spectrometer coupled to the HPLC system. High resolution mass spectral data was collected in-house using an Agilent 6210 time-of-flight mass spectrometer, also coupled to an Agilent Technologies 1200 series HPLC system. If needed, products were purified via a Waters semi

preparative HPLC equipped with a Phenomenex Luna C18 reverse phase (5 µm, 30mm X 75 mm) column having a flow rate of 45 mL/min. The mobile phase was a mixture of acetonitrile (0.025% TFA) and H_2O (0.05% TFA), and the temperature was maintained at 50°C.

1. (5-Iodo-thiophen-2-yl)-oxo-acetic acid ethyl ester (3):

To a stirred solution of **1** (20 g, 95.69 mmol), compound-**2** (14.3 mL, 105.2 mmol) are in 200 ml DCM, was cooled to 0°C, To this $AlCl_3$ (25.5 g, 191.4 mmol) was added in portions wise over the period of 25 min, then the reaction mixture was stirred at RT for 12 hrs. The progress of the reaction was monitored by TLC. The reaction medium was poured in crushed ice water, The aqueous phase was extracted twice with DCM (2x100 ml). The organic phases are combined and are washed with 2 N HCl solutions. The organic phase is dried over $NaSO_4$ and the filtrate is concentrated under reduced pressure. The crude compound was purified by column chromatography (SiO_2 60-120 mesh) eluting with 15% EtoAc/Hexane to afford compound **3** (17.2 g, 57.00 mmol, 59%) as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (d, *J*= 8.5 Hz, 1H), 7.39 (d, *J* = 8.2 Hz, 1H), 4.20 (m, 2H), 1.39 (m, 2H); MS (ESI): *m/z* 311 $[M+1]^+$.

2. 2-Hydroxy-2-(5-iodo-thiophen-2-yl)-propionic acid ethyl ester (4):

To a stirred solution of **3** (10 g, 32.36 mmol) in diethyl ether (100 mL) was added Methyl magnesium chloride (32.3 mL, 64.72 mmol, 2 M in THF) at 0 °C. After being stirred for 30 min at 0 °C, the stirring was continued for another 3 h at RT under inert atmosphere. The progress of the reaction was monitored by TLC. The reaction was quenched with saturated NH_4Cl solution (50 mL) and extracted with ethyl acetate (3 x 75 mL). The combined organic layers were washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by column chromatography (SiO_2, 100-200 mesh) eluting with 25% EtOAc/Hexane to afford Compound- 6.4 g, 19.6 mmol, 66%) as off white solid. ^{1}H NMR (400 MHz, CDCl3): δ 7.19 (d, *J*= 8.5 Hz, 1H), 6.77 (d, *J* = 8.2 Hz, 1H), 4.20 (m, 2H), 4.01 (bs, OH), 1.79 (S, 3 H), 1.39 (m, 2H); MS (ESI): 327 $[M+1]^+$.

3. 2-Hydroxy-2-(5-iodo-thiophen-2-yl)-propionic acid hydrazide (5):

To a solution containing the **4** (6 g, 18.40 mmol) in Obsolute Ethanol (60 ml) was added Hydrazine hydrate (4.6 g, 92.02 mmol) in drop wise manner over the period of 20 min at 0 °C. After being stirred for 30 min at 0 °C, the stirring was continued for another 10 h at RT under inert atmosphere. The progress of the reaction was monitored by TLC. The reaction mixture was concentrated under reduced pressure, obtained crude was purified by column chromatography (SiO_2 60-120 mesh) eluting with 70% EtoAc/Hexane to afford compound **5** (4.2 g, 13.46 mmol, 75%) as off white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.02 bs, 1 H), 7.30 (d, *J*= 8.5 Hz, 1H), 6.78 (d, *J* = 8.2 Hz, 1H), 4.20 (bs, 2H), 1.61 (S, 3 H); MS (ESI): 313 $[M+1]^+$.

4. 1-(5-Amino-[1,3,4]oxadiazol-2-yl)-1-(5-iodo-thiophen-2-yl)-ethanol (6):

To a stirred solution of **5** (4 g, 12.82 mmol) in 1,4-Dioxane and water (2:1 80 : 40 mL) was added Sodium bi carbonate (1 g, 12.8 mmol) followed by cynogen bromide (2.0 g, 19.23 mmol) at RT. The reaction was continued stirred for 16 h. The progress of the reaction was monitored by TLC .The reaction mixture was concentrated under reduced pressure; the obtained residue was diluted with water (100 mL) and extracted with EtOAc (2 X 75 mL). The combined organic layers were collected, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude compound was washed with ether to get desired brown solid (3.4 g, 10.89 mmol, 79%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.16 (d, *J* = 8.5 Hz, 1H), 7.10 (S, 1H), 6.79 (S, 1 H),6.23 (d, J = 8.0 Hz, 1 H), 1.85 (S, 3 H); MS (ESI): 338 $[M+1]^+$.

5. N-{5-[1-Hydroxy-1-(5-iodo-thiophen-2-yl)-ethyl]-[1,3,4]oxadiazol-2-yl}-benzamide (9):

(a). Benzoyl Chloride preparation:

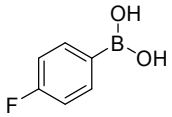
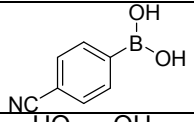
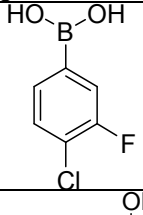
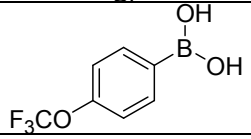
Benzoic acid (1.5 g, 12.22 mmol) was taken in clean RB- flask under inert atmosphere, was added Thionyl chloride (1.8 mL, 24.44 mL), and heated to reflux for 4 hr, after that solvent was removed under reduced pressure under inert atmosphere, the abtained crude was used for next step.

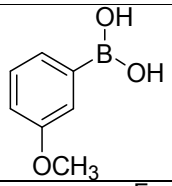
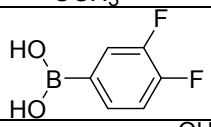
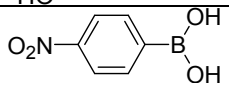
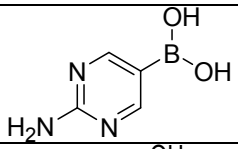
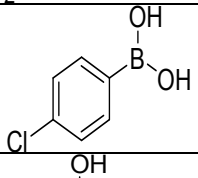
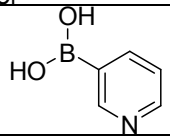
To a stirred solution of **6** (1.5 g, 4.45 mmol) in dry pyridine (2 mL) was added to above acid chloride solution in drop wise over the period of 10 min at 0°C. After being stirred for 30 min at 0°C, the stirring was continued for another 3 h at RT under inert atmosphere. The progress of the reaction was monitored by TLC. The reaction was quenched with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic layers were washed 2N HCl solution, brine, The organic layer collected and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by column chromatography (SiO_2, 100-200 mesh) eluting with 70% EtOAc/Hexane to afford **7**. (1.2 g, 2.72 mmol, 56%) as off white solid. ^{1}H NMR (500 MHz, DMSO-d_6): □δ 9.12 (□□bs, 1 H),□ 7.16 (d, *J* = 8.5 Hz, 1H), 7.82-7.43 (m, 5 H), 6.23 (d, J = 8.0 Hz, 1 H), 1.85 (S, 3 H); MS (ESI): 442 $[M+1]^+$.

6. N-{5-[1-Hydroxy-1-(5-phenyl-thiophen-2-yl)-ethyl]-[1,3,4]oxadiazol-2-yl}-benzamide (10):

To a stirred solution of **7** (150 mg, 0.34 mmol) in THF (20 mL) and water (4 mL) were added Phenyl boronic acid (45.4 mg, 0.37 mmol), K_2CO_3 (141 mg, 1.02 mmol), at RT under inert atmosphere. After purged with nitrogen for a period of 20 min, $Pd(dppf)_2Cl_2$ (42 mg, 0.15 mmol), then the reaction mixture was heated to 80°C and stirring was continued for 3 h. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to RT and filtered through a pad of celite. The filtrate was concentrated under reduced pressure; obtained residue was dissolved in ethyl acetate (3 x 20 mL). The organic layer was washed with water, brine and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by column chromatography (SiO_2, 100-200 mesh) eluting with 40% EtOAc/Hexane to afford **8** **(**80 mg, 0 .20 mmol, 60%) as white solid. ^{1}H NMR (500 MHz, DMSO-d_6):□ δ □9.15 (□□bs, 1 H),□ 7.04 (d, *J* = 8.5 Hz, 1H), 7.82-7.22 (m, 10 H), 6.67(d, J = 8.0 Hz, 1 H), 1.90 (S, 3 H); MS (ESI): 442 $[M+1]^+$. HPLC: 98.2%; m.p : 143-147°C.

TABLE-1: 10a-10f derivatives:

Entry	Product	R=Benzoic acid Derivatives.	Yield (%)
1	10a		73%
2	10b		71%
3	10c		55%
4	10d		63%

5	10 e		72%
6	10 f		71%
7	10 g		87%
8	10 f		55%
9	10 i		66%
10	10 j		77%

7. N-(5-{1-[5-(4-Fluoro-phenyl)-thiophen-2-yl]-1-hydroxy-ethyl}-[1,3,4]oxadiazol-2-yl)-benzamide (**11a**, Eantry **1**):

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.20 (bs, 1 H), 7.62-7.44 (m, 5 H), 7.46 (d, *J* = 9.0 Hz,2 H), 7.04 (d, *J* = 8.5 Hz, 1H), 7.03 (m, 2 H), 6.70 (d, J = 8.0 Hz, 1 H), 1.85 (S, 3 H). MS (ESI): 410 [M^++1]. HPLC ; 97.6% m.p : 137-142^{o}C.

8. N-(5-{1-[5-(4-Cyano-phenyl)-thiophen-2-yl]-1-hydroxy-ethyl}-[1,3,4]oxadiazol-2-yl)-benzamide (**11b**, Entry **2**):

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.12 (bs, 1 H), 7.70-7.44 (m, 5 H), 7.46 (d, *J* = 9.0 Hz,2 H), 7.35 (d, *J* = 9.0 Hz,2 H), 7.07 (d, *J* = 8.5 Hz, 1H), 6.65 (d, J = 8.0 Hz, 1 H), 1.85 (S, 3 H). **MS (ESI):** 417 [M^++1]. HPLC ; 95.5 % m.p : 147-155^{o}C.

9. N-(5-{1-[5-(4-Chloro-3-fluoro-phenyl)-thiophen-2-yl]-1-hydroxy-ethyl}-[1,3,4]oxadiazol-2-yl)-benzamide (**11C**, entry **3**):

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.12 (bs, 1 H), 7.83-7.51 (m, 5 H), 7.23 (d, *J* = 9.0 Hz,2 H), 7.13 (d, *J* = 8.5 Hz, 1H), 7.08 (m, 1H), 7.15 (d, *J* = 9.0 Hz,1 H), 6.65 (d, J = 8.0 Hz, 1 H), 1.74 (S, 3 H). MS (ESI): 444 [M^++1]. HPLC ; 95.7 % m.p : 127-133^{o}C.

10. N-(5-{1-Hydroxy-1-[5-(4-trifluoromethoxy-phenyl)-thiophen-2-yl]-ethyl}-[1,3,4]oxadiazol-2-yl)-benzamide (**11d**, Entry **4**):

^{1}H NMR (500 MHz, DMSO-d_6): δ 9 (bs, 1 H), 7.83-7.51 (m, 5 H), 7.35 (d, *J* = 9.0 Hz,2 H), 7.13 (d, *J* = 8.5 Hz, 1H), 6.80 (d, *J* = 9.0 Hz, 1 H), 6.63 (d, J = 8.0 Hz, 1 H), 1.74 (S, 3 H).
MS (ESI): 476.4 [M^++1]. HPLC ; 93.7 % m.p : 128-132 ^{0} C.

11. N-(5-{1-Hydroxy-1-[5-(3-methoxy-phenyl)-thiophen-2-yl]-ethyl}-[1,3,4]oxadiazol-2-yl)-benzamide (**11e**, Entry **5**):

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.2 (bs, 1 H), 7.78-7.43 (m, 5 H), 7.20 (t, *J* = 9.0 Hz,2 H), 7.20 (d, *J* = 8.5 Hz, 1H), 7.02 (d, *J* = 9.0 Hz, 1 H), 6.91 (S, 1H), 6.63 (d, J = 8.0 Hz, 1 H), 3.82 (S. 3H), 1.63(S, 3 H). MS (ESI): 422 [M^++1]. HPLC ; 98.1 % m.p : 125-128^{o}C.

12. N-(5-{1-[5-(3,4-Difluoro-phenyl)-thiophen-2-yl]-1-hydroxy-ethyl}-[1,3,4]oxadiazol-2-yl)-benzamide (**11f**, entry **6**):

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.19 (bs, 1 H), 7.78-7.43 (m, 5 H), 7.23 (t, *J* = 9.0 Hz,1 H), 7.17-7.05 (m, 2H), 6.70 (d, J = 8.0 Hz, 1 H), 1.63 (S, 3 H). MS (ESI): 428 [M^++1]. HPLC ; 96.3 % m.p : 115-117 ^{0} C.

13. N-(5-{1-Hydroxy-1-[5-(4-nitro-phenyl)-thiophen-2-yl]-ethyl}-[1,3,4]oxadiazol-2-yl)-benzamide (**11g**, Entry **7**):

^{1}H NMR (400 MHz, DMSO-*d_6*): δ 9.24 (bs, 1 H), 8.20 (t, *J* = 9.0 Hz,2 H), 7.78-7.43 (m, 5 H), 7.73 (t, *J* = 9.0 Hz, 2 H), 6.70 (d, J = 8.0 Hz, 1 H), 1.71 (S, 3 H). MS (ESI): 437 [M^++1]. HPLC ; 92.1 % m.p : 127-135^{o}C.

14. N-(5-{1-[5-(2-Amino-pyrimidin-5-yl)-thiophen-2-yl]-1-hydroxy-ethyl}-[1,3,4]oxadiazol-2-yl)-benzamide (**11h**, Entry **8**):

^{1}H NMR (400 MHz, DMSO-*d_6*): δ 9.07 (bs, 1 H), 8.68 (S, 2 H), 7.78-7.43 (m, 5 H), 6.82 (bs, 2 H), 6.60 (d, J = 8.0 Hz, 1 H), 1.71 (S, 3 H). MS (ESI): 409 [M^++1]. HPLC ; 93.2 % m.p : 137-141^{o}C.

15. N-(5-{1-[5-(4-Chloro-phenyl)-thiophen-2-yl]-1-hydroxy-ethyl}-[1,3,4]oxadiazol-2-yl)-benzamide (**11I**. Entry **9**):

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.14 (bs, 1 H), 7.78-7.43 (m, 5 H), 7.42 (d, J=9.0 Hz, 2 H), 7.33 (d, J=9.0 Hz, 2 H), 6.65 (d, J = 8.0 Hz, 1 H), 1.63 (S, 3 H). MS (ESI): 427 [M^++1]. HPLC ; 93.2 % m.p : 141 – 146^{o}C.

16. N-{5-[1-Hydroxy-1-(5-pyridin-3-yl-thiophen-2-yl)-ethyl]-[1,3,4]oxadiazol-2-yl}-benzamide (**11J**: Entry-**10**):

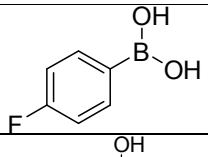
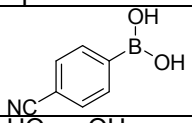
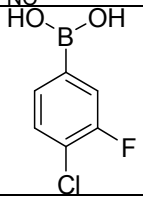
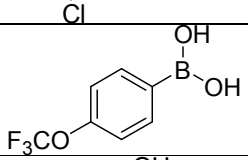
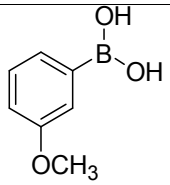
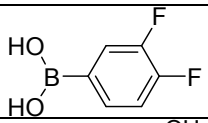
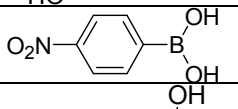
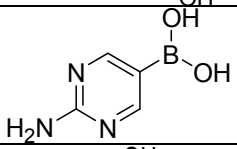
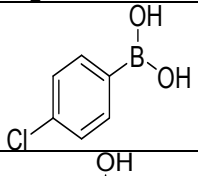
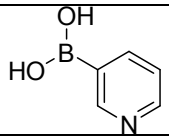
^{1}H NMR (400 MHz, DMSO-d_6): δ 9.17 (bs, 1 H), 8.81 (Σ 1 H), 8.65 (d, J = 8.5 Hz, 1 H), 7.97 (d, J = 8.5 Hz, 1 H), 7.78-7.43 (m, 5 H), 7.42 (t, J=9.0 Hz, 2 H), 6.71 (d, J = 8.0 Hz, 1 H), 1.69 (S, 3 H). MS (ESI): 393 [M^++1]. HPLC ; 96.6 % m.p : 117-119^{o}C.

17. N-(5-(1-hydroxy-1-(5-phenylthiophen-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)-2-methylbenzamide (**14**):

To a stirred solution of **13** (200 mg, 0.43 mmol) in THF (35 mL) and water (6 mL) were added Phenyl boronic acid (58.9 mg, 0.48 mmol), K_2CO_3 (167 mg, 1.02 mmol), at RT under inert atmosphere. After purged with nitrogen for a period of 20 min, $Pd(dppf)_2Cl_2$ (53.8 mg, 0.065 mmol), then the reaction mixture was heated to 80°C and stirring was continued for 3 h. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to RT and filtered through a pad of celite. The filtrate was concentrated under reduced pressure; obtained residue was dissolved in ethyl acetate (3 x 20 mL). The organic layer was washed with water, brine and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by column chromatography (SiO_2, 100-200 mesh) eluting with 40% EtOAc/Hexane to afford **8 (**80 mg, 0 .20 mmol, 60%) as white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.2 (bs, 1 H), 7.91–7.24 (μ, 10 H 7.17 (d, *J* = 8.5 Hz, 1H), 6.63 (d, J = 8.0 Hz, 1 H), 2.55 (S, 3 H), 1.90 (S, 3 H). MS (ESI): 442 [M^++1]. HPLC; 99.7% m.p : 153-156^{o}C.

Table-II:

14 a – 14 f derivatives:

Entry	Product	R 2-methyl benzoic acid derivatives	Yield (%)
1.	14a		73%
2.	14b		67%
3.	14c		72%
4.	14d		54%
5.	14 e		66%
6.	14 f		83%
7.	14 g		74%
8.	14 f		63%
9.	14 i		54%
10.	104j		76%

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SYNTHESIS AND CHARECTRARISATION OF 2- DIFLUORO 1,3,4-OXADIAZOLE-5-THIOL DERIVATIVES ARE POTENT AND SELECTIVE INHIBITORS OF HUMAN RETICULOCYTE 15-LIPOXYGENASE-1

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ABSTRACT

A novel synthetic approach of 5-difluoro substituted 1,3,4-oxadiazoles 2-thiols chemotypes which are potent and selective inhibitors of 15-hLO-1. This novel scaffolds are chemically tractable and amenable to chemical modificationat various positions of themolecule, allowing for rapid exploration of the SAR profile.

KEYWORDS

1,3,4-oxadiazole-2-thiols, pyridine-pipezinemesylate.

INTRODUCTION

Lipoxygenases (LOsa) are nonheme, iron-containing enzymes found in both the plant and animal kingdoms. Los catalyze the dioxygenation of 1,4-*cis*-pentadiene-containing polyunsaturated fatty acids (e.g., linoleic acid (LA) and arachidonic acid (AA)) to form hydroperoxy-fatty acids[1]. The mechanism for this reaction is the abstraction of a hydrogen atom from the 1,4-cis,cis-pentadiene by an active site ferric ion[2]. Inhibitors of lipoxygenases can target this unique reaction via a variety of mechanisms such as reductive, chelation, competitive, and/or allosteric[3]. Human lipoxygenases (hLOs) have been implicated in several diseases involving inflammation, immune disorders, and various types of cancers [4-6]. 5-hLO[7] has been implicated in cancer[3e,8] and asthma[9], while platelet-type 12-LO[10] has been implicated in psoriasis[11] and pancreatic[12], breast [13,14] and prostate cancers [15, 16]. Reticulocyte 15-hLO-1 (15-hLO-1) is less straightforward since it has been implicated both in resolving and promoting human disease[17]. In prostate tumors, cancer cells have a higher expression of 15-hLO-1 compared with normal adjacent tissue, and this expression positively correlates with the virulence of the tumor[18-20].

In contrast, 15-hLO-2 is expressed in normal prostate tissue, but poorly expressed in prostate tumors, with an inverse correlation with the virulence of the tumor[21]. This opposing effect between 15-hLO-1 and 15-hLO-2 is thought to be due to the difference in product generation. While one of the major 15-hLO-1 products, 13-(S)-hydroperoxy-9,11-(Z,E)-octadecadienoic acid (13-HPODE) from LA, up-regulates the MAP kinase signaling pathway, the major 15-hLO-2 product,

15-(S)-HPETE from AA, down-regulates MAP kinase. In colon cancer, however, 15-hLO-1 has been proposed to have a beneficial role. Down regulation of 15-hLO-1 is linked to colorectal tumorigenesis and restoring 15-hLO-1 expression in colon cancer in vivo xenografts down regulates antiapoptotic proteins and inhibits cell growth[22]. Other potential therapeutic benefits of 15-hLO-1 inhibitors include asthma[23], cardiovascular disease[24] and minimizing the brain damage that occurs after a stroke.

One of the major features of neuronal cell death after a stroke event is the accumulation of reactive oxygen species (ROS)[25]. Recently, it has been reported that 15-hLO-1 damages the mitochondria, which leads to the breakdown of the membrane potential, the production of ROS, and cytochrome c release, suggesting that 15-hLO-1 is the central executioner in an oxidative stress related neuronal death program[26]. These broad implications in disease regulation underscore the need for small molecule inhibitors against 15-hLO-1. However, to date, relatively few inhibitors have been reported that are both potent and selective. The most promising 15-hLO-1 inhibitors thus far are the tryptamine[27] (**3**) and imidazole-based[28] (**4**) derivatives, which have low nanomolar potency and selectivity against both 5-hLO and 12-hLO (FIG-I).

Figure I

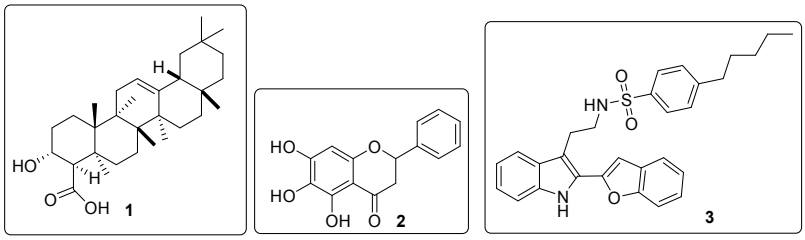
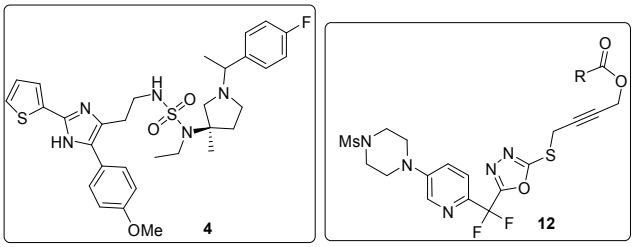
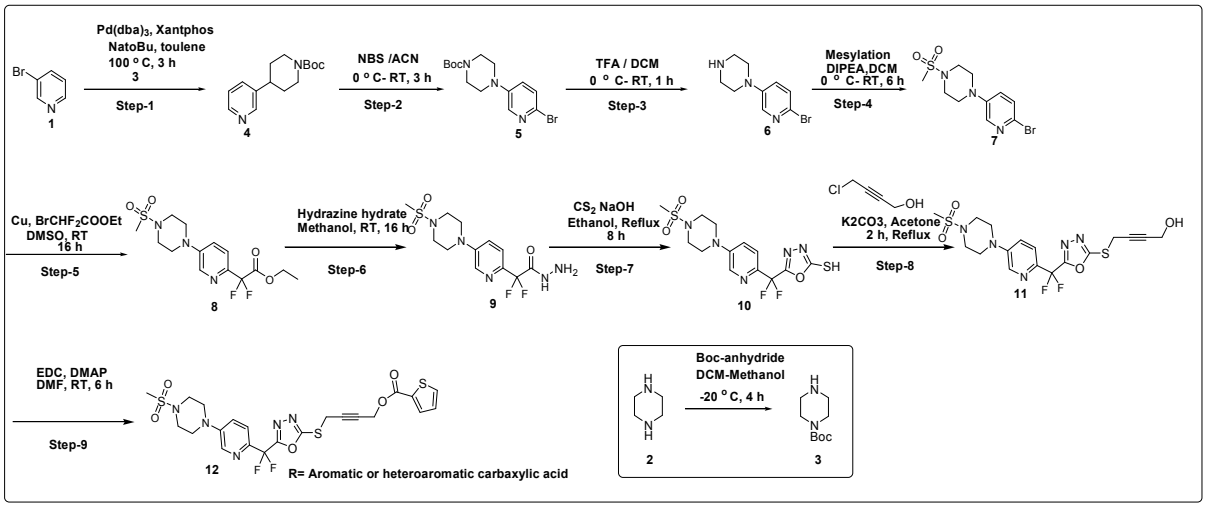


Figure II



Moreover, this novel scaffold is chemically tractable and amenable to chemical modificationat various positions of themolecule, allowing for rapid exploration of the SAR profile.Our initial round of analogues was focused around modification of the 2-thiophene carboxylic acid moiety in our lead compound **12**. As such, the synthesis commenced with treatment of the Ethyl 2,2-difluoro-2-{5-[4-(methylsulfonyl) piperazin-1-yl] pyridin-2-yl} acetate with anhydrous hydrazine in ethanol at reflux to afford the desired hydrazide**9** in high yield (Scheme 1). Cyclization was accomplished using carbon disulfide in the presence of KOH in ethanol followed by acidification of the resulting thiolate to provide **10**[30a,30b]. Alkylation of **10** with 4-chlorobut-2-yn-1-ol with K_2CO_3 in acetone provided the key propargylic alcohol intermediate from which a wide variety of ester derivatives (11a-20f) were prepared in a facile manner using EDC and cat. DMAP in DMF at room temperature[31].

Scheme I

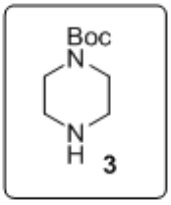


EXPERIMENTAL SECTION:

GENERAL CHEMISTRY

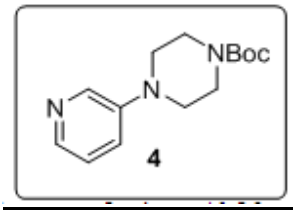
All reactions were carried out under an atmosphere of dry nitrogen in dried glassware. Indicated reaction temperatures refer to those of the reaction bath, while room temperature (RT) is noted as 25°C. Commercially available starting materials and reagents were used as received. Thin layer chromatography (TLC) was performed with Merck TLC plates (20 X20 cm). Visualization was accomplished by irradiation under a 254 nm UV lamp. Chromatography on silica gel was performed using forced flow (liquid) of the indicated solvent system on Biotage KP-Sil prepacked cartridges and using the Biotage SP-1 automated chromatography system. ^{1}H and ^{13}C NMR spectra were recorded on aGemini Varian 300 MHz spectrometer. Chemical shifts are reported in ppm, with the solvent resonance as the internal standard ($CDCl_3$ 7.26 ppm, 77.00 ppm, DMSO-d6 2.49 ppm, 39.51 ppm for ^{1}H, ^{13}C, respectively). Data are reported as follows: chemical shift, multiplicity (s=singlet, d=doublet, t = triplet, q = quartet, brs = broad singlet, m = multiplet), coupling constants, and number of protons. Low resolution mass spectra (electrospray ionization) were acquired on an Agilent Technologies 6130 quadrupole spectrometer coupled to the HPLC system. High resolution mass spectral data was collected in-house using an Agilent 6210 time-of-flight mass spectrometer, also coupled to an Agilent Technologies 1200 series HPLC system. If needed, products were purified via a Waters semi preparative HPLC equipped with a Phenomenex Luna C18 reverse phase (5 µm, 30mm X 75 mm) column having a flow rate of 45 mL/min. The mobile phase was a mixture of acetonitrile (0.025% TFA) and H_2O (0.05% TFA), and the temperature was maintained at 50°C.

1. Tetra-butyl piperizine-1-carbaxylate (3)



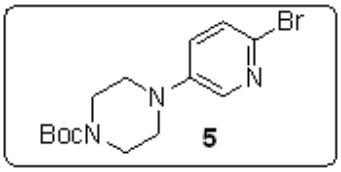
To piperzine (50 g, 581.19 mmol) was dissolved in DCM (500 ml) and methanol (40 ml). Then Boc anhydride (25 g, 116.23 mmol) was added drop wise over a period of two hours at -20^{0}c under nitrogen atm. Reaction mixture was maintained at -20^{0}c for 3 h. bring the reaction temperature to RT, diluted with water. Separate the aqueous layer and extracted with dichloromethane. Combine organic layer wash with water and brine. Organic layer was dried over Na_2SO_4 and concentrated in vacuum. Obtained crude compound was purified by triturated with pentane to get compound as white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm): 3.38 (t, *J*=5Hz, 4H,), 2.80 (t, *J*=4.5Hz, 4H), 1.45 (S, 9H). MS (ESI): 187$[M+1]^+$.

2. Tetra butyl-4 (pyridine-3-yl)piperzine- 1-carbaxylate (4):



Sodium 2-methylpropan-2-olate (6.30 g, 65.62 mmol), 3-Bromompyridine (10 g, 63.29 mmol), and tetr-butyl piperazine-1-carbaxylate (12.36 g, 66.42 mmol), were combined in 100 ml of anhydrous toluene. Ar gas was bubbled through the solution briefly for 20 mins before adding 4, 5-bis (diphenylphosphino)-9, 9-dimethyl-9H-Xanthene (1.27 g, 2.20 mmol), and Pd (dba) $_3$ (1.15 g, 1.26 mmol). The reaction mixture was heated to 85 ^{0}C for 12 h. The progress of the reaction was monitored by TLC. The solution was cooled to room temperature and diluted with saturated NH_4Cl solution and then extracted with DCM (2 X 100 ml). The organic layer was dried over Na_2SO_4 and then concentrated under vaccum. The crude compound was purified by column chromatography (SiO_2 60-120 mesh) eluting with 35% EtoAc/Hexane to afford compound **4** (12 g, 45.67 mmol, 72%) as brown solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 8.55 (s, 1 H), 8.23 (d, *J*= 8.5 Hz, 1H), 7.16 (t, *J*= 8.0 Hz,1H), 7.26 (d, *J* = 8.0 Hz, 1H), 3.43-3.49 (m,4H), 3.11-3.20 (m, 4H), 1.47 (S, 9H); MS (ESI): 264$[M+1]^+$.

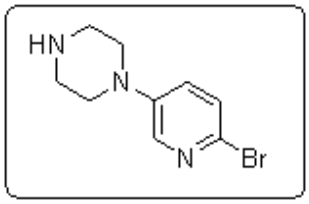
3. Tetr butyl-4-(6-Bromo pyridine-3-yl)-piperazine-1-carbaxylate (5):



6.12 g (34.22 mmol) of N-bromosuccinimide was added portion wise to a solution of4 (9 g, 34.22 mmol) in 100 ml of acetonitrile cooled to 0^{0}C. Stirring is continued 0^{0}C for 15 min and then at RT for 2 h. The progress of the reaction was monitored by TLC. 100 ml of aq. NaOH (1M) solution and 100 ml EtOAc

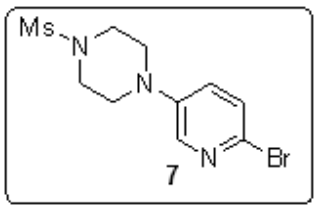
are added to the reaction medium. The aqueous phase is separated out and extracted with EtOAc (2 x 100 ml), the combined organic phases are washed with saturated NaCl solution and dried over Na_2SO_4 then concentrated under vaccum. The crude compound was purified by column chromatography (SiO_2 60-120 mesh) eluting with 20% EtoAc/Hexane to afford compound **4** (10 g, 29.23 mmol, 85%) as orange-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 8.57 (S, 1H), 7.81 (d, *J*=7.5 Hz, 1H), 7.43 (d, *J*=7.0 Hz, 1H), 3.59 (m,4H), 3.13 (m, 4H), 1.47 (S, 9H). MS (ESI): 344$[M+1]^+$.

4. 1-(6-Bromo pyridine-3-yl)-piperazine (6):



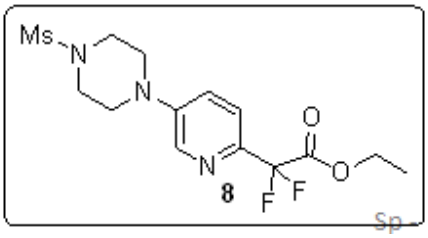
19.53 ml (263.1 mmol) of TFA are slowly added to a suspention of **5** (9g, 26.31 mmol) in 125 ml of DCM. Stirring is continued for 16 hrs. The progress of the reaction was monitored by TLC. The reaction mixture is concentrated under reduced pressure, the residue is taken in 100 ml of chloroform and 10 ml of aqueous NaOH solution (10 M) are then slowly added. The aqueous phase is separated out and then extracted twice with chloroform. The organic phases are combined and are washed with saturated NaCl solution. The organic phase is dried over $NaSO_4$ and the filtrate is concentrated under reduced pressure yielded orange-coloredoillycrude **6** (8.20 g). This product-**6** is used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 8.43 (s, 1H), 7.73 (d, *J*=7.5 Hz, 1H), 7.40 (d, *J*=7.0 Hz, 1H), 3.58 (m, 4H), 3.12 (m, 4H), 3.12 (bs, 1H); MS (ESI): 243 $[M+1]^+$.

5. 1-(6-Bromo pyridine-3-yl)-4-(methylsulfonyl)piperazine (7):



To a stirred solution of compound-6 (8 g, 33.05 mmol) in 80 ml DCM, was cooled to 0^0 C, To this DIEPA (16.2 ml, 99.15 mmol) was added in drop wise over the period of 10 min, then to this mesyl chloride (3.9 ml, 49.57 mmol) was added, then the reaction mixture was stirred at RT for 6 hrs. The progress of the reaction wasmonitored by TLC. The reaction medium was diluted with water, The aqueous phase was separated out and then extracted twice with DCM (2x100 ml).The organic phases are combined and are washed with saturated NaCl solution. The organic phase is dried over $NaSO_4$ and the filtrate is concentrated under reduced pressure. The crude compound was purified by column chromatography (SiO_2, 60-120 mesh) eluting with 30% EtoAc/Hexane to afford compound **7** (8.7 g, 27.18 mmol, 82%)as off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 8.55 (S, 1H), 7.80 (d, *J*=7.5 Hz, 1H), 7.45 (d, *J*=7.0 Hz, 1H), 3.59 (m,4H), 3.13 (m, 4H), 2.84 (S, 3H); MS (ESI): 321 $[M+1]^+$.

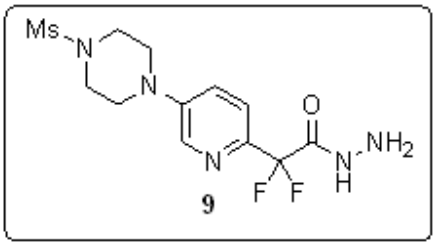
6. Ethyl 2,2-difluoro-2-{5-[4-(methylsulfonyl)piperazin-1-yl]pyridin-2-yl}acetate (8).



8

To a suspension of copper powder (6.4 g, 100.78 mmol) in DMSO (80 ml) was added ethyl bromodifluoroacetate (6.4 ml, 50.0 mmol) and stirred for 1 h at RT. Compound-7 (8 g, 25.00 mmol) was added and then continued stirring for another 15 h at RT. The progress of the reaction was monitored by TLC. The reaction was quenched with saturated NH_4Cl solution (100 ml) and extracted with DCM (3 x 100 ml). The combined organic layers were washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford crude product. The crude compound was purified by column chromatography (SiO_2 60-120 mesh) eluting with 30% EtoAc/Hexane to afford compound-8 (5.2 g, 14.10 mmol, 56%)as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 8.50 (s, 1H), 7.43 (d, *J*=7.5 Hz, 1H), 7.32 (d, *J*=7.0 Hz, 1 H), 3.59 (m,4H), 3.13 (m, 4H), 4.12 (q, *J*= 5.5 Hz , 2H), 2.87 (S, 3H), 1.30 (t, *J*=5.0 Hz, 3H); MS (ESI): 364$[M+1]^+$.

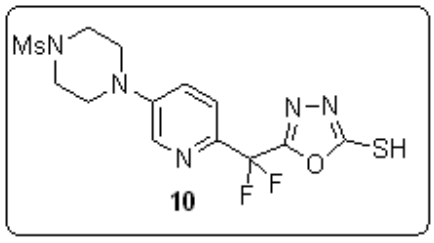
7. 2,2-difluoro-2-{5-[4-(methylsulfonyl)piperazin-1-yl]pyridin-2-yl}acetohydrazide (9):



9

To a solution containing the difluoroester**8** (5 g, 13.73 mmol) in methanol (50 ml) was cooled to 0 ^{0}C, to this anhydrous hydrazine (3.9 ml, 68.65 mmol) was added and the reaction mixture was stirred at RT for overnight. The progress of the reaction wasmonitored by TLC. From the reaction mixture solvent was removed under reduced pressure, and the obtained crude was purified by column chromatography (SiO_2 60-120 mesh) eluting with 70% EtoAc/Hexane to afford compound **9** (4.1 g, 11.74 mmol, 85.5%) as off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6)**:** δ (ppm): 8.53 (s, 1 H), 8.02 (brs, 1H), 7.42 (d, *J*=8.5 Hz, 1H), 7.33 (d, *J*= 8.5 Hz, 1H), 3.41 (m, 4H), 3.29 (m, 4H), 2.84 (s, 3H), 2.0 (brs, 2H); MS (ESI): 350 $[M+1]^+$.

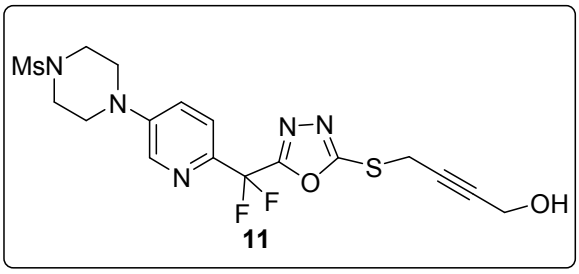
8. 5-{difluoro{5-[4-(methylsulfonyl)piperazin-1-yl]pyridin-2-yl}methyl}-1.3.4-oxadiazole-2-thiol (10):



10

To a solution containing the Compound **8** (10 g, 28.65 mmol) in absolute ethanol (100 ml) was added CS_2 (12.8 g, 168.42 mmol), followed by the addition NaOH (11.4 g, 285.0 mmol), and the reaction mixture was heated to 70^oC for 8 h.The progress of the reaction wasmonitored by TLC. From the reaction mixture solvent was removed under reduced pressure, and the obtained crude was dissolved in water (150 mL), acidified with 1N-HCl solution (PH=2), extracted with EtOAc (3 X 100 mL), The organic layer was dried over Na_2SO_4, after concentration obtained crude was purified by column chromatography (SiO_2 60-120 mesh) eluting with 45% EtoAc/Hexane to afford compound 10 **(**7.6 g, 67.8%) as light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 8.55 (s, 1H), 7.40 (d, *J*=9.5 Hz, 1 H), 7.27 (d, *J*= 9.0 Hz, 1 H) 4.3, 4.2 (m, 4H), 3.31 (m, 4H), 3.12 (s, 1H), 2.79 (s, 3H); MS (ESI): 392 $[M+1]^+$; HPLC= 97%.

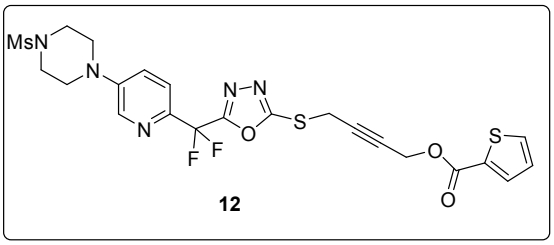
9. 4-(5-{Difluoro-[5-(4-methanesulfonyl-piperazin-1-yl)-pyridin-2-yl]-methyl}-[1,3,4]-oxadiazol-2-ylsulfanyl)-but-2-yn-1-ol (11):



11

A solution of compound 10 (7 g,17.9 mmol), 4-chloro-2-yn-1-ol (1.86 g, 17.90 mmol), and potassium carbonate (12.3 g, 89.51 mmol) in acetone (70 ml) was refluxed for 1 h, and the filtrate was evaporated via reduced pressure. The crude residue was purified by column chromatography (SiO_2, 60-120 mesh) eluting with 45% EtoAc/Hexane to afford compound 11 **(**7.6 g, 67.8%) as light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 8.51(s, 1H), 7.42 (d, *J*=9 Hz, 1H), 7. 30 (d, *J*=8.5 Hz, 1H), 4.22 (s, 2H), 3.60 (s, 2H),3.40 (m,4H), 3.31 (m, 4H), 2.79 (s, 3H), 2.10 (s, 1H); MS (ESI): 460 $[M+1]^+$; HPLC= 95%.

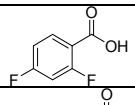
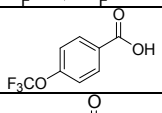
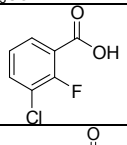
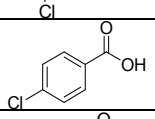
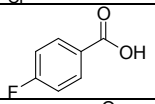
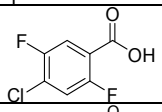
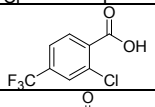
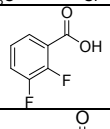
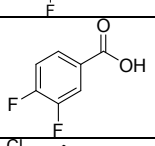
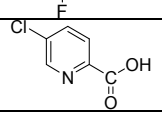
10. Thiophene-2-carboxylic acid 4-(5-{difluoro-[5-(4-methanesulfonyl-piperazin-1-yl)-pyridin-2-yl]-methyl}-[1,3,4]-oxadiazol-2-ylsulfanyl)-but-2-ynyl ester (12):



12

A solution of compound 11 (250 mg, 0.54 mmol), 2-thiophene carboxylic acid (78 mg, 0.60 mmol), EDC (169 mg, 1.08 mmol) and DMAP (33 mg, 0.27 mmol) in DMF (2 mL), was stirred at RT for 4 h. The progress of the reaction wasmonitored by TLC. Diluted with water and extracted with EtOAc (3X 50 mL), followed by brine solution wash, organic layer was dried over sodium sulphate, obtained crude was purified by column chromatography (SiO_2, 60-120 mesh) eluting with 60 % EtOAc/Hexane to afford compound 12 **(**122 mg, 40%)as off white solid. ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm):8.50(s, 1H), 7.66 (d, *J*=7.5 Hz, 1H), 7.40-7.32 (m,3 H), 6.90 (t, *J*= 6.8 Hz, 1H), 4.98 (s, 2H), 3.67 (s, 2H), 3.18 (d, *J*=6.5 Hz, 4H), 2.78 (d, *J* =6.0 Hz, 4H), 2.91 (s, 3H); MS (ESI): 570 $[M+1]^+$. m.p: 92-99 ^oC; HPLC= 98%.

Table 1
12a-20f derivatives

Entry	Product	R	Yield (%)
1.	12a		97
2.	12 b		90
3.	2c		80
4.	2d		88
5.	2e		86
6.	2f		97
7.	2g		85
8.	2h		98
9.	2i		85
10.	2j		91

11. 2,4-Difluoro-benzoic acid 4-(5-{difluoro-[5-(4-methanesulfonyl-piperazin-1-yl)-pyridin-2-yl]-methyl}-[1,3,4]oxadiazol-2-ylsulfanyl)-but-2-ynyl ester (12a): {Entry-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 8.42(s, 1H), 7.93 (m, 1H), 7.40 (d, *J*=7.5 Hz, 1H), 7.32 (d, *J*=7.0 Hz, 1H), 6.85-6.79 (m, 2H), 4.98 (s, 2H), 3.67 (s, 2H), 3.18 (d, *J*=6.5 Hz, 4H), 2.78 (d, *J* = 6.0 Hz, 4H), 2.93 (s, 3H); MS (ESI): 600 $[M+1]^+$. HPLC= 96.3%.Off white solid: m.p: 124-131 ^{0}C.

12. 4-Trifluoromethoxy-benzoic acid 4-(5-{difluoro-[5-(4-methanesulfonyl-piperazin-1-yl)-pyridin-2-yl]-methyl}-[1,3,4]-oxadiazol-2-ylsulfanyl)-but-2-ynyl ester {(12b), Entry-2}:

^{1}H NMR (400 MHz, $CDCl_3$)**:** δ (ppm): 8.47 (s, 1H), 7.90 (d, *J*= 8.5 Hz, 2H), 7.40 (d, *J*= 7.5 Hz, 1H), 7.32 (d, *J*=7.0 Hz, 1H), 6.86 (d, *J*= 8.5 Hz, 2H), 4.98 (S, 2H), 3.67 (s, 2H), 3.18 (d, *J* = 6.5 Hz, 4H), 2.78 (d, *J* = 6.0 Hz, 4H), 2.99 (s, 3H); MS (ESI): 600 $[M+1]^+$. HPLC= 99.2%. White solid: m.p: 98-102 ^{o}C.

13. 3-Chloro-2-fluoro-benzoic acid 4-(5-{difluoro-[5-(4-methanesulfonyl-piperazin-1-yl)-pyridin-2-yl]-methyl}-[1,3,4]oxadiazol-2-ylsulfanyl)-but-2-ynyl ester {(12C,) ,Entry-3}:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm):8.62 (s, 1H), 7.83 (d, *J*= 8.5 Hz, 1H), 7.40 (d, *J*= 7.5 Hz, 1H), 7.32 (d, *J*=7.0 Hz, 1H), 7.46 (d, *J*= 8.0 Hz, 1H), 7.08 (t. *J*= 8.0 Hz, 1H), 4.98 (s, 2 H), 3.67 (S, 2H), 3.18 (d,

J = 6.5 Hz, 4H), 2.75 (d, *J* = 6.0 Hz, 4H), 2.87 (S, 3H), MS (ESI): 617 $[M+1]^+$. Thicky syrup (colure less). HPLC= 99.0%.

14. 4-Chloro-benzoic acid 4-(5-{difluoro-[5-(4-methanesulfonyl-piperazin-1-yl)-pyridin-2-yl]-methyl}-[1,3,4]oxadiazol-2-ylsulfanyl)-but-2-ynyl ester: { (12 D), Entry-4}:
White solid. M.p : 100-109 ^oC.^1H NMR (400 MHz, $CDCl_3$): δ (ppm): 8.62 (s, 1H), 7.91 (d, *J*= 8.5 Hz, 2H), 7.40 (d, *J*= 7.5 Hz, 1H), 7.32 (d, *J*=7.0 Hz, 1 H), 7.38 (d, *J*= 8.0 Hz, 2H), 4.95 (s, 2H), 3.67 (s, 2H), 3.22 (d, *J* = 6.5 Hz, 4H), 2.80 (d, *J* = 6.0 Hz, 4H), 2.75 (s, 3H**),** MS (ESI): 599 $[M+1]^+$. HPLC= 94.3%.

15. 4-Fluoro-benzoic acid 4-(5-{difluoro-[5-(4-methanesulfonyl-piperazin-1-yl)-pyridin-2-yl]-methyl}-[1,3,4]oxadiazol-2-ylsulfanyl)-but-2-ynyl ester: {(12 E), Entry-5}:
Yellowish solid. M.p: 137-140 ^oC.^1H NMR (400 MHz, $CDCl_3$): δ (ppm): 8.54 (s, 1 H), 7.95 (d, *J*= 8.5 Hz, 2 H), 7.42 (d, *J*= 7.5 Hz, 1 H), 7.33 (d, *J*=7.0 Hz, 1 H), 7.08 (m, 2H), , 4.90 (S, 2 H), 3.69(S, 2H), 3.27 (d, *J* = 6.5 Hz, 4H), 2.86 (d, *J* = 6.0 Hz, 4H), 2.90 (S, 3H), MS (ESI): 582.5 $[M^+ +1]$. HPLC= 97.7%.

16. 4-Chloro-2,5-difluoro-benzoic acid 4-(5-{difluoro-[5-(4-methanesulfonyl-piperazin-1-yl)-pyridin-2-yl]-methyl}-[1,3,4]-oxadiazol-2-ylsulfanyl)-but-2-ynyl ester:{(12 F), Entry-6}:
Off white solid. M.p : 135-138 ^oC. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm): 8.50 (s, 1H), 7.60 (m, 1 H), 7.45 (d, *J*= 7.5 Hz, 1H), 7.36 (d, *J*=7.0 Hz, 1H), 7.08 (m, 1H), , 4.90 (s, 2 H), 3.70(s, 2H), 3.27 (d, *J* = 6.5 Hz, 4H), 2.86 (d, *J* = 6.0 Hz, 4 H), 2.84 (s, 3H), MS (ESI): 635.2 $[M^+ +1]$ & 636.2 $[M^+ + 2]$,HPLC= 98.1%.

17. 2-Chloro-4-trifluoromethyl-benzoic acid 4-(5-{difluoro-[5-(4-methanesulfonyl-piperazin-1-yl)-pyridin-2-yl]-methyl}-[1,3,4]-oxadiazol-2-ylsulfanyl)-but-2-ynyl ester: {(12 g), Entry-7}:
Thick light green syrup. ^1H NMR (500 MHz, $CDCl_3$): δ (ppm): 8.61 (s, 1 H), 7.84 (d, *J*= 8.0 Hz,1H), 7.45 (d, *J*= 7.5 Hz, 1H), 7.33 (d, *J*=7.0 Hz, 1H), 7.57-7.44 (m, 2H),4.91 (s, 2 H), 3.73 (s, 2H), 3.29 (d, *J* = 6.5 Hz, 4H), 2.88 (d, *J* = 6.0 Hz, 4H), 2.89 (s, 3H), MS (ESI): 667$[M^+ +1]$ & 668.2 $[M^+ + 2]$, HPLC= 97.4%.

18. 2,3-Difluoro-benzoic acid 4-(5-{difluoro-[5-(4-methanesulfonyl-piperazin-1-yl)-pyridin-2-yl]-methyl}-[1,3,4]oxadiazol-2-ylsulfanyl)-but-2-ynyl ester: {(12 h), Entry-8}:
Off white solid. M.p: 117-121^oC. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm): 8.57 (s, 1 H), 7.72 (d, *J*= 8.0 Hz,1H), 7.48 (d, *J*= 7.5 Hz, 1H), 7.38 (d, *J*=7.0 Hz, 1H), 7.17-7.13 (m, 2H), 4.97 (s, 2 H), 3.74 (s, 2H), 3.30 (d, *J* = 6.5 Hz, 4H), 2.91 (d, *J* = 6.0 Hz, 4H), 2.90 (s, 3H), MS (ESI): 600.5 $[M+1]^+$, HPLC= 93.7%.

19. 3,4-Difluoro-benzoic acid 4-(5-{difluoro-[5-(4-methanesulfonyl-piperazin-1-yl)-pyridin-2-yl]-methyl}-[1,3,4]oxadiazol-2-ylsulfanyl)-but-2-ynyl ester: {(12 I), Entry-9}:
White solid. 119-125 ^oC.^1H NMR (400 MHz, $CDCl_3$) : δ (ppm): 8.65 (s, 1H), 7.73 (d, *J*= 8.0 Hz,1H), 7.66 (d, *J*= 7.5 Hz, 1H),7.45 (d, *J*= 7.5 Hz, 1H), 7.32 (d, *J*=7.0 Hz, 1H), 7.06 (m, 1H), 4.92, (s, 2H), 3.74 (s, 2H), 3.30 (d, *J* = 6.5 Hz, 4H), 2.91 (d, *J* = 6.0 Hz, 4H), 2.92 (s, 3H), MS (ESI): 600.0 $[M+1]^+$, HPLC= 95.7%.

20. 5-Chloro-pyridine-2-carboxylic acid 4-(5-{difluoro-[5-(4-methanesulfonyl-piperazin-1-yl)-pyridin-2-yl]-methyl}-[1,3,4]oxadiazol-2-ylsulfanyl)-but-2-ynyl ester: {(12 J), Entry-10}:
White solid : m.p : 98-102^oC. ^1H NMR (400 MHz, $CDCl_3$)**:** δ (ppm): 9.21 (s, 1 H), 8.65 (S, 1 H), 8.54 (d, *J*= 8.5 Hz,1H), 8.42 (d, *J*=8.0 Hz, 1H) ,7.45 (d, *J*= 7.5 Hz, 1H), 7.32 (d, *J*=7.0 Hz, 1H), 4.94 (s, 2 H),

3.74 (s, 2H), 3.30 (d, J = 6.5 Hz, 4H), 2.91 (d, J = 6.0 Hz, 4H), 2.80 (s, 3H), MS (ESI): 600.0 $[M+1]^+$, 602 $[M+2]^+$, HPLC= 99.34%.

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AN EFFICIENT METHOD FOR THE PREPARATION OF 2′-O-(2-METHOXYETHYL)-AND 2′-DEOXY NUCLEOSIDE-5′-CARBOXYLIC ACIDS

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ABSTRACT

Modified nucleosides have become increasingly useful agents for the preparation of biologically active molecules, especially in the treatment of cancer and viral diseases[1]. In continuation of our research, we intended to use oligonucleotides having radio actively labelled hydrogen at C-5' position of one of the nucleoside units, which can be followed throughout the body of an animal to study the drug mechanism. For this purpose we wished to use 2'-O-(2-Methoxyethyl)-nucloside-5'carboxylic acid methyl ester, and 2-deoxynucleoside-5'-carboxylic acid methyl ester, which can be finally reduced with a deutirated reducing agent to afford the 5'- D- radio labelled nucleosides.

KEYWORDS

2′-O-(2-Methoxyethyl)-nucleosides, 2-deoxynucleoside-5′-carboxylic acid methyl ester, 2′-O-(2-Methoxyethyl)-nucloside-5′carboxylic acid methyl ester

INTRODUCTION

The preparation of these compounds involves a multi step synthesis following protection and deprotection strategies. We, herein, report an efficient method for the preparation of 2′-O-(2-Methoxyethyl) and 2′-Deoxynucleoside-5′-carboxylicacids. For this purpose, we used the partially protected nucleosides as starting materials. The first step, we chose, was to protect the 3′-hydroxyl group as stable silyl ether, which will withstand the subsequent deprotection and oxidation. Thus, treatment of 1a-8a with t-butyl diphenyl silyl chloride, using imidazole and dry dimethylformamide at 35-40°C, to our surprise, found only 2a, 4a, 6a, 8a to undergo a smooth silylation to afford 2b, 4b, 6b, 8b in 88, 90, 91 and 94 % yields respectively. The reason for the failure of silylation of 1a, 3a, 5a, and 7a, may probably be attributed to the steric hindrance by the substituents

present in 2′ and 5′ positions. These, substrates, however, were smoothly converted to the corresponding *t*-butyldimethylsilylethers, 1b, 3b, 5b, 7b in 93, 92, 93 and 86% yields respectively. The subsequent DMT- deprotection of these nucleosides was also studied in various conventional reported reaction conditions, such as 10% Pd-C, *p*-TSA, 90% acetic acid and HCl-Dioxane. Unfortunately none of them were of general use but found to be selective for some particular nucleosides. Thus treatment of 1b, 5b, and 7b, with p-TSA in dry methanol at 10-30°C afforded 1c, 5c, 7c in 80, 76, and 63% yields respectively after column chromatographic purification.

Similarly, The DMT deprotection of 3b, 4b, 6b and 8b by 90 % acetic acid at 30-35°C, afforded 3c, 4c, 6c, 8c in 62, 56, 70 and 75 % yields respectively. Interestingly, compound 2b, under the similar reaction conditions, resulted in the formation of complex mixture of products. The same compound, however, underwent deprotection using a 5% solution of HCl-Dioxane at − 50°C to yield 2c in 75% yield after column chromatographic purification. In none of these acid mediated deprotection reactions, there was no evidence of formation of other by-product resulting from silylgroup migration from C-3′to C-5′ position. Our next objective was oxidizing the alcohols to their corresponding nucleoside 5′-carboxylicaicds using a suitable oxidation system. Our initial attempt to oxidize **1c** using Platinum catalyst[2,3] was incomplete and afforded the carboxylic aicds in very low yield. Potassium permanganate mediated oxidation[4] of **1c** under strongly basic conditions provides complex mixture of products. In another approach, attempt to oxidized by CrO_3 and Acetic anhydride followed by oxidation with *m*-CPBA[5,6] were also unsuccessful. As per a recently reported method a mixture of ruthenium trichloride and sodiumperiodate[7] however failed to yield the 5′-carboxylicacids and instead depurination was observed under these conditions. As a result, these methods have not been a method of choice for the preparation of nucleoside-5′-carboxylicacids.In a recent publication there reported an efficient method for the preparation of 2,3-Isopropylidine-protected nucleoside-5′-carboxylicacid using higher concentration of water, catalytic amounts of 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) and stoichiometric amount of an oxidizing agent, namely, bis-acetoxyiodobenzene (BAIB) [8,9]. This method seemed to be useful in the sense that the active oxidant N-ox ammonium salt is generated by the dismutation of TEMPO that is used in catalytic amounts and is regenerated during the reaction by BAIB. The by products acetic acid and iodobenzene are easily purified by means of simply organic solvent washing. Taking advantage of the efficacy of this strategy, we further wished utilize this method for the preparation of 3′-O-silylnucleoside-5′-carboxylicacids. Thus, oxidation of **1c** with TEMPO-BAIB system at 10 ^{0}C for 2 hours precipitated the crude carboxylic acid, which was filtered and triturated with ether - acetone system to afford **1d** in high purity and 90.5% yield.

Similarly, the efficiency of this methodology was further proved by the preparation of 2′-deoxy-3′-O-silylated-5′-carbxylicacids. Thus, oxidation of **2c** at room temperature furnished the crude which was freed from the traces TEMPO impurities by washing with diethyl ether to obtain carboxylic acid **2d** as a white powder in 83% yield. Further, oxidation products of thymine related nucleosides behaved differently from that of adenosine related carboxylic acids. Thus, the product **3d** did not precipitate and remained in the solution. Attempts to precipitate the product as the corresponding sodium salts by the addition of sodium bicarbonate, as per the reported procedure, were also unsuccessful.

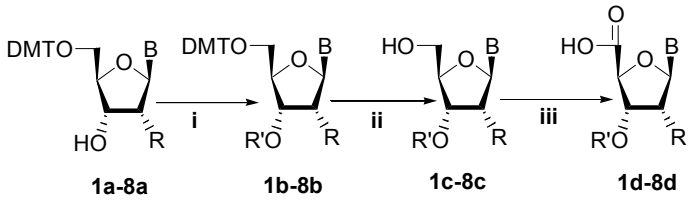
A slight modification in the work up procedure thus was necessary to obtain the product. The reaction mixture was reduced to half of its originals volume and to was added 5% toluene/hexane and stirred at room temperature 30 minutes. The resulting precipitate was filtered and stirred with n-hexane and filtered to give **3d** in 86.7% yield with high purity. Similarly, the oxidation of **4c** at 15-20°C for 3 hours afforded the carboxylic acid **4d**.

However, the isolation of the product was not similar to that of **3d**, and got precipitated directly from the reaction medium. The crude was stirred with toluene for 30minutes and it was filtered to furnish **4d** in 87.62 % yield. Oxidation of guanosine and cytosine derivatives has also been undertaken to assess the efficacy of this methodology. We found that, the oxidation chemistry and the isolation process were little different from that of other nucleoside derivatives and as a result some procedural modification were required to obtain the carboxylic acids in good yields and purity.

Initial attempt to oxidize **5a** using the TEMPO-BAIB system at 15-20°C resulted in the formation of complex mixture of products. However, running the reaction at 0-5°C for 5 hrs afforded the carboxylic acid, which did not however precipitate from the reaction mixture. Attempt to precipitate as the corresponding sodium carboxylate salt also was unsuccessful. The solvents were then stripped off under diminished pressure and the product was isolated by column chromatographic purification, and finally purified by trituration with toluene- hexane to afford **5d** in 60% yield. In contrast to the above observed fact, the 3-O-silyl-2-deoxyguanosine-5′-carboxylicacid **6d** formed at 15-20°C and precipitated out from the reaction mixture. However, the compound was finally obtained in high purity in 71% yield by passing through a small bed of silica gel. The oxidation and isolation behavior of cytosine derivatives was also similar to that of guanosine derivatives.

Thus, oxidation of **7c** with the TEMPO-BAIB system at 15-20 deg gave mixture of complex products. However, oxidation at 0-5°C for 6 hrs afforded the product **7d** in 50% yield after chromatographic purification. Interestingly, the reaction mode and isolation behavior was different in case of cytosine also. Oxidation of 2′-deoxy-3′-O-silylcytosine derivative **8c** at 15- 20°C furnished the corresponding crude carboxylic acid which finally purified after recrystallisation from diethyl ether to furnish **8d** in 78% yield. In summary, we have developed an efficient, general and mild method for the preparation of 3’-O-silylated-nucleoside 5′-carboxylicacids. The generality of this methodology lies in the fact that, in most of the cases the corresponding nucleoside 5′-carboxylicacids precipitated themselves in good yields and purity directly from the reaction mixture, which were conveniently purified by trituration with suitable organic solvents. The efficacy and mildness of this reaction was further proved by the tolerance of acid, base and oxidative labile functional groups to the reaction conditions thus making it suitable for large-scale productions.

Scheme:



1a = $A^{Bz;}$ R=OMOE
2a = A^{Bz}; R=H
3a = T; R=OMOE
4a = T; R=H
5a = G^{Ibu}; R=OMOE
6a = G^{Ibu}; R=H
7a = C^{Bz}; R=OMOE
8a = C^{Bz}; R=H

1d =A^{Bz}; R=OMOE; R’=TBDMS
2d = A^{Bz}; R=H; R’=TBDPS
3d = T; R=OMOE; R’=TBDMS
4d = T; R=H; R’=TBDPS
5d = G^{Ibu}; R=OMOE; R’=TBDMS
6d = G^{Ibu}; R=H; R’=TBDPS
7d = C^{Bz}; R=OMOE; R’=TBDMS
8d = C^{Bz}; R=H; R’=TBDPS

i) TBDMS-Cl or TBDPS-Cl, Imidazole, DMF.ii) *p*-TSA / MeOH (for 1b, 5b, 7b), 90% AcOH, 0→ RT (for 3b, 4b, 6b, 8b), 1,4-Dioxane- HCl (for 2b) iii) TEMPO / BAIB, CH_3CN: Water (1:1), 0→RT ,A^{BZ} = N-Benzoyl Adénosine; G^{Ibu} = N-Isobutyryl Guanosine,T = Thymine; CBz = N-Benzoyl Cytidine

EXPERIMENTAL SECTION

General Methods.

All the reactions were carried out under anhydrous conditions as and when necessary. All the Starting materials and solvents were dried as per the known standard procedures. All the reactions were performed under nitrogen atmosphere. Thin Layer Chromatography (TLC) using Merck silicagel 60 F254 0.25 mm plates followed the progress of the reactions. Visualization was performed using ultraviolet light and 5% sulfuric acid in methanol. All the products were purified either by crystallization or by chromatography using Acme 60-120 mesh silica gel only. Concentration of solvents referes to the evaporation under reduced pressure using Heidolph rotary evaporator and an industrial vacuum line. ^1H and ^{13}C NMR spectra were recorded on Varian Gemini spectrometers at 200 or 400 MHz for proton and at 50 or 100 MHz for carbon and are indicated. The chemical shift data is reported in parts per million (delta) down field from TMS.

General Procedure for the silylation of partially protected nucleosides:

The dried nucleoside (1.0 mmol) and Imidazole (4 mmol) were dissolved under stirring in dry N,N-dimethylformamide (1- 2 ml) and a chlorosilane reagent (2.5-3.0 mmol) Were added portions over 5 minutes period at 30-40°C and the resulting solution was stirred at that temperature for the required period (3-8 hrs). TLC monitored the progress of the reaction. Reaction mixture was further diluted with ethyl acetate and washed successively with water, aqueous sodium bicarbonate solution, and water and finally followed by brine. The organic layer was dried over anhydrous sodiumsulphate and evaporated under reduced pressure to yield the silylated nucleoside derivative individually described below.

1. 5′-O-(4,4′-Dimethoxytrityl)-3′-O-t-butyldimethylsilyl-2′-O-(2-methoxyethyl)-N-6-benzoyladenosine (1b):

Silylation of 1a (25.0 gms, 34.17 mmol), dry DMF (200 ml), Imidazole (9.297 gms, 136.72 mmol), and t-Butyldimethylchlorosilane (12.85 gms, 85.45 mmol) for 3 hrs, as per the above-described procedure afforded 1b as a semi solid (27.0 gms, 93.4% yield).^1H NMR ($CDCl_3$): δ (ppm): 0.1 & 0.2 (6H, 3H X2, 2S), 0.9 (9H, s, t-Bu), 3.2 (3H, s, OCH_3), 3.2-3.5 (4H, m), 3.6-3.8 (2H, m), 3.80 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 4.25 (1H , dd, *J*=7.8 ,1.4 Hz), 4.45 (1H, dd, *J*=7.3, 1.6 Hz), 4.65 (1H, dd, *J*=7.8, 2.3 Hz), 6.2 (1H, d, *J*=8.8 Hz), 6.8 (4H, d, *J*=10.5 Hz, Ph), 7.2-7.6 (14 H, m, Ph), 8.25 (1H, s, H-3), 8.8 (1H, s, H-8), 9.1 (1H, s, Br , NH). ^{13}C NMR ($CDCl_3$): δ (ppm): -4.9, -4.7, -3.6, -3.0, 18.0, 25.6, 25.9, 55.1, 58.7, 62.7, 70.1, 71.0, 71.9, 81.4,84.6, 86.5, 87.4, 113.1, 123.5, 126.8, 127.7, 127.8, 128.1, 128.6, 130.0, 130.9, 132.5, 133.7, 135.7, 142.1, 144.4, 149.5, 151.5, 152.5, 158.5.

2. 2-Deoxy-5′-O-(4,4′-dimethoxytrityl)-3′-O-t-butyldiphenylsilyl-N-6-benzoyladenosine (2b):

Silylation of **2a** (10.0 gms, 15. 1 m.moles), dry DMF (50 ml), Imidazole (4.1 gms, 60.6 m.moles), and t-Butyldiphenylylchlorosilane (12.4 gms, 45.5 mmol) for 8 hrs, as per the above-described procedure afforded 2b as a thick syrup (27 gms, 93.4% yield). ^1H NMR ($CDCl_3$): δ (ppm): 1.1 (9H, s, *t*- Bu), 2.3 (1H, m), 2.8 (1H, m), 3.7 (6H, s, 2 X OCH3), 4.3 (1H , d , *J*=8.2 Hz), 4.7 (1H, m), 5.3 (1H, d, *J*=9.2 Hz), 6.5 (1H, t), 6.7 (2H, d, *J*=5.3 Hz), 7.1-7.4 (10H, m), 7.67.8 (8H, m), 8.0 (2H, t), 8.7 (1H, s). ^{13}C NMR ($CDCl_3$): δ (ppm): 13.9, 18.83, 20.731, 26.4, 40.0, 54.9, 60.26, 63.3, 73.7, 76.3, 77.6, 84.9, 86.2, 112.5, 122.5, 126.6, 127.4, 127.9, 128.3, 1129.2, 132.2, 133.3, 134.6, 135.4, 140.9, 144.3, 149.6, 150.9, 152.3, 158.2, 164.

3. 5′-O-(4, 4′-Dimethoxytrityl)-3′-O-t-butyldimethylsilyl-2′-O-(2-methoxyethyl) Thymine riboside (3b):

Silylation of **3a** (25 gms, 40.46 mmol), dry DMF (150 ml), Imidazole (11.0 gms, 161.82 mmol), and *t*-Butyldimethylchlorosilane (18.2 gms, 121.36 mmol) for 4 hrs, as per the above described procedure afforded 3b as a semi solid (27.5 gms, 92.74 % yield). ^{1}H NMR ($CDCl_3$): δ (ppm): 0.1 (6H, 3H X s, Si $(CH)_3$), 0.9 (9H, s, Si-t Bu), 3.2 (1H , dd , *J*=7.8,2.5 Hz), 3.3 (3H, S), 3.52-3.58 (2H, m), 3.8 (6H, S , OCH_3 X 2), 4.1 (2H , m), 4.4 (1H, dd , *J*=6.5 , 2.3 Hz), 6.0 (1H, d , *J*= 6.2 Hz), 6.8 (2H, d , *J*=7.5 Hz), 7.2 –7.4 (16H, m, Ph), 7.8 (1H, S), 8.6 (1H, S, Br, NH). ^{13}C NMR ($CDCl_3$): δ (ppm): 5.12, 4.7, 11.6, 17.9, 25.5, 58.8 , 61.8, 69.8, 70.0, 72.0, 82.5, 83.3, 86.7, 87.9, 110.6, 113.1, 127.0, 127.7, 128.1, 130.0, 135.3, 135.5, 144.1, 150.4, 158.6, 164.2.

4. 2-Deoxy-5′-O-(4,4′-dimethoxytrityl)3′O-t-butyldiphenylsilyl-thymine riboside (4b):

Silylation of **4a** (20.0 gms, 37.76 mmol), dry DMF (100 ml), Imidazole (7.5 gms, 110.30 mmol) t-butyldiphenylylchlorosilane (20.15 gms, 73.53 mmol) for 3 hrs, as per the described procedure afforded 4b as a semi solid (27.0 gms, 93.4% yield). ^{1}H NMR (DMSO-d_6): δ (ppm): 0.9 (9H, s, Si- t-Bu) ,1.45 (3H, s), 2.2 (1H, ddd, *J*=13.5, 7.5, 6.3 Hz), 2.45 (1H, dd, *J*=13.5, 5.5 Hz), 2.8 (1H, dd, *J*=10.5, 2.2 Hz), 2.9-3.2 (1H, dd, *J*=10.1, 2.0 Hz), 3.8 (3H, s) , 4.0 (1H, s), 4.5 (1H, m), 6.3 (1H, dd, *J*=7.0, 5.1 Hz), 6.8 (2H, d, *J*=8.1 Hz), 7.1-7.8 (21H, m, Ph), 11.4 (1H, S, Br). ^{13}C NMR ($CDCl_3$): δ (ppm): 11.5, 15.0, 18.9, 26.5, 26.8, 41.0, 55.0, 63.2, 73.6, 84.8, 86.7, 110.9, 113.1, 126.9, 127.5, 127.9, 129.0, 129.4, 129.9, 132.9, 134.7, 135.3, 135.6, 144.2, 150.2, 158.5, 163.8.

5. 5′-O-(4,4′-Dimethoxytrityl)-3′-O-t-butyldimethylsilyl-2′-O-(2-methoxyethyl)-N-2-isbutyrylguanosine (5b):

Silylation of **5a** (25 gms, 35.04 mmol), dry DMF (200 ml) Imidazole (9.52 gms, 140.12 mmol) and t-Butyldimethylsilylchloride (15.77 gms, 105.19 mmol) for 5hours, as per the described procedure to afford **5b,** as a semi-solid (27 gms, 93.1% yield).^{1}H NMR ($CDCL_3$): δ (ppm): 0.1 & 0.2 (6H, 2s, $Si(CH_3)_2$ X 2), 0.90 (9H, s, t-Bu), 1.0 (6H, d, 2 X CH_3), 3.0 (1H, m, $CH(CH_3)_2$), 3.3 (3H, S, OCH_3), 3.4-3.6 (4H, m, CH_2 X2), 3.75 (2H, m), 3.8 (6H, S, $(OCH_3)_2$), 4.1 (1H, dd, *J*=5.7, 1.85 Hz), 4.4 (1H, dd, *J*=5.6, 1.3 Hz), 4.7 (1H, m), 5.9 (1H, d, *J*=5.9 Hz), 6.8 (5H, d, Ph), 7.3-7.6 (8H, m, 2 X Ph), 7.8 (1H, S), 7.9 (1H, S, Br, NH), 11.95 (1H, S, Br, NH). ^{13}C NMR ($CDCL_3$): δ (ppm): -4.9, -4.7, -3.6 , -2.9, 18.0, 18.5, 25.6, 36.1, 55.2, 63.1, 70.0, 70.7, 72.0, 81.4, 85.3 , 86.4, 86.7, 113.2, 127.0, 127.9, 128.0, 129.9, 135.6, 135.9, 138.3, 144.7, 147.1, 155.4, 158.7, 178.1.

6. 2-Deoxy-5′-O-(4,4′-dimethoxytrityl)-3′-O-t-butyldiphenylsilyl-N-2-isobutyrylguanosine (6b):

Silylation of **6a** (25 gms, 39.87 mmol), dry DMF (200 ml), Imidazole (10.84 gms, 159.48 mmol) and t-Butyldiphenylylchlorosilane (32.77gms, 119.61 mmol) for 4 hrs, as per the described procedure afforded 6b (6% MeOH/$CHCl_3$: Rf=0.5) semisolid (27.0 gms, 93.4% yield). ^{1}H NMR ($CDCl_3$): δ (ppm): 1.1 (9H, s, tBu), 1.2 (6H, d, 2 x CH_3); 2.3 (2H, M), 2.6 (1H, M, CH $(CH_3)_2$); 3.1 (1H, t); 3.7 (1H, d), 3.8 (6H, s, $(OMe)_2$, 4.1 (1H, s), 4.6 (1H, s), 5.0 (1H, d) 6.2 (1H, dd), 6.8 (5H, d, Ph), 7.3-7.6 (8, m, 2x Ph), 7.8 (1H, s, br NH), 11.95 (1H, s, br NH), ^{13}C NMR ($CDCl_3$): δ (ppm): 18.8, 26.9, 36.3, 40.9, 62.5, 74.4, 86.5, 89.0, 122.4, 127.8, 128.3, 128.9, 130.0, 133.1, 135.1, 135.31, 143.8, 144.2, 147.4, 155.1, 178.

7. 5′-O-(4,4′-Dimethoxytrityl)-3′-O-t-butyldimethylsilyl-2′-O-(2-methoxyethyl)-N-4-benzoylcytosine (7b):

Silylation of **7a** (10.0 gms, 13.8 mmol), dry DMF (50 ml), Imidazole (3.7 gms, 5.5 mmol), and t-Butyldimethyl chlorosilane (6.2 gms, 41.4 mmol) for 6 hrs, as per the above-described procedure afforded 7b as a semi solid (10.0 gms, 86 % yield). ^{1}H NMR ($CDCl_3$): δ (ppm): 0.2, 0.4 (6H, 2xs, $Si(Me)_2$, 0.9 (9H, s, tBu), 1.5 (3H, s, CH_3), 3.15 (3H, s, OCH_3), 3.65 (4H, m), 3.8 (1H, d, *J*=8.2 Hz), 3.9 (6 H, s, 2 X OCH_3), 4.0 (2H, m), 4.2 (1H, d, *J* =8.5 Hz), 4.4 (1H, m), 6.0 (1H, d, *J* =9.0 Hz), 6.8 (4H, dd, *J* =9.5 Hz, 9.8 Hz), 7.3,7.6 (13H, m), 8.0 (1H, s), 8.3 (2H, d). ^{13}C NMR ($CDCl_3$): δ (ppm): 2.9, 12.782, 18.026, 25.630, 55.219, 58.943, 61.762, 69.981, 72.139, 82.836, 88.488, 111.780, 113.227, 127.158, 128.018, 129.843, 130.191, 132.291, 135.354, 136.919, 137.209, 144.226, 147.883, 158.780, 159.796, 179.483.

8. 2-Deoxy-5′-O-(4,4′-dimethoxytrityl)-3′-O-t-butyldiphenylsilyl-N-4-benzoylcytidine (8b):

Silylation of **8a** (25.0 gms, 39.49 mmol), dry DMF (150 ml), Imidazole (10.8 gms, 157.6 mmol), and t-Butyldiphenylylchlorosilane (32.4 gms, 118.3 mmol) for 4 hrs, as per the above-described procedure afforded 8b as a thick syrup (27gms, 94 % yield).

^{1}NMR ($CDCl_3$): δ (ppm): 1.1 (9H. S. tBu), 1.3 (1H, m), 1.8 (2H. s), 2.4 (1H.S), 2.8 (1H, m), 2.9 (1H, m), 3.25 (1H, d, *J* =7.3, 1.6 Hz), 3.6 (1H, s), 4.05 (1H, dd, *J* =6.7, 1.4 Hz), 6.5 (1H, s), 6.8 (4H, dd), 7.3 (9H, m), 7.6 (9H, s, Ph), 7.8 (5H, t, Ph), 7.9 (2H, d, *J* =7.6, 1.4 Hz), 7.3 (9H, M,) ^{13}CNMR ($CDCl_3$): δ (ppm): 14.8, 18.69, 21.00, 26.3, 26.5, 41.7, 54.6, 65.2, 72.1, 86.5, 86.9, 96.0, 112.9, 124.9 126.7, 127.4, 127.5, 127.8, 128.3, 128.5, 128.9, 129.7, 132.5, 134.5, 134.9, 135.0, 135.3, 135.5, 143.8, 144.2, 154.1, 158.3, 162.0, 166.2.

General procedure for the deprotection of the Dimethoxytrityl group:

Method A: Using p-TSA in methanol.

The nucleoside 5′-O- DMT derivative (1.0 mmol) was dissolved in dry methanol (8-10 ml) and anhydrous p-TSA (0.6 mmol) was added in ice-cold condition. The solution was stirred at 10^0C till the TLC showed no starting material. The reaction mixture was extracted in to ethyl acetate, washed with saturated sodium bicarbonate solution, and finally with water. The organic layer was dried over sodium sulphate and concentrated under reduced pressure to afford the crude, which was purified by 60 - 120 mesh silica gel to afford the pure deprotected compound.

Method B: Using 90% Acetic acid.

The nucleoside DMT derivative was dissolved in 90% dilute acetic acid. The solution was stirred at RT for 6-7 hrs. TLC monitored the progress of the reaction. The reaction mixture was diluted with ethyl acetate and washed with saturated sodium bicarbonate solution and finally with water. The organic layer was dried over sodium sulphate and concentrated to afford syrup, which was purified by silica gel chromatography to furnish the title compound.

Method C: Using $HCl_{(g)}$ / 1,4 - Dioxane:

A solution of Hydrogen chloride in 1,4-dioxane (2.5 ml) was added to a stirred solution of nucleoside DMT derivative in dry dichloromethane at –50^{o}C. After 2 min an aliquot (1 ml) of the reaction mixture was quenched by adding it to a mixture of pyridine-methanol (1:1, 1ml) at –50 deg. C. The reaction mixture was diluted with dichloromethane and washed with saturated sodium bicarbonate solution and finally with water. The organic layer was dried on anhydrous sodium sulphate and concentrated to give thick syrup, which was purified by silica gel chromatography to furnish the depredated compound.

9. 2′-O-(2-Methoxyethyl)-3′-O-t-butyldimethylsilyl-2′-6-N–benzoyladenosine (1c):

Deprotection of **1b** (27.3 gms, 31.9 mmols), dry methanol (200ml) anhydrous p-TSA (3.64 gms, 19.15 mmols) following the method A for 10 hrs, afforded 1c (14.0 gms, 80.7% yield) after column chromatographic purification, as a solid, m.p 123-5 ^{0} C. ^{1}H- NMR (DMSO-d_6): δ (ppm): 0.1 & 0.2 (6H, s), 0.9 (9H, s, t-Bu), 3.05 (3H, s, OCH_3), 3.2-3.4 (4H, m), 3.6-3.8 (2H, m) 3.9 (1H, m), 4.2 (1H, s), 4.55 (1H , d , *J* = 5.12 Hz), 4.8 (1H ,dd, *J* =5.12,7.5 Hz), 5.9 (1H, d, *J* =7.5 Hz), 6.2 (1H, dd, *J* =1.5, 7.2 Hz), 7.4-7.6, 8.0 (5H, m, Ph), 8.1(1H, s, H-3), 8.8 (1H, s, H-8), 9.2 (1H, s, Br. NH). ^{13}C NMR ($CDCl_3$)**:** δ (ppm): -4.9 , -4.8 , 17.99, 25.5, 58.5, 62.5, 69.8, 71.6, 81.2, 89.1, 89.4, 124.4, 127.7, 128.5, 132.5, 133.3, 143.2, 150.1, 150.5, 151.7, 164.5.

10. 2′-O-(2-Methoxyethyl)-3′-O-t-butyldimethylsilyl-N-2-isobutyrylguanosine (5c):

Deprotection of **5b** (27.0gms, 32.61 m.moles), anhydrous p-TSA (3.72 gms, 19.56 mmols) following method A for 5 hours, afforded the title compound as a white powder (13gms, 75.8% yield), m.p 88-92^{0}C. ^{1}H NMR ($CDCl_3$): δ (ppm): 0.1 & 0.2 (6H, 2s, $Si(CH_3)_2$ X 2) ,0.95 (9H, s, t-Bu), 1.3 (6H, d, 2 XCH_3), 2.6 (1H, m, $CH(CH_3)_2$), 3.1 (3H, s, OCH_3), 3.3-3.9 (6H, m, CH_2 X3), 4.1(1H, s), 4.4 (2H, m), 5.3 (1H, d, 5^1-OH), 5.8 (1H , dd , *J*=9.5 , 1.7) , 7.8 (1H, S, H-8), 8.3 (1H, S, Br, NH), 12.05 (1H , S , Br , NH). ^{13}C NMR ($CDCl_3$) :

-4.9 , -4.7, 18.1, 18.8, 25.6,36.01, 58.7, 61.9, 70.0, 71.3, 71.9, 81.7, 87.3, 88.4, 96.0,139.0, 147.7, 155.3, 179.3.

11. 2′-O-(2-Methoxyethyl)-3′-O-t-butyldimethylsilyl-4-benzoylcytosine (7c):

Deprotection of **7b** (10 gms, 11.9 mmols), anhydrous p-TSA (2.5 gms, 13.15 mmols) and methanol (50 ml) at room temperature following method A for 6 hrs, afforded the product 4.0 gms (62.7%), as a white solid, m.p 153-5^{0}C. ^{1}H- NMR (CDCl3): δ (ppm): (0.1 & 0.2 (6H, 2Xs, $Si(Me)_2$), 0.9 (9H, s, t-Bu), 2.0 (3H, s, CH_3), 3.2 (3H, s, OCH_3), 3.4-3.6 (4H, m), 3.8-4.0 (2H, m), 4.2 (2H, m), 5.5 (1H, d, *J*=5.6 Hz), 7.2-7.4 (3H, m, Ph), 7.6 (1H, s), 8.2 (2H, d, Ph), 13.0 (1H, s, br). ^{13}C-NMR (CDCl3): δ (ppm): -4.9, -4.7, 13.4, 18.1, 25.6, 58.8, 61.1, 69.9, 71.9, 81.3, 85.6, 91.9, 111.5, 128.0, 129.8, 132.4, 137.0, 139.3, 148.0, 159.6, 179.5.

12. 2′-Deoxy-3′-O-t-butyldiphenylsilyl-N-6-benzoyladenosine (2c):

Deprotection of **2b** (8.95 gms, 10.0 mmol), dichloromethane (200 ml), and a solution of HCl-Dioxane (25 ml) as per the method C for 2 minutes, furnished the titled compound **2c** as a white solid , 4.4 gms(75% yield), m.p 153-5 ^{0}C. ^{1}H - NMR ($CDCl_3$): δ (ppm): 1.05 (9H, S, t-Bu), 2.3 (1H, dd , *J*=5.2, 10.0 Hz, H-2^1) , 2.8 (1H, ddd, *J* = 1.8, 3.5, 7.8 Hz, H-2^{11}), 3.2 (1H, m), 3.7 (1H, d, *J*=12Hz), 4.2 (1H, S), 4.8 (1H, d, *J*=6.0 Hz), 5.5 (1H, d, *J* =9.5 Hz) , 6.4 (1H, dd, *J* =5.2 , 10.1 Hz ,H-1^1) , 7.4-8.0 (15H , m, Ph), 8.1 (1H, S, H-3), 8.7 (1H, S, H-8), 9.1 (1H, S-Br, NH). ^{13}C - NMR ($CDCl_3$): δ (ppm): 18.93, 26.84, 40.9, 62.6, 74.6, 87.4, 89.9, 124.22, 127.8, 128.56, 129.99, 132.55, 133.09, 133.3, 135.5, 142.3, 150.1, 150.5, 151.7, 164.8.

13. 2′-O-(2-Methoxyethyl)-3′-O-t-butyldimethylsilylthymineriboside (3c):

Deprotection of 3b (27.5 gms), 90% acetic acid (250 ml), as per the method B for 3 hrs , furnished the titled compound **3c** as a white solid, 10.0 gms(62% yield), m.p 92-5^{0}C. ^{1}H- NMR ($CDCl_3$): δ (ppm): -0.05-0.06 (6H, 3H X S, Si $(CH_3)_2$), 0.9 (9H, S, t-Bu), 1.9 (3H, S), 3.2 (1H, d, *J*=1.52 Hz), 3.24 (3H, S), 3.4 (2H, d, *J*=1.64 Hz), 3.6 (3H, m), 3.8 (1H, d, *J*=1.55 Hz), 4.0 (1H, S), 4.2-4.4 (2H, m), 5.45 (1H, d, *J*=5.5 Hz), 7.4 (1H, S), 8.9 (1H, S, Br. NH). ^{13}C- NMR($CDCl_3$): δ (ppm): -5.0, -4.8, 12.2, 18.0, 25.6, 58.8, 61.3, 69.8, 70.0, 71.9, 81.0 , 85.6, 91.6, 110.5, 138.4, 150.5, 164.1.

14. 2′-Deoxy-3′-O-t-butyldiphenylsilylthymineriboside (4c):

Deprotection of **4b** (26.02 gms, 10.0 mmol), 90% acetic acid (250 ml), as per the method B for 2 hrs , furnished the titled compound **4c** as a white solid , 9 gms (56.5 % yield), m.p 104-6^{0}C. ^{1}H - NMR ($CDCl_3$) **:** δ 1.1(9H, S, Si (t-Bu), 1.85 (3H , S), 2.1-2.3 (2H, 2 X ddd , *J*=13.5, 7.5, 6.3 Hz), 3.2 (1H, dd, *J*=10.5, 2.2 Hz), 3.6 (1H, dd, *J*=9.8, 2.5 Hz), 4.0 (1H, d, *J*=7.5 Hz), 4.5 (1H, dd, *J*=9.5, 2.3 Hz), 6.2 (1H, dd, *J*=8.5, 4.5 Hz), 7.2-7.8 (11H, m, Ph) , 8.7 (1H, S, Br). ^{13}C- NMR (DMSO-d_6)**:** δ (ppm): 12.3, 18.9, 26.8, 40.3, 61.9, 72.9, 86.4, 87.6, 110.8, 127.8, 130.2, 133.0, 133.2, 135.6, 136.7, 150.3, 163.8.

15. 2′-Deoxy-3′-O-t-butyldiphenylsilyl-N-2-isobutyrylguanosine (6c):

Deprotection of **6b** (32 gms, 36.42 mmol), 90% acetic acid (100ml), as per the method B for 5 hrs , furnished the titled compound **8c** as a white solid , 15 gms(75 % yield), m.p 110-115^0 C. ^{1}H-NMR ($CDCl_3$): δ (ppm): 0.9 (9H, S, t-Bu), 1.2 (6H, 2xd, 2xCH_3), 1.6 (1H, M, H-2'), 2.2 (1H, M, H-2"), 2.8 (1H, M, H), 3.2 (1H, d, *J*=10 Hz), 3.7 (1H, d, *J*=10 Hz), 4.1 (1H, S) 4.6 (1H, S), 5.0 (1H, d, *J*=8 Hz), 6.2 (1H, dd, *J*=7.0, 2.1 Hz), 7.4 (5H, m, Ph), 7.6 (1H, S, H8), 7.8 (5H, S, Ph), 8.9 (1H, S, br. NH), 12.1 (1H, Sbr, NH). ^{13}CNMR ($CDCl_3$): δ (ppm): 18.8, 26.8, 36.1, 40.8, 62.5, 74.4, 86.5, 89.0, 122.4, 127.8, 130.0, 133.0, 135.6, 138.5, 147.4, 147.5, 155.1, 178.8.

16. 2′-Deoxy-3′-O-t-butyldiphenylsilyl-N-4-benzoylcytosine (8c):

Deprotection of **8b** (32 gms, 36.69 mmol), 90% acetic acid (100 ml), as per the method B for 6 hrs , furnished the titled compound **8c** as a white solid , 15 gm s(75 % yield), m.p 80-88^{0}C. ^{1}HNMR ($CDCl_3$): δ (ppm): 1.1 (9H. S. tBu), 2.3 (1H ,M ,H2'), 2.6 (1H, M, H2"), 3.25 (1H, d, *J*=7.5, 2.1 Hz), 3.6 (1H, d, *J*=8.1, 2.2 Hz), 4.05 (1H, dd, *J*=5.9, 2.3 Hz), 4.4 (1'H, M) 6.3 (1H', dd, J=7.8, 1.2 Hz), 7.4 (9H. S. Ph), 7.6 (6H, T,

Ph), 7.9 (2H, d, *J*=6.8, 1.4 Hz), 8.2 (1H, d), ^{13}CNMR ($CDCl_3$) δ (ppm): 19.03, 26.9, 41.5, 61.6, 72.4, 76.3, 77.0, 77.6, 88.2, 88.5, 96.6, 127.5, 127.8, 128.9, 130.1, 133.0, 135.6, 145.4, 162.1.

General procedure for the preparation of protected nucleoside-5'carboxylicacids:

The protected nucleoside (1.0 mmol) was dissolved in a 1:1 mixture of acetonitrile and water and the solution was cooled to 0 deg. C. Bisacetoxyiodobenzene (BAIB) (-2.5-3.0 moles), followed by 2,2', 6,6'-Tetramethyl-1-piperidinyloxyl (TEMPO) (0.4 mmols) was added to the cooled solution and stirring was continued at ~ 10- 35 deg. C and the respected products were isolated individually as described below.

17. 2-O-(Methoxyethyl)-3′-O-t-butyldimethylsilyl-N-6-benzoyladenosine-5′-carboxylicacid (1d):

1c (14.0 gms, 25.782 mmoles), BAIB (20.78 gms, 64.45 mmols) and TEMPO (1.61 gms, 10.31 mmols) and 140 ml of acetonitrile water (1:1) as per the described procedure. The resulting precipitate was filtered, triturated with ether acetone (10:1), filtered and dried in vaccuo to furnish 13.0 gms of **1d** (90.5% yield) as a white solid, m.p 204–6^{0}C. ^{1}**H**- NMR (DMSO-d_6): δ (ppm): 0.2 (6H, s, 2X Si $(CH_3)_2$, 1.0 (9H, s, Si –(t-Bu)), 3.2 (3H, s, OMe), 3.4-3.6 (4H, m), 4.45 (1H, s), 4.7 (2H, m), 6.3 (1H, d, *J*=9Hz), 7.50, 7.62, 8.1 (5H, m, Ph), 8.8 & 8.85 (2 X 1H, 2 X s, H-2, H-8). ^{13}C- NMR ($CDCl_3$): δ (ppm): -4.9, 18.08, 25.65, 58.68, 70.09, 72.03, 74.0, 81.88, 85.01, 88.4, 123.78, 128.03, 128.75, 132.88, 133.26, 142.95, 150.23, 150.69, 151.68, 164.8, 171.6 .

18. 2′-Deoxy-3′-O-t-butyldiphenylsilyladenosine-5′-carboxylicacid (2d):

2c (10.0 gms, 16.80 mmoles), BAIB (16.2 gms, 50.61 mmols) and TEMPO (1.0 gm, 6.0 mmols) and 120 ml of acetonitrile water (1:1) as per the described procedure. The resulting precipitate was filtered, triturated with ether acetone (10:1), filtered and dried in vaccuo to furnish **2d** 10.2 gms (83% yield) as a white solid, m.p 177 – 9 ^{0}C. ^{1}H -NMR ($CDCl_3$): δ (ppm): 1.05 (9H, S, t-Bu), 2.3 (2H, m), 4.8 (2H, m, H-3^1& 4^1), 6.65 (1H, dd, *J*=6.7, 2.2 Hz, H-1^1) , 7.4-8.0 (15H, m, Ph), 8.5 (1H, S, H-3^1), 8.7 (1H, S, H-8); ^{13}C - NMR ($CDCl_3$): δ (ppm): 18.43, 26.24, 40.21, 84.78, 84.92, 123.29, 127.25, 127.65, 127.85, 129.5, 131.83, 133.07, 134.97, 141.78, 149.25, 151.30, 164.8, 171.76.

19. 2′-O-(2-Methoxyethyl)-3′-O-t-butyldimethylsilylthymineriboside-5′-carboxylicacid (3d):

3c (10.0 gms, 23.20 mmoles), BAIB (22.42 gms, 69.6 mmols) and TEMPO (1.45 gms, 9.28 mmols) and 150 ml of acetonitrile water (1:1) as per the described procedure. The resulting precipitate was filtered, triturated with ether acetone (10:1), filtered and dried in vacuo to furnish **3d** 9.0 gms (86.7% yield) as a white solid, m.p 223 – 5^{0}C; ^{1}**H**- NMR ($CDCl_3$) : δ (ppm): 0.1-0.2 (6H, S, Si $(CH_3)_2$), 0.9 (9H, S, Si-t Bu), 1.8 (3H, S), 3.2-3.6 (4H, m), 4.2-4.4 (3H, m), 5.5 (1H, d, *J*=7.5 Hz), 7.3 (1H, S), 8.9 (1H, S, Br). ^{13}C – NMR ($CDCl_3$): δ (ppm): -5.0, -4.8, 12.3, 18.1, 25.6, 58.9, 69.9, 72.1, 73.8, 80.8, 83.3, 89.3, 111.4, 138.1, 150.9, 164.8, 173.5.

20. 2′-Deoxy-3-O-t-butyldiphenylsilylthymineriboside-5′-carboxylicaicd (4d):

4c (10.0 gms, 20.83 mmoles), BAIB (16.77 gms, 52.08 mmols) and TEMPO (0.98 gms, 6.25 mmols) and 100 ml of acetonitrile water (1:1) as per the described procedure. The resulting precipitate was filtered, triturated with ether acetone (10:1), filtered and dried in vacuo to furnish **4d** 9.2 gms (87.62% yield) as a white solid, m.p 234–6^{0}C. ^{1}H NMR ($CDCl_3$)**:** δ (ppm): 1.0 (9H, S, Si (t-Bu)), 1.8 (3H, S, t-CH_3), 1.8-2.2 (2H, m), 4.4 (1H, S), 4.6 (2H, d, *J*=4.6 Hz), 6.4 (1H, dd, *J*=9.5, 5.0 Hz), 7.4-7.7 (10H, m, Ph), 8.0 (1H, s), 11.3 (1H, S, Br). ^{13}C - NMR (DMSO - d_6)**:** δ (ppm): 12.3, 18.6, 26.6, 38.7, 76.5, 84.4, 85.5, 109.5, 127.9, 130.1, 132.4, 132.5, 135.2, 136.2, 150.5, 163.6, 171.

21. 2′-O-(2-Methoxyethyl)3′-O-t-butyl-dimethylsilyl-N2-isbutyrylguanosine-5′-carboxylic-acid (5d):

5c (13.0 gms, 24.74 mmoles), BAIB (23.92 gms, 74.22 mmols) and TEMPO (1.54 gms, 9.9 mmols) and 140 ml of acetonitrile water (1:1), at 0^{0}C as per the described procedure. The solvents were stripped off under diminished pressure, and the residue was purified by column chromatography to yield the titled compound as a white solid (8.0 gms, 60.1% yield), m.p 115-120^{o}C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.1& 0.2 (6H, 2s, Si$(CH_3)_2$ X 2), 0.9 (9H, s, t-Bu), 1.3 (6H, d, 2 XCH_3), 2.7 (1H, m, $CH(CH_3)_2$), 3.20 (3H, S, OCH_3),

3.43.65 (4H, m, 2 XCH_2), 4.3 (1H, m), 4.6 (2H, S), 6.1 (1H, dd, J=8.6, 1.75 Hz), 7.3 (1H, S), 8.5 (1H, S, H-8), 8.6 (1H, S, Br, NH) , 12.1 (1H, S, Br, NH). ^{13}C- NMR - ($CDCl_3$) δ (ppm): -4.9, 18.0, 18.8, 25.6, 29.6, 36.3, 58.7, 69.9, 71.9, 73.7, 82.7, 84.6, 87.1, 139.4, 147.8, 155.0, 178.7.

22. 2′-Deoxy-3-O-t-butyldiphenylsilylguanosine-5′-carboxylicaicd (6d):

6c (14.0gms, 25.74 m.moles), BAIB (19.60 gms, 60.86mmols) and TEMPO (1.52gms, 9.72 mmols) and 150ml of acetonitrile water (1:1) as per the described procedure. The resulting precipitate was filtered, washed with ether acetone (10:1) Dried in vaccuo to furnish **6c** 10.0 gms (71% yield) as a white solid, m.p 148– 152 ^{o}C. ^{1}HNMR($CDCl_3$) δ (ppm): 1.1 (s, 9H, tBu), 1.2 (d, 6H, 2xCH_3), 2.3 (2H, M, H2′,2′′), 2.6 (1H, M, $CH(CH_3)_2$, 4.6 (1H, S), 4.8(1H, S), 6.5 (1H, dd, J=8.0, J=1.8), 7.4 (5H, S, Ph), 7.6 (5H, S, Ph), 7.7 (1H, S, H8), 8.74 (1H, S, brNH), 12.1 (1H, S, NH). ^{13}CNMR ($CDCl_3$): δ (ppm): 19.0, 26.8, 27.3, 36.0, 127.7, 129.9. 132.6, 133.1, 135.5, 135.66, 155.65.

23. 2′-O-(2-Methoxyethyl)-3′-O-t-butyldimethylsilyl-N-4-benzoylcytosine-5'-*carboxylicacid (7d*):

7c (10.0gms,18.72 m.moles), BAIB (18.06 gms, 56.08mmols) and TEMPO (1.16gms, 7.4 mmols) and 50ml of acetonitrile water (1:1) as per the described procedure. The reaction mixture was concentrated under reduced pressure and the resulting residue was purified by column chromatography to yield **7d** as a solid (5.0gms, 50%yield), m.p125-9 ^{0}C. ^{1}HNMR($DMSO_6$) δ (ppm): 0.1 & 0.2(6H, 2Xs, $Si(Me)_2$, 0.9 (9H, s, tBu), 2.1 (3H, s, CH_3), 3.3 (3H, s, OCH_3), 3.45 (2H, m), 3.65 (2H, m), 4.2 (1H,m), 4.45 (1H, m), 4.6(1H, d, J=3.2Hz), 5.9(1H, d, J=8.3Hz), 7.4(5H, m, Ph), 8.2(2H, d), 8.4(1H,s), 13.0(1H,s,br); ^{13}CNMR($CDCl_3$): 4.9, 13.65, 18.11, 25.65, 58.92, 70.0, 72.1, 73.9, 81.1, 83.4, 89.4, 112.3, 127.3, 128.0, 128.6, 129.8, 132.3, 132.4, 136.8, 138.7, 148.6, 159.5, 169.8, 171.0, 173.4, 179.3.

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24. 2′-Deoxy-3-O-t-butyldiphenylsilyl-N-4-benzoylcytosine-5′-carboxylicaicd (8d):

8c (15.0gms, 26.32 m.moles), BAIB (21.21 gms, 65.84mmols) and TEMPO (1.64gms, 10.53 mmols) and 150ml of acetonitrile water (1:1), at 10-15 deg.C as per the described procedure. The Resulting gprecipitatewasfiltered,triturated with ether and acetone (10:1),filtered and dried in vacuo to afford **8d** 12.0 gms (78% yield) as a white solid, m.p 210 – 13^{o}C. ^{1}HNMR($CDCl_3$): 1.1(9H, S, tBu), 1.8 (1H, M, H2'), 2.6 (1H, M, H2"), 4.7 (2H, S), 6.5 (1H, dd, J=6.5, 2.3Hz), 7.4 (9H, S, Ph), 7.7 (5H, T, Ph), 7.9 (2H, d, J=7.3, 2.2Hz), 8.6 (1H, d, J=8.2, 2.4Hz).; ^{13}CNMR ($CDCl_3$) δ ppm: 19.05, 26.8, 40.5, 76.3, 76.9, 77.6, 85.8, 89.9, 96.7, 127.7, 128.7, 130.1, 132.6, 132.9, 133.4, 135.7, 146.5, 154.3, 162.7, 174.39.

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AN EFFICIENT CHROMATOGRAPHY FREE DIRECT SYNTHESIS OF 2'-O-(2-METHOXY ETHYL) DERIVATIVES OF UNMASKED MODIFIED HETEROCYCLIC NUCLEOSIDES USING METHOXYETHYL MESYLATE.

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ABSTRACT

An eco-friendly efficient and chromatography-free direct synthesis of 2'-O-methoxyethyl derivatives of unmasked nucleosides in high overall yields has been developed.

KEYWORDS

Methoxy ethyl mesylate, 2'-O-MOE-nucleosides, 2'-O-alkylation methoxyethyl mesylate, unmasked nucleosides, antisense technology chromatography free synthesis

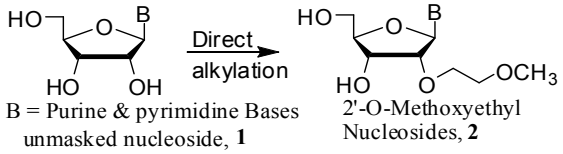
INTRODUCTION

A disease in human body is either manifestation of improper gene fiction or attack by pathogens. Determination of genetic code sequence by human genome project has unravelled the role of genes in major human diseases[1,2,3,4]. Inactivating or silencing the gene responsible for genetic disorders is a key technology for successful cure or prevention of the disease. Antisense technology is one such form of treatment which inactivates the responsible gene by synthesizing a strand (antisense) of nucleic acid (DNA/RNA) that will bind to the mRNA produced by that gene and inactivates it (Gene silencing).[5]Structural modification of oligonucleotides is an essential feature of antisense technology to prevent them from cellular nuclease degradationwith non-bridging sulphur atom[6a], modification of sugar residues[6b] and modification of bases[6c,6d,6e] are some of the successful structural modifications in antisense technology. These modified oligonucleotides are effective inhibitors of gene expression in cancer treatment. In the process of structural modifications, introduction of an alkyl group at 2'-OH position of various nucleosides has gained significant importance in the recent past due to its better chemotherapeutic properties[7]. Among different alkyl groups tested, 2'-O methoxyethyl group (2'-O-MOE) emerged as a potent alkyl group owing to strong hybridization properties and high nuclease resistance.[8] Consequently, 2'-O-(2-methoxyethyl)-nucleosides emerged as potent second generation oligonucleotide hybridization

based agents[9,10,11]. An efficient synthesis of these nucleosides in good overall yields is therefore necessary and a challenging task for an organic chemist.

RESULTS AND DISCUSSION

Various syntheses of 2'-O-alkyl nucleosides were reported in the literature. For example Morten Groti et al. reported the synthesis of 2'-O-methoxyethyl nucleosides involving concurrent protection of 3' and 5'-OH groups by a *bis*-silylating reagent TIPSCl followed by 2'-OH alkylation and final global deprotection with TBAF yielded 2'-O-methoxyethyl nucleosides.[12] Starting from D-ribose and by applying protection-deprotection strategy and alkylation reaction, Martyn P. et al. synthesized 2'-O-methoxyethyl nucleosides in 10 steps with an overall yield of 33%[13]. Later progress by C. B. Reese et al. revealed a convenient synthesis of 2-O-methoxyethyl pyrimidine nucleosides using aluminium 2-O-methoxyethoxide and 2, 2'-anhydropyrimidine nucleosides in *N,N*-dimethylacetamide[6]. In a recent report E. A. Theodorakis et al. used a new silicon based protecting group, methylene-bisdiisopropylsilylchloride (MDPSCL2), for masking 3' and 5' hydroxy groups followed by 2'-OH alkylation with 2-methoxyethyl bromide and NaHMDS to afford 2'-O-(methoxyethyl) guanosine in 65% overall yield[15]. Mc Gee and Zhai et al. reported a convenient and efficient procedure for the synthesis of 2'-O-alkyl pyrimidine nucleosides relying on ring opening of corresponding 2,2'-anhydropyrimidine analogues with magnesium methoxide in DMF at 100^0C[16]. However this strategy is limited only to the preparation of 2'-O-methoxy nucleosides as the other higher alkoxides gave poor yields. All the above multistep syntheses of 2'-O-alkyl nucleosides are expensive with low yields, due to involvement of protections-deprotections and chromatographic purifications.



Scheme 1
Direct methoxyethylation of unmasked nucleosides

These drawbacks propelled us to design a novel strategy for the synthesis of 2'-O-alkyl nucleosides. Direct alkylation of unmasked nucleosides was considered as the best possible pathway for the production of 2'-O-alkyl nucleosides in high overall yields. There is a only one report on direct methylation of unmasked nucleosides which too suffers from economic producivity[19c]. There is no report on the direct alkylation of unmasked nucleosides particularly for methoxyethylation. Taking the advantage of high reactivity of 2'-OH towards hard electrophiles, we wish to report a direct 2'-O-alkylation and cost effective strategy for the preparation of 2'-O-alkyl nucleosides without resorting to chromatographic purification (Scheme 1).

Table 1
2'-O- Methoxyethylation of adenosine 3 with methoxyethyl bromide

MOE-Br	Reaction Conditions	Yield %
1.2 equiv	Basic Alumina, CH3CN, rt to 60^0C, 24 h	No reaction
1.2 equiv	KOH, DMF, 0°C to rt, 12 h	Complex mixture
2.2 equiv	KOH, DMSO, rt, 12 h	Complex mixture

2.2 equiv	KOH, TBAB, DMF, 45°C , 12 h	20% of 3a
2.2 equiv	NaH, DMSO, 0°C to rt, 12 h	Complex mixture,20% of 3a
3.2 equiv	aq. NaOH, TBAB, 50^0C, 48 h	30% of 3a
4.8 equiv	aq. NaOH, H_2O, 60^0C, 12 h	20% of 3a
4.8 equiv	aq. NaOH, H_2O, 60^0C, 24 h	25% of 3a

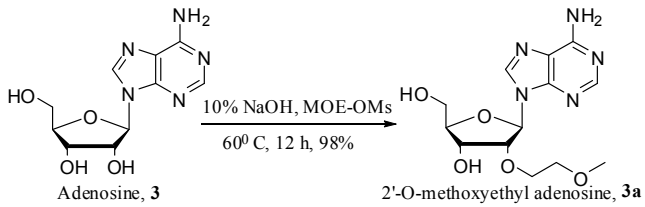
Our quest for the 2'-O-methoxyethylation started with initial studies of identifying a suitable alkylating agent bearing an efficient leaving group. In this direction, we identified 2-O-methoxyethyl bromide (prepared by modified procedure developed in our laboratory[16]) as an electrophile and adenosine **3** as a model substrate for direct methoxyethylation. Treatment of adenosine **3** with methoxyethyl bromide using various bases like KOH, NaOH, NaH etc in different solvents like CH_3CN, DMSO, DMF at diverse temperatures 0 to 60^0C led to the disappointing fallout as there was no reaction or polyalkylations leading to a complex mixture of products with minimum amount of desired product (**Table 1**). Addition of catalytic amount of TBAB in aqueous media and using an excess of MOE-Br (4.8 eq.) resulted in the formation of good amount of product along with other side products. Later we shifted our attention towards strong electrophilic alkylating reagent 2-methoxyethyl mesylate **(**MOEMs, **6** this was indigenously prepared in our laboratory[17] and used for chemoselective S-methoxyethylation of 2-mercapto benzimidazoles) for better alkylation considering the high reactivity of 2'-OH group with hard-electrophiles. Encouraging results (~50% product), with fairly good selectivity, were obtained when adenosine was treated with aq. solution of NaOH, 2-methoxyethyl mesylate **6** and catalytic amount of TBAB in H_2O at 50-60 ^{0}C. But still the reaction needed further optimization as other alkylated products were also obtained

.

Table 2
2'-O- Methoxyethylation of adenosine 3 with methoxyethyl mesylate

MOE-Ms	Reaction Conditions	Yield %
1.2 equiv	Basic Alumina, CH3CN, rt to 60	No reaction
1.2 equiv	0C, 24 h KOH, DMF, 0 °C to rt, 12 h	Complex mixture
2.2 equiv	KOH, TBAB, DMF, 45 °C , 24 h	40% of 3a
2.2 equiv	KOH, TBAB, DMSO, 45 °C , 12 h	30% of 3a
4.8 equiv	aq. NaOH, TBAB, 50 0C, 48 h	50% of 3a
4.8 equiv	aq. NaOH, TBAB, 60 0C, 12 h	98% of 3a
4.8 equiv	aq. NaOH, TBAB, 60 0C, 24 h	90% of 3a

Based on our practical observation, that the selectivity can be enhanced by adding an excess of MOEMs (4.8 eq.) in portions to diluted reaction mixture. Fortunately this strategy worked well when the reaction was performed at 50-60^0C with 4.8 equivalents of MOE-Ms adding in three portions over a period of 12 h (**Table 2**). The progress of the reaction was monitored by checking the worked up aliquots of the reaction mixture by HPLC at different intervals and comparing the peaks with respective chromatograms of authentic samples which were prepared by reported procedures[18]. The reaction was finally terminated by treatment with cataion exchange resin IR-120 and worked up to provide the sticky crude which was difficult to crystallize in various solvents especially lower alcohols.



Scheme 2
Synthesis of 2'-O-methoxyethyl adenosine 3a

Use of other solvents like acetone and acetonitrile could only remove the traces of other alkylated products as the desired product has poor solubility in these solvents. With reasonably good yield of the crude (98% HPLC) in hand, we subsequently paid attention on the purification part. Exploiting the insoluble nature of the product, we sought to utilize the Soxhlet extraction technique for isolation of the desired product.

The crude was adsorbed on to silica gel and the resulted slurry was then subjected to continuous extraction by a Soxhlet apparatus initially with hot toluene to remove undesired nonpolar impurities and finally the desired product was isolated by extraction with hot DCM which was concentrated under diminished pressure to directly yield the desired product with 93–95% purity by HPLC. This material was further purified (98–99% by HPLC) by crystallization from hot 2-propanol. By using this protocol we successfully synthesized the 2'-O-methoxyethyl derivatives of adenosine **3a**, uridine **4a** and cytidine **5a** except guanosine which was practically insoluble in water. The products were identical to authentic sample by HPLC, UV, ^{1}H NMR and ^{13}C NMR-spectroscopy[18].

Although the precise reason for the regioselective alkylation in not yet known we thought that the selectivity would arise from both the substrate as well as the reagent. The reagent MEOMs being a hard electrophile and smaller in size prefers to react with a hard nucleophilic site therefore prefers to react with 2'-OH group of adenosine instead of other hydroxyl groups particularly the primary hydroxyl which is soft nucleophile as it is involved in extensive hydrogen bonding.

It is for the first time that a novel protocol for isolation of nucleosides is reported and creates an opportunity for the large scale production of 2'-O-methoxyethyl and 2'-O-methyl nucleosides. In summary, we have developed an environmentally benign, convenient scalable and direct method for the manufacturing 2'-O-methoxyethyl derivatives of adenosine, cytidine, and uridine from their respective unmasked nucleosides without resorting to protection-deprotection strategy and chromatographic purifications. Further studies are directed towards methoxyethylation of guanosine and direct methylation of these nucleosides.

Typical Experimental procedure for direct methoxyethylation

Adenosine **3** (5.0 g, 20.23 mmol) was dissolved in 50 ml of aq. 10% NaOH solution (4.0 g of NaOH dissolved in 40 ml of deminaralized water), The temperature of the reaction mass was raised to 55-60 ^{0}C and 2'-O-methoxyethylmesyalte (15 ml, 97.4 mmol) was added in three portions (7.5 ml + 3.8 ml + 3.8 ml), the reaction mass was stirred at 60^{0}C for 12 h. The progress of the reaction was monitored by the HPLC for every one hour. After 12 h the complete consumption of starting material was shown by HPLC. The temperature of the reaction mixture was brought to rt and PH of the reaction mass was adjusted to 7.0 by using 20 g of IR-120H^{+} resin, after neutralization the resin was filtered and washed with demineralised water (50 ml). The water was distilled under reduced pressure to 1/3 of the total volume and the resulted crude product was washed with toluene (25 ml) to remove the un reacted methoxyethylsylate and non-polar impurities. The aqueous layer was distilled under reduced pressure to get thick syrup. Methanol (35 ml) was added and stirred for 30 minutes at

25-30^0 C to get white solid that was filtered and washed with methanol. The methanol was evaporated and crude was on 60-120 mesh silica-gel. The adsorbed silica-gel was loaded on soxhlet and extraction was carried out with hot toluene (25 ml) to remove non-polar and bis-alkylated impurities. It was followed by DCM (25 ml) extraction in hot condition to get the product. The DCM was collected and evaporated to dryness, from which only 95% pure product was isolated. The product was further purified by recrystalization from hot 2-propanol to get 99% pure product 5.7 g of **4** (95% yield).

EXPERIMENTAL

1. 2'-O-(2-methoxyethyl)adenosine (3a):

MP: 203-204.5^{0}C (Lit. 203-204 ^{0}C); ^{1}H NMR(DMSO-d_6): δ(ppm) 3.14 (s, 3H), 3.38 (t, 2H), 3.49 -3.57 (m, 2H), 3.61-3.63 (m, 2H), 3.95 (d, 1H), 4.28 (d, 1H), 4.46 (t, 1H), 5.11 (d, 1H), 5.53 (t, 1H), 5.82 (d, 1H), 5.86 (s, 2H), 6.9 (s, 2H), 7.97 (s, 1H); ^{13}C NMR (DMSO-d6, δ in ppm) 58.1, 61.7, 68.9, 69.2, 71.1, 80.97, 85.1, 86.1, 113.5, 136.2, 151.4, 156.3, 160.1; LC-MS required 325.32 found 325.3270; Anal. Calcd for $C_{13}H_{19}N_5O_5$ C, 48.00; H, 5.89; N, 21.53; Found C, 48.21; H, 5.93; N, 21.23;The product was identical to authentic sample by HPLC, UV, ^{1}H NMR, ^{13}C NMR –spectroscopy.

2. 2'-O-(2-Methoxyethyl)uridine (4a)

MP: 123-125 ^{0}C (Lit. 124-125 ^{0}C); ^{1}H NMR (DMSO-d6):δ(ppm) 3.22 (s, 3H), 3.44 (m, 2H), 3.55 (m, 1H), 3.60-3.71 (m, 3H), 3.85 (m, 1H), 3.95 (m, 1H), 4.09 (m, 1H), 5.06 (d, *J* = 5.7 Hz, 1H), 5.15 (t, *J* = 5.0 Hz, 1H), 5.65 (d, *J* = 8.1 Hz, 1H), 5.84 (d, *J* = 5.0 Hz, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 11.37 (brs, 1H); ^{13}C NMR (DMSO-d6, δ in ppm): 58.0, 60.4, 68.3, 68.9, 71.1, 81.3, 84.9, 86.0, 101.7, 140.4, 150.5, 163.0. LC-MS required 302.28 found 302.2842; Anal. Calcd for $C_{I2}H_{I8}N_20_7$ C, 47.68; H, 6.00; N, 9.27; Found C, 47.69; H, 5.96; N, 9.25. The product was identical to authentic sample by HPLC, UV, ^{1}H NMR and ^{13}C NMR spectroscopy.

3. 2'-O-(2-Methoxyethyl)cytidine (5a)

MP: 154-156^0 C (Lit 154-156 ^{0}C); ^{1}H NMR (DMSO-d6):δ(ppm) 3.23 (s, 3H), 3.45 (t, *J* = 4.8 Hz, 2H), 3.55 (m, 1H), 3.64- 3.77 (m, 3H), 3.81 (m, 2H), 4.04 (m, 1H), 4.96 (d, *J* = 6.1 Hz, 1H), 5.12 (t, *J* = 5.1 Hz, 1H), 5.72 (d, *J* = 7.4 Hz, 1H), 5.83 (d, *J* = 3.9 Hz, 1H), 7.22 (brs, 2H), 7.90 (d, *J* = 7.4 Hz, 1H); ^{13}C NMR (DMSO-d6, δ in ppm) 58.0, 60.1, 68.0, 68.8, 71.1, 81.9, 84.0, 87.2, 93.8, 141.1, 155.1, 165.5. LC-MS required 301.30 found 301.3108; Anal. Calcd for $C_{12}H_{19}N_30_6$ C, 47.84; H, 6.36; N, 13.95; Found C, 47.79; H, 6.24; N, 13.78. The product was identical to authentic sample by HPLC, UV, ^{1}H NMR and ^{13}C NMR spectroscopy.

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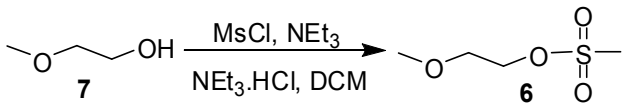
17. Preparation of 2'-O-methoxyethyl mesylate**:** To an ice cold solution of 2'-O-methoxy ethanol in DCM was added NEt_3 and NEt_3.HCl and stirred for 5 min. Into this was introduced methane suponyl chloride slowly over a period of 10 min and the reaction mixture stirred at rt for 1 h. Solvent was evaporated under vacuum and resulted methoxyethyl mesylate is stored at rt for several days without any noticed decomposition.



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SYNTHESIS AND CHARACTERIZATION OF POTENTIAL IMPURITY OF NATEGLINIDE

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ABSTRACT

Nateglinide, an oral ant hyperglycemic agent used in the treatment of non-insulin-dependent diabetes mellitus (NIDDM). It is from the meglitinide class of short-acting insulin secretagogues, which act by binding to β cells of the pancreas to stimulate insulin release. Herein, we wished to discuss identification, synthesis and characterization of cis Nateglinide impurity of Nateglinide

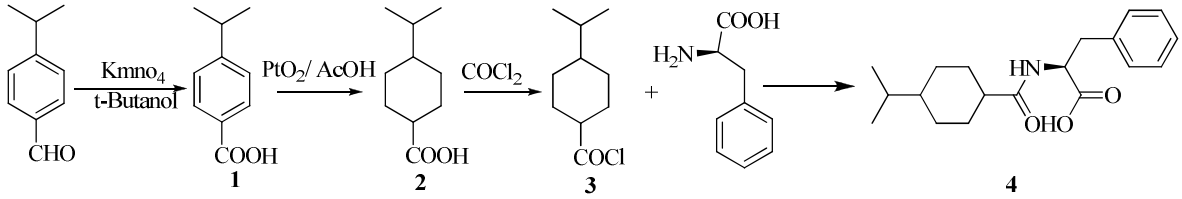
KEYWORDS

4-Isopropyl-benzoic acid, 4-Isopropyl-benzaldehyde, 4-Isopropyl-cyclohexanecarboxylic acid, 2 - [(4 - Isopropyl- cyclohexanecarbonyl) - amino] -3-phenyl-propionic acid

INTRODUCTION

Now Drugs have become an important part of human life to combat with various diseases. Unlike ancient days, most of the drugs in recent years are purely synthetically made. Unambiguously, the synthetic drugs certainly contain various impurities such as chemical or microbial [1]. But of course most of the impurities are chemical only. The presence of impurities, also called as, related substances in an active pharmaceutical ingredient (API) can have a significant impact on the quality and safety of the drug products[1]. Therefore, it is necessary to study the impurity profile of any API[2-6] and control it during the manufacturing of a drug product[1]. As per the ICH guidelines for any impurities[1], which are forming at a level of ≥ 0.10% with respect to the API should be identified, synthesized and characterized thoroughly.

Scheme.



MATERIALS AND METHODS

EXPERIMENTAL:

All commercial reagents were used without purification and all the solvents used were reagent grade. Melting points were reported on a Buchi R-535 apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT / IR – 240°C spectrometer with KBr optics. ^{1}H & ^{13}C NMR spectra were recorded on a Varian Gemini 200 spectrometer in $CDCl_3$ using TMS as an internal standard. Mass spectra were recorded on Finnigan-MAT 1020 Mass Spectrum opening at 70 eV.

General Procedure:

1. Preparation of 4-isopropylbenzoic acid (1):

To well stirred t-butanol, $KMNO_4$ was added at 0°C and later aldehyde was added slowly while maintaining the temperature at 0°C and then the reaction mass was allowed to stir for 8 hrs or till the reaction completes (by TLC check). Then the t-Butanol was distilled and ethyl acetate was added to the residue and filtered through celite. The organic layer was washed with water followed by brine then distilled to get a crude which was purified by column chromatography in Hexane and Ethyl acetate. ^{1}H-NMR ($CDCl_3$) δ ppm: 1.30 (s, 6H, CH_3), 3.10 (m, 1H, CH), 7.30 (d, 2H, CH), 8.05 (d, 1H, 2H), 11.0 (s, 1H, OH); ^{13}C NMR δ ppm : 24.6, 24.6, 31.0, 153, 126.2, 129.8, 127, 129.8, 126.2.

2. Preparation of 4-isopropylcyclohexanecarboxylic acid (2):

In a pressure reactor charged Stage I then added acetic acid followed by PtO_2. The reaction mass was stirred at 100°C the reaction was monitored by TLC after completed of the reaction after 10hrs cool the reaction mass, now filtered the methanol containing compound was filtered through celite pad then concentrated under vaccum. The crude was purified by high vacuum distillation to get 3:1 ration of *cis-trans* 4-isopropyl cyclohexyl carboxylic acid was obtained. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.01 (s, 6H, CH_3), 1.82 (m, 1H. CH), 1.42 (m, 1H, CH), 1.40 (m, 4H, CH2), 1.67 (m, 4H, CH2), 2.31 (q, 1H, CH). ^{13}C NMR δ (ppm) : 20.0, 30.5, 26.2, 39.0, 24.7, 41.8, 181.

3. preparation of 4-isopropylcyclohexanecarbonyl chloride (3):

4-Isopropyl-cyclohexanecarboxylic acid Stage III was added to a clean RB in Dichloride methane and dimethyl form amide under nitrogen and cooled to 0°C. Then added oxalyl chloride to the reaction mixture for 1 hr and stir for another one hour followed by refluxing the reaction mass for 2-3hrs or till the reaction completed by TLC check, distilled the dichloromethane under nitrogen this crude used as such further. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.01 (s, 6H, CH_3), 1.82 (m, 1H, CH), 1.42 (m, 1H, CH), 1.40 (m, 4H, CH2), 1.67 (m, 4H, CH2), 2.31 (q, 1H, CH). ^{13}C NMR δ (ppm) : 20.0, 30.5, 25.4, 25.0, 53.0, 176

4. preparation of (S)-2-(4-isopropylcyclohexanecarboxamido)-3-phenylpropanoic acid (4) :

Charged DPA in 10% sodium hydroxide solution in a clean RB and added acetone at this stage acid chloride Stage III in acetone was added slowly in half an hour. Cool the reaction mass by adding sodium hydroxide 10% solution for 30 min. the PH should not increased more than 10 during the reaction and reaction mass was allowed to room temperature after 30 min, check the TLC and after completion of the reaction acidify with 10% HCl and extracted with ethyl acetate, the organic layer was evaporated under vaccum the product obtained in brown oily liquid. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.01 (s, 6H, CH_3), 1.82 (m, 1H, CH), 1.42 (m, 1H, CH), 1.40 (m, 4H, CH2), 1.67 (m, 4H, CH_2), 2.31 (q, 1H, CH), 3.04 (m, 2H, CH_2), 4.85 (t,1H, CH), 7.12 (d, 2H, CH), 7.21 (d, 1H, 2H), 7.08, ^{13}C NMR δ (ppm): 25.8, 25.3, 40.3, 25.6, 25.8, 38.7, 30.6, 20.1, 178, 60, 36.5, 140, 127, 128, 125.0, 127.4, 177.0. SOR -6.3. mp 116 °C.

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SYNTHESIS AND CHARACTERIZATION OF A POTENTIAL IMPURITY OF LORATIDINE

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ABSTRACT

Loratadine[1], [Ethyl-4- (8-chloro-5, 6-dihydro- 11H-benzo [5,6] cyclohepta [1,2-b]pyridin-11-ylidene) piperidine-1-carboxylate] and Desloratidine[2] are non sedating antihistamine drugs used to treat allergic disorders, especially rhinitis and urticaria. According to the literature desloratadine is 2.5–4 times more active orally than loratidine and antihistaminic activity lasts for 24 hrs [3-4]. Various degradation products [5-8] have been reported for both of these drugs. The present research relates to a process for the synthesis of 7-chloro derivatives of Loratidine these are degradation products, which may retains as an impurity in the final drug in a very small quantity. The preparation of both contaminants has been necessary to prepare references for quality control analysis and validation. IR, NMR, MS spectral studies have been also discussed for these contaminants.

KEYWORDS

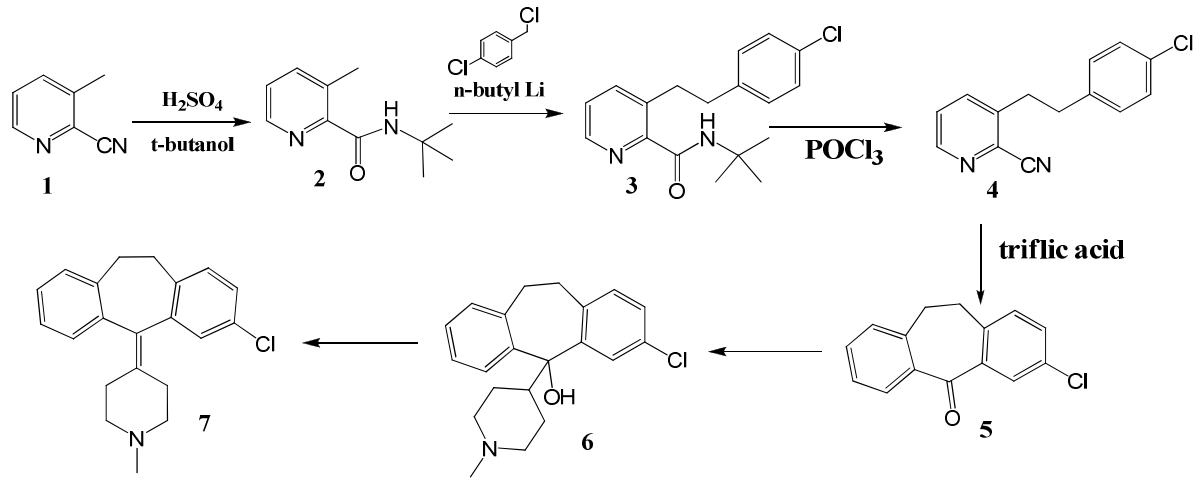
Fused pyridines, Loratadine, synthesis, contaminant

INTRODUCTION

There is an ever increasing interest in impurities present in API's. Recently, not only purity profile but also impurity profile has become essential as per various regulatory requirements. In the pharmaceutical world, an impurity is considered as any other organic material, besides the drug substance, or ingredients, arise out of synthesis or unwanted chemicals that remains with API's. The impurity may be developed either during formulation, or upon aging of both API's and formulated API's in medicines. A good illustration of this definition may be identification of impurity in API's like 1-(1, 2, 3, 5, 6, 7-hexahydro-s-indacen-4-yl)-3-4[- 1-hydroxy-1-methyl-ethyl)-furan-2-sulphonylurea using Multidisciplinary approach[9]. The presence of these unwanted chemicals, even in small amount, may influence the efficacy and safety of the

pharmaceutical products. Impurity profiling (i.e., the identity as well as the quantity of impurity in the pharmaceuticals), is now gaining critical attention from regulatory authorities. The different Pharmacopoeias, such as the British Pharmacopoeia (BP), United States Pharmacopeia (USP), and Indian Pharmacopoeia (IP) are slowly incorporating limits to allowable levels of impurities present in the API's or formulations. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has also published guidelines for validation of methods for analyzing impurities in new drug substances, products, residual solvents and microbiological impurities[10-13]. A number of articles[14-16] have stated guidelines and designed approaches for isolation and identification of process-related impurities and degradation products, using Mass spectrometry (MS), Nuclear Magnetic Resonance (NMR), High Performance Liquid.

Scheme I



MATERIALS AND METHODS

Experimental:

All commercial reagents were used without purification and all the solvents used were reagent grade. Melting points were reported on a Buchi R-535 apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT / IR – 240C spectrometer with KBr optics. ^{1}H & ^{13}C NMR spectra were recorded on a Varian Gemini 200 spectrometer in $CDCl_3$ using TMS as an internal standard. Mass spectra were recorded on Finnigan-MAT 1020 Mass Spectrum opening at 70 ev.

Synthesis procedure:

1. 3-Methyl-pyridine-2-carboxylic acid tert-butylamide (2):

100 g (0.847 mol) of 3-methyl-2-cyanopyridine, 151.08 g (1.695 mol) of t-Butanol and 5.77 g of anhydrous H_2SO_4 was added slowly in 1 hr the temperature is raised to 75°C and is maintained at 140°C for 15-18 hours. The end of the reaction is monitored by TLC. At the end of the reaction the mixture is cooled to 60°C and, at this point, it is filtered through a Gooch crucible. After completion of the reaction mass the reaction mass is diluted with water and toluene is added about 500ml then adjust the PH to 10 with ammonia

then separated organic layer this organic layer was evaporated under nitrogen The toluene solution is evaporated to give 166.5 g of a pale red oil Compound I: ^{1}H-NMR (200 MHz, $CDCl_3$) δ (ppm): 1.30 (s, 9H, CH_3), 2.32 (dd, 9H, CH_3), 7.75 (dd, 1H, CH), 8.87 (s, 1 H). ^{13}C NMR δ (ppm): 30.8, 30.8, 41.7, 167, 13.7, 137, 131.4, 151.2, 146.1, 125.6.

2. 3-[2-(4-Chloro-phenyl)-ethyl]-pyridine-2-carboxylic acid tert-butylamide (3):

To a stirred mass of stage I in THF at -78ºC was added chloro compound slowly for one hr to this added sodium bromide then stir for 10 min then allowed the reaction mass to stir for 1 hr and the reaction completed was check by GC after the reaction completes the reaction mass was quenched with water. The reaction mass was extracted with ethyl acetate and given water followed by brine washing to the organic layer, then dried and concentrated under vacuum, the crude obtained was purified by column chromatography. : ^{1}H-NMR (200 MHz, $CDCl_3$) δ (ppm): 1.40 (s, 9H, CH_3), 2.50 (dd, 9H, CH_3), 7.80 (dd, 1H, CH), 8.05 (s, 1H). ^{13}C NMR δ (ppm) : 126.0, 145.5, 149.5, 137.9, 136.6, 30.7, 167.3, 41.7, 30.8, 30.8, 37.5, 138.0, 129.3, 131.0, 128.8, 129.3.

3. 3-[2-(4-Chloro-phenyl)-ethyl]-pyridine-2-carbonitrile (4):

To a solution of stage II (10 mmoles) in dry dimethylformamide (50 mmoles) freshly distilled dry phosphorus oxychloride (10 mmoles) was added with constant stirring at 0 ºC. The reaction mixture was kept overnight and then poured onto crushed ice. The yellow compound which separated out was filtered and washed with water. The solid product which was purified by column chromatography over silica gel by eluting with petroleum ether gave 3-[2-(4-Chloro-phenyl)-ethyl]-pyridine-2-carbonitrile in 70-85 % yields. Compound was recrystallized from methanol to give pale yellow needles. ^{1}H-NMR (200 MHz, $CDCl_3$) δ (ppm): 2.88 (s, 4H, CH_2), 7.22 (dd, 2H, CH), 7.06 (dd, 2H, CH), 8.09 (d, 1H, CH), 7.84 (dd, 1H, CH), 8.96 (d, 1H, CH). ^{13}C NMR δ (ppm): 126.8, 148.8, 133.5, 144.1, 136.7, 30.6, 37.8, 138.3, 128.8, 131.0, 128.8, 129.3, 118.0.

4. 3-Chloro-10,11-dihydro-dibenzo[a,d]cyclohepten-5-one (5):

Stage compound III is placed in a 250 ml reactor, 6 volumes of triflic acid was added drop wise at room temperature and the mixture is brought to 55-60 °C and left to react for three hours. The disappearance of the starting material is monitored by TLC and the excess triflic acid was quenched in crushed ice water to give 6 g of a dark residue. This mass was basified by 50% Sodium hydroxide solution, then extracted with ethyl acetate and given water followed by brine washings, the obtained organic layer was distilled under vacuum. This crude material was recrystallized in MTBE to obtain white solid. ^{1}H-NMR (200 MHz, $CDCl_3$) δ (ppm): 7.16 (s, 1H, CH), 7.41 (dd, 1H, CH), 7.66 (dd, 1H, CH), 7.22 (d, 1H, CH), 7.18 (dd, 1H, CH), 7.40 (d, 1H, CH), 2.88 (s, 4H, CH_2). ^{13}C NMR δ (ppm): 132.1, 125.4, 130.0, 137.2, 141.8, 127.6, 31.2, 139.9, 138.6, 130.4, 130.78, 132.5, 129.0, 187.0

5. 3-Chloro-5-(1-methyl-piperidin-4-yl)-10,11-dihydro-5H-dibenzo [a, d]cyclohepten-5-ol (6) :

In a 4N round bottom flask added Magnesium turnings in THF and heated to reflux till the Mg dissolves and a suspension forms. Reflux should maintain while adding 1-Chloro-4-hloromethyl-benzene and maintained for 1 hr, then Stage IV dissolved in THF was added in 1 Hr, now cool the Grignard mass to 0°C and check the TLC. The reaction mass was diluted with water and extracted with ethyl acetate and dried and distilled the ethyl acetate the crude obtained was purified in column chromatography. ^{1}H-NMR (200 MHz, $CDCl_3$) δ (ppm): 7.16 (s, 1H, CH), 7.41 (dd, 1H, CH), 7.66 (dd, 1H, CH), 7.22 (d, 1H, CH), 7.18 (dd, 1H, CH), 7.40 NMR δ (ppm): 138.2, 143.8, 73.2, 73.2, 142.4, 140.1, 32.0, 128.7, 131.5, 126.3.

6. 4-(3-Chloro-10,11-dihydro-dibenzo[a,d]cyclohepten-5-ylidene)-1-methyl-piperidine (7):

Charge triflic acid in a neat round bottom flask and added stage V slowly in the acid and heated to reflux to 100°C the reaction completes was monitored by HPLC after completion the reaction mass was cooled to 0°C, then quenched in ice water then basified with sodium hydroxide solution and extracted with

ethyl acetate the obtained organic layer was distilled under vacuum the grown oily liquid was purified by column chromatography. ^{1}H-NMR (200 MHz, $CDCl_3$) δ (ppm): 7.00 (s, 1H, CH), 7.31 (dd, 1H, CH), 7.55 (dd, 1H, CH), 7.210 (d, 1H, CH), 7.20 (dd, 1H, CH), 7.30 (d, 1H, CH), 2.88 (s, 4H, CH_2), 2.34, (m, 1H, CH), 1.46 (dd, 4CH_2), 2.24 (dd, 4H, CH_2), ^{13}C NMR δ (ppm): 138.2, 143.8, 73.2, 73.2, 142.4, 140.1, 32.0, 128.7, 131.5, 126.3.

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SYNTHESIS AND CHARACTERIZATION OF NOVEL 4-[3-(4-SUBSTITUTED BENZYL-PIPERAZIN-1-YL)-PROPOXY]-7-METHOXY-3-PHENYL-CHROMEN-2-ONE ANALOGUS.

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ABSTRACT

A series of some new 4-[3-(4-substituted piperazin-1-yl) propoxy]-7-methoxy-3-phenyl-*2H*-chromen-2-one (4a-j) were synthesized in good yields by reductive amination with different substituted aromatic aldehydes using sodium cynoborohydride in methanol. The structures of newly synthesized derivatives (4a-4j) were characterized by ^{1}H-NMR, ^{13}C-NMR and FT-IR spectroscopy.

KEYWORDS

Coumarine, piperizine, sodium cynoborohydride and aldehydes.

INTRODUCTION

The piperizines are extensive class of chemical compounds with several vital pharmacological properties. These compounds have been shown to potent analgesics, psychotolytic[1] and antifungal activities[2,3]. N-substituted piperizines have been reported to possess various activities like, local anesthetic, anti hyper lipidemic, anticoagulant[4] and also antihelmenthic, anticancer[5-7], antihistamic[8], antidepressant[9]. Some aryl piperizine derivatives possess antienteroviral activity[10,11], anti- HIV properties[12] also of great importance to many different biological targets, especially central nervous system receptors. In the case of serotonin, (5-HT) receptors, compounds containing this moiety represent the biggest and thoroughly examined class of 5-HT_{1A} receptor ligands[13,14].

The incorporation of different halo substitutes in piperizine moiety is a significant synthetic strategy-it is highly used in medicinal chemistry due to its wide range of biological applications, proper alkality, solubility nature in water and physiocochemical properties[15,16].

Compounds of the coumarin series constitute a group of more than 40 drugs, which are widely used in medicine as anticoagulant, spasmolytic, hypotensive, antiarrhythmic, antiviral, and immunomodulant agents[17,18]. Coumarins and its derivatives are distinguished, they are anticoagulant, antimicrobial[19], anti-HIV, antioxidant[20], antiallergic, anticancer[21] and antiviral activities[22]. In this connection we report the synthesis of new molecules which contain

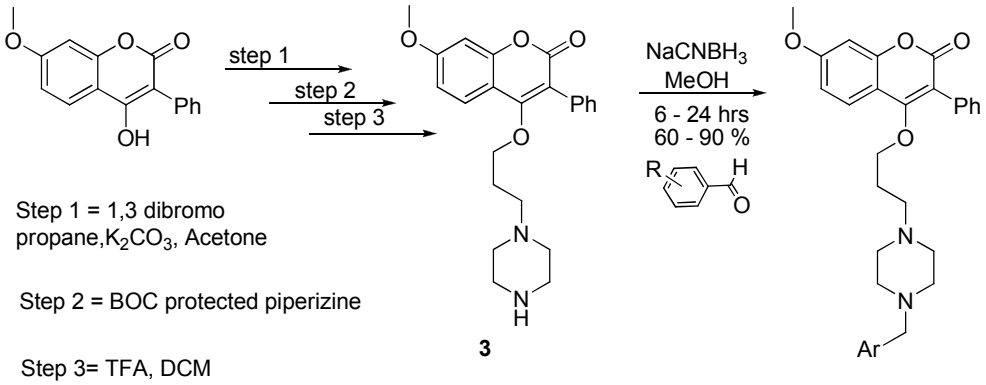
piperizine and coumarin moieties within the framework.

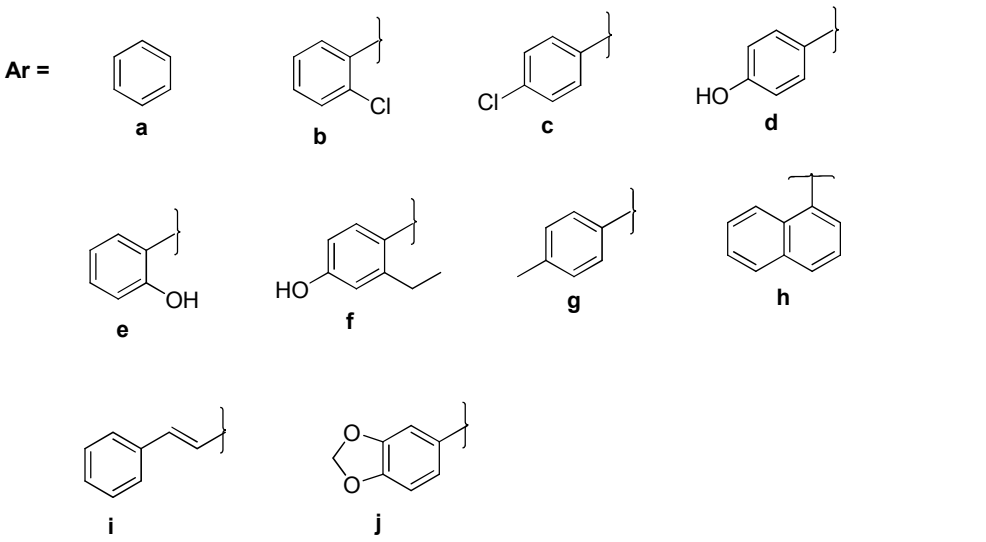
In continuation of our research on the synthesis of bioactive heterocycles and their biological evaluation we have synthesized some of coumarin based piperazine derivatives to screen their activity against the antimicrobial activity. Several studies provided an evidence that the introduction of such groups like substituted aryl groups could increase antimicrobial activity by enhancing lipophilicity of the molecule, which may result in more penetration into cells.

RESULTS & DISCUSSIONS

Some 4-[3-(4-piperazin-1-yl) propoxy]-7-methoxy-3- phenyl-*2H*-chromen-2-one derivatives (4a-j) were prepared by the reaction of 7-methoxy-3-phenyl-4-(3-piperizin-1-yl-propaxy)-chromen-2-one (**3**) and different substituted aromatic aldehydes. Deprotection of compound 4-[3-(7-methoxy-2-oxo-3-phenyl-*2H*-chromen-4-yloxy)-propyl]-piperazine-1-carboxylic acid *tert*-butyl ester with trifluoroaceticacid in dichloromethane at room temperature to give 7-methoxy-3-phenyl-4-(3-piperizin-1-yl-propaxy)-chromen-2-one (**3**). The compound **3** reaction with different substituted aromatic aldehydes in presence of catalytic amount of acetic acid in methanol at room temperature to produce the imine product it was insitu reduced by using mild reducing agent sodium cyanoborohydride to yielded N-substituted 7-methoxy-3-phenyl-4-(3-piperizin-1-yl-propaxy) chromen-2-one derivatives (4a-4j). All the above sequences of reactions are summarized in Scheme-

Scheme 1





EXPERIMENTAL SECTION

General preparation method for 4-[3-(4-piperzin-1-yl)-propoxy]-7-methoxy-3-phenyl-chromen-2-one derivatives (**4a-j**):

A solution of 4-(3-piperzin-1-yl-propoxy) 7-methoxy-3-phenyl *2H*-chromen-2-one (3) (0.1 mol), different substituted aromatic aldehydes (individually) (0.1 mol) and catalytic amount of acetic acid (2 drops) in methanol (10 vol) were stirred for 3 h at room temperature. After completion of reaction as per monitored by TLC, slowly added $NaCNBH_3$ (0.15 mol) at 0°C and stirred again for another 3-16hrs at room temperature. Then the progress of reaction monitored by TLC, solvent was evaporated by rotary, quenched with saturated ammonium chloride solution, extracted with ethyl acetate. The combined organic layers were washed with water and brine solution dried over Na_2SO_4 and concentrated to afford crude products which were purified by Prep. TLC using methanol in chloroform as mobile phase to afford corresponding products (**4a-4j**). All the purified products were confirmed by TLC, FT-IR and ^{1}H-NMR spectral analysis.

1. 4-[3-(4-Benzyl-piperazin-1-yl)-propoxy]-7-methoxy-3-phenyl-chromen-2-one (**4a**):

The compound 4-(3-piperzin-1-yl-propoxy) 7-methoxy-3-phenyl *2H*-chromen-2-one (3) was reacted with benzaldehyde and isolated the title compound 4a (71%) as off white color solid. R_f = 0.50 (MeOH: CH_2Cl_2= 05: 95). ^{1}H-NMR-(400 Hz) in $CDCl_3$: δ (ppm) ^{1}H-NMR (400 Hz) in $CDCl_3$: δ (ppm) 1.82-1.74 (m, 2H, -CH_2 propyl), 2.34 (t, 4H, Piperazine), 2.60 (t, 4H, Piperazine), 3.47 (t, 2H, -NCH_2 propyl), 3.69 (t, 2H, -OCH_2 propyl), 3.55 (s, 3H, -OCH_3), 4.40 (s, 2H, -NCH_2Ar-benzylic), 6.55 (s, 1H, Ar-H), 7.12 (d, 2H, Ar-H), 7.46-7.36 (m, 5H, Ar-H), 7.55-7.50 (m, 3H, Ar-H), 7.66-7.58 (m, 2H, Ar-H). MS-*m/z:* 485$(M+H)^+$; ESI-HRMS: *m/z* calcd. For $C_{30}H_{32}N_2O_4$ $[M+H]^+$485.2385; Found: 485.2379

2. 4-{3-[4-(2-Chloro-benzyl)-piperazin-1-yl]-propoxy}-7-methoxy-3-phenyl-chromen-2-one (**4b**):

The compound 4-(3-piperzin-1-yl-propoxy) 7-methoxy-3-phenyl *2H*-chromen-2-one (3) was reacted with 2-clorobenzaldehyde and isolated the title compound 4b (66 %) as a light brown color solid. R_f = 0.62 (MeOH: CH_2Cl_2); ^{1}H-NMR-(400MHz) in $CDCl_3$: δ (ppm) 1.80-1.74 (m, 2H, -CH_2 propyl), 2.38 (t, 4H, Piperazine), 2.60 (t, 4H, Piperazine), 3.45 (t, 2H, -NCH_2 propyl), 3.78 (t, 2H, -OCH_2 propyl), 3.62 (s, 3H, -OCH_3), 4.28 (s, 2H, -NCH_2Ar-benzylic), 6.94 (s, 1H, Ar-H), 7.18 (d, 2H, Ar-H), 7.56-7.45 (m, 5H, Ar-H), 7.53 (s, 4H, Ar-H); ^{13}C-NMR in $CDCl_3$ (75 MHz): δ (ppm) 169.26, 165.88, 161.32, 153.26, 135.87, 13.65, 131.74, 129.78, 128.60, 126.18, 122.32, 111.62, 110.64, 107.18, 100.10, 62.01, 58.10, 55.08, 52.12, 48.90, 28.80.

3. 4-{3-[4-(4-Chloro-benzyl)-piperazin-1-yl]-propoxy}-7-methoxy-3-phenyl-chromen-2-one (**4c**):

The compound 4-(3-piperzin-1-yl-propoxy) 7-methoxy-3-phenyl *2H*-chromen-2-one (3) was reacted with 4-chlorobenzaldehyde and isolated the title compound 4c (81 %) as a off white solid. R_f = 0.43 (MeOH: CH_2Cl_2 = 10:90). ^{1}H-NMR-(400MHz) in $CDCl_3$: δ (ppm) 1.78-1.73 (m, 2H, -CH_2 propyl), 2.50-2.42 (m, 6H, Piperazine), 2.62 (t, 2H, Piperazine), 3.48 (t, 2H, -NCH_2 propyl), 3.82 (t, 2H, -OCH_2propyl), 3.55 (s, 3H, -OCH_3), 4.22 (s, 2H, -NCH_2Ar-benzylic), 6.96 (s, 1H, Ar-H), 7.10 (d, 2H, Ar-H), 7.48-7.42 (m, 5H, Ar-H), 7.72 (d, 4H, Ar-H). ^{13}C-NMR in $CDCl_3$ (75 MHz): δ (ppm) 165.96, 162.33, 162.72, 151.96, 138.37, 135.65, 135.74, 131.86, 129.60, 127.58, 126.32, 111.62, 109.44, 106.78, 96.99, 64.28, 59.34, 56.18, 52.12, 38.60, 27.10.

4. 4-{3-[4-(4-Hydroxy-benzyl)-piperazin-1-yl]-propoxy}-7-methoxy-3-phenyl-chromen-2-one (**4d**):

The compound 4-(3-piperzin-1-yl-propoxy)-7-methoxy-3-phenyl-*2H*-chromen-2-one (3) was reacted with 4-hydroxy benzaldehyde and isolated the title compound 4d (73 %) as a yellow solid. R_f = 0.45 (MeOH : CH_2Cl_2 = 05 : 95); ^{1}H-NMR-(400MHz) in $CDCl_3$: δ (ppm) 1.80-1.74 (m, 2H, -CH_2 propyl), 2.32 (t, 4H, Piperazine), 2.56 (t, 4H, Piperazine), 3.45 (t, 2H, -NCH_2propyl), 3.73 (t, 2H, -OCH_2propyl), 3.95 (s, 3H, -

OCH_3), 4.36 (s, 2H, -NCH_2Arbenzylic), 6.1 (s, 1H, Ar OH), 6.95 (s, 1H, Ar-H), 7.10 (d, 2H, Ar-H), 7.56-7.45 (m, 5H, Ar-H), 7.61 (d, 4H); ^{13}C-NMR in $CDCl_3$ (75 MHz): δ (ppm) 167.26, 165.88, 161.32, 150.26, 146.55, 139.80, 133.65, 131.74, 129.78, 128.60, 125.18, 121.32, 112.62, 110.64, 108.18, 55.68, 61.71, 58.10, 55.24, 53.18, 50.24, 28.60; MS-*m/z*: 564 $(M+H)^+$ +Ve Scan.

5. 4-{3-[4-(2-Hydroxy-benzyl)-piperazin-1-yl]-propoxy}-7-methoxy-3-phenyl-chromen-2-one (**4e**):

The compound 4-(3-piperzin-1-yl-propoxy) 7-methoxy-3-phenyl *2H*-chromen-2-one (3) was reacted with 2-hydroxy benzaldehyde and isolated the title compound (4e) (80 %) as a solid. R_f = 0.57 (MeOH: CH_2Cl_2 = 10:90); ^{1}H-NMR-(400MHz) in $CDCl_3$: δ (ppm) 1.78-1.73 (m, 2H, -CH_2 propyl), 2.51-2.44 (m, 6H, Piperazine), 2.64 (t, 2H, Piperazine), 3.46 (t, 2H, -NCH_2 propyl), 3.82 (t, 2H, -OCH_2 propyl), 3.55 (s, 3H, -OCH_3), 4.57 (s, 2H, -NCH_2Ar-benzylic), 6.0 (s, 1H), 6.95 (s, 1H, Ar-H), 7.18 (d, 2H, Ar-H), 7.48-7.42 (m, 5H, Ar-H), 7.52 (m, 4H), ^{13}C-NMR in $CDCl_3$ (75 MHz): δ (ppm) 168.66, 165.43, 163.87, 1521.95, 138.35, 137.65, 135.54, 132.46, 131.90, 129.18, 123.42, 111.92, 109.34, 107.78, 55.12, 63.88, 58.34, 54.76, 52.12, 35.64, 26.20.

6. 4-{3-[4-(2-Ethyl-4-hydroxy-benzyl)-piperazin-1-yl]-propoxy}-7-methoxy-3-phenyl-chromen-2-one (**4f**):

The compound 4-(3-piperzin-1-yl-propoxy) 7-methoxy-3-phenyl *2H*-chromen-2-one (3) was reacted with 2-ethyl, 4-hydroxybenzaldehyde and isolated the title compound 4f (85 %) as a off white solid. R_f = 0.44 (MeOH: CH_2Cl_2 = 05:95); ^{1}H-NMR-(400MHz) in $CDCl_3$: δ (ppm) 1.22 (t, 3H, -CH_2CH_3), 1.80-1.74 (m, 2H, -CH_2), 2.38 (t, 4H, Piperazine), 2.60 (t, 4H, Piperazine), 3.40 (t, 2H, -NCH_2propyl), 3.78 (t, 2H, -OCH_2propyl), 3.92 (s, 3H –OCH_3), 4.22 (s, 2H, -NCH_2-benzylic)), 6.1 (s, 1H, Ar OH), 6.94 (d, 2H, Ar-H), 7.18 (s, 1H, Ar-H), 7.46-7.36 (m, 5H, Ar-H), 7.58 (d, 2H, Ar-H), 7.62 (s, 1H, Ar-H).

7. 7-Methoxy-4-{3-[4-(4-methyl-benzyl)-piperazin-1-yl]-propoxy}-3-phenyl-chromen-2-one (**4g**):

The compound 4-(3-piperzin-1-yl-propoxy) 7-methoxy-3-phenyl *2H*-chromen-2-one (3) was reacted with 4-methyl benzaldehyde and isolated the title compound 4g (65 %) as a white solid. R_f = 0.56 (MeOH: CH_2Cl_2= 10: 90); ^{1}H-NMR-(400MHz) in $CDCl_3$: δ (ppm) 1.04 (t, 3H, -$OCH_2CH_2CH_3$), 1.80-1.74 (m, 4H, -CH_2 propyl & -$OCH_2CH_2CH_3$), 2.38 (t, 4H, Piperazine), 2.60 (t, 4H, Piperazine), 3.40 (t, 2H, -NCH_2 propyl), 3.78 (t, 2H, -OCH_2propyl), 3.50 (s, 3H, -CH_3 -), 4.18 (s, 2H, -NCH_2Ar-benzylic), 6.96 (d, 2H, Ar-H), 7.22 (d, 2H, Ar-H), 7.46-7.32 (m, 5H, Ar-H), 7.53 (d, 2H, Ar-H), 7.68 (s, 1H, Ar-H); ^{13}C-NMR in $CDCl_3$ (75 MHz): δ (ppm) 165.55, 161.32, 155.78, 151.26, 147.86, 133.65, 131.10, 128.78, 126.24, 115.25, 112.62, 109.24, 107.78, 74.43, 60.34, 57.80, 56.08, 52.12, 30.61, 26.55, 22.70, 12.02.

8. 7-Methoxy-4-[3-(4-naphthalen-1-ylmethyl-piperazin-1-yl)-propoxy]-3-phenyl-chromen-2-one (**4h**):

The compound 4-(3-piperzin-1-yl-propoxy) 7-methoxy-3-phenyl *2H*-chromen-2-one (3) was reacted with 1-napthaldehyde and isolated the title compound 4h (59 %) as a solid. R_f = 0.34 (MeOH : CH_2Cl_2= 10 : 90); ^{1}H-NMR-(400MHz) in $CDCl_3$: δ (ppm) ^{1}H-NMR-(400MHz) in $CDCl_3$: δ (ppm) 1.82-1.74 (m, 2H, -CH_2 propyl), 2.34 (t, 4H, Piperazine), 2.60 (t, 4H, Piperazine), 3.47 (t, 2H, -NCH_2 propyl), 3.69 (t, 2H, -OCH_2 propyl), 3.55 (s, 3H, -OCH_3), 4.20 (s, 2H, -NCH_2Ar-benzylic), 6.55 (s, 1H, Ar-H), 7.12 (d, 2H, Ar-H, 7.46-7.38 (m, 5H, Ar-H), 7.5 (m, 7H, nap-H).

9. 7-Methoxy-3-phenyl-4-{3-[4-(3-phenyl-allyl)-piperazin-1-yl]-propoxy}-chromen-2-one (**4i**):

The compound 4-(3-piperzin-1-yl-propoxy) 7-methoxy-3-phenyl *2H*-chromen-2-one (3) was reacted with cinnamadehydeld and isolated the title compound 4i (68 %) as a light brown color solid. R_f = 0.41 (MeOH : CH_2Cl_2= 10 : 90); ^{1}H-NMR-(400MHz) in $CDCl_3$: δ (ppm) ^{1}H-NMR-(400MHz) in $CDCl_3$: δ (ppm)

1.80-1.74 (m, 2H, -CH_2 propyl), 2.36 (t, 4H, Piperazine), 2.58 (t, 4H, Piperazine), 3.40 (t, 2H, -NCH_2 propyl), 3.65 (t, 2H, -OCH_2 propyl), 3.55 (s, 3H, -OCH_3), 4.28 (s, 2H, -NCH_2Ar-benzylic)), 6.96 (s, 1H, Ar-H), 6.5&7.1 (d, 2H) 7.20 (d, 2H, Ar-H), 7.46-7.38 (m, 5H, Ar-H) 7.5 (m, 5H, Ar-H).

10. 4-[3-(4-Benzo[1,3]dioxol-5-ylmethyl-piperazin-1-yl)-propoxy]-7-methoxy-3-phenyl-chromen-2-one (**4j**):

The compound 4-(3-piperzin-1-yl-propoxy) 7-methoxy-3-phenyl *2H*-chromen-2-one (3) was reacted with pipernaldehyde and isolated the title compound4j (75 %) as a brown color solid. R_f = 0.42 (MeOH : CH_2Cl_2= 10 : 90); ^{1}H-NMR-(400MHz) in $CDCl_3$: δ (ppm) ^{1}H-NMR-(400MHz) in $CDCl_3$: *δ* 1.78-1.73 (m, 2H, -CH_2 propyl), 2.19 (s, 2H, –OCH2O-), 2.50-2.45 (m, 6H, Piperazine), 2.58 (t, 2H, Piperazine), 3.48 (t, 2H, -NCH_2 propyl), 3.67 (t, 2H, -OCH_2propyl), 3.96 (s, 3H, -OCH_3), 4.12 (s, 2H, -NCH_2Ar-benzylic)), 6.76 (s, 1H, Ar-H), 7.13 (d, 2H, Ar-H), 7.48-7.40 (m, 5H, Ar-H), 7.88 (t, 1H, Ar-H), 8.30 (d, 1H, Ar-H), 8.76 (d, 1H, Ar-H); ^{13}C-NMR in $CDCl_3$ (75 MHz): δ (ppm) 165.22, 163.76, 161.72, 146.44, 147.65, 139.37, 136.65, 133.74, 130.12, 127.86, 125.90, 122.88, 121.52, 113.62, 109.44, 108.78, 97.22, 62.28, 57.17, 56.34, 53.18, 48.12, 27.80.

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SYNTHESIS AND CHARACTERIZATION OF SOME NOVEL SUBSTITUTED 4,7-DIHYDROXY-8-(4-METHYL-1H-BENZO[B][1,4] DIAZEPIN-2-YL)-3-PHENYL-CHROMEN-2-ONE ANALOGUES

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ABSTRACT

A series of substituted 1,5-benzdiazepines which has 4-hydroxy-7-methoxy-3-phenyl-chromen-2-one moiety were synthesized in a new methodology using 1-(2-hydroxy-4-methoxy-phenyl)-2-phenyl-ethanone. The 1,3-diketone system has been developed on at C-8 position of coumarin nucleus by Backer-Venkatraman Rearrangement reaction. It is further converted into substituted benzodiazepines under appropriate conditions using ethanol as solvent. All the reactions are monitored by TLC, all the compounds were purified by column chromatography and structures were analyzed by FT-IR, ^{1}H-NMR and Mass spectral data.

KEYWORDS

Coumarin; 1,5-Benzodiazepines; 1,2-Diaminobenzenes; 1,2-Diaminopyridines

INTRODUCTION

1,5-Benzodiazepines and their derivatives have been investigated extensively by organic chemists due to their medicinal properties such as analgesic and anti-inflammatory activities[1]. These compounds are widely used as anticonvulsant, antianxiety, analgesic, sedative, antidepressive, hypnotic agents as well as anti-inflammatory agents[2]. Moreover 1,5-benzodiazepine derivatives are valuable synthons that can be used in the preparation of other fused ring compounds such as triazolo-, pyrolo-, oxadiazolo-, oxazino- or furano-benzodiazepines[3]. As a result, research in this area is still very active and is directed toward the synthesis of compounds with enhanced pharmacological activity[4]. Lofendaza[5] is an organic molecule which is a benzodiazepine derivative. Lofendazam is a 1,5-benzodiazepine with the nitrogen atoms located at

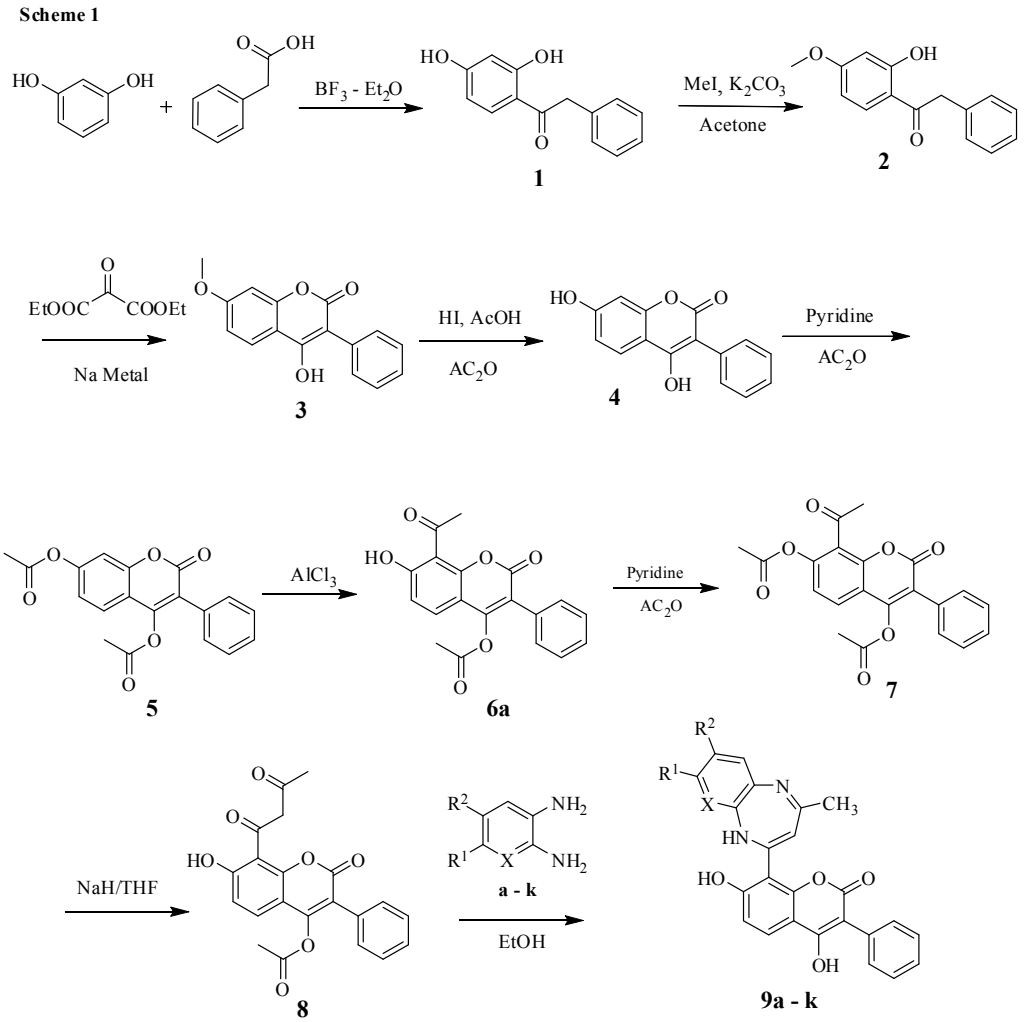
positions 1 and 5 of thediazepine ring; therefore, lofendazam is most closely related to other 1,5-benzodiazepines such as clobazam[6,7].Lofendazam as a human pharmaceutical has sedative and anxiolytic effects similar to those produced by other benzodiazepine derivatives. It is an active metaboliteof another benzodiazepine, arfendzam[8].

Benzodiazepines are a class of agents that work on the central nervous system, acting selectively on gamma-aminobutyric acid-A (GABA-A) receptors in the brain. It enhances response to the inhibitory neurotransmitter GABA, by opening GABA-activated chloride channels and allowing chloride ions to enter the neuron, making the neuron negatively charged and resistant to excitation[9]. Coumarin heterocyclics are one of the major classes of naturally occurring compounds and constitute a family of pharmaceutically active agents. A number of coumarin derivatives endowed with a large number of biological activities such as antihelmenthic, hypnotic insecticidal anti coagulant and coronary vasodilator. Coumarin derivatives have been reported to serve as antibacterial[10-12], antioxidant[13,14], anti-inflammatory[15,16] and antitumoragents[17,18]. Some of the Coumarins have displayed CNS depressant and anti-HIV[19]activities. These pharmacological properties of Coumarin and benzodiazepines aroused our interest in synthesizing some novel benzodiazepines containing Coumarin nuclei within the same molecule with the aim of testing their microbiological activity.

RESULTS AND DISCUSSION

1-[2,4-dihydroxyphenyl]phenylethanone[20,21](**1**) has been prepared by heating of resorcinol and phenylaceticacid in the presence of $BF_3.Et_2O$. The compound **1**was methylated with CH_3I/ K_2CO_3in acetone to obtain compound (**2**). This reaction is chemo selective, it offered the product**2** as major, this compound **2**confirmed by ^{1}H NMR and mass spectral data, in which the broad singlet at δvalue 5.38 ppm, corresponds to normal phenolic proton is disappeared and observed m/z: 243, is also supported. The compound **2**is allowed to react with sodium in diethylcarbonate to yield corresponding substituted coumarin[22], compound **3**. Disappearance of benzylic protons and appearance of enolic protons in^{1}H NMR spectra of **3**, confirms the formation coumarin ring. This compound **3** is treated with HI/AcOH in AC_2O followed by acetylated in AC_2O/Pyridine to afford deacetylated product **5**.The compound **5** lacks the both OH protons in ^{1}H-NMR spectra suggesting that, it is deacetylated. In the next step compound **5** is subjected to Fries Migration with $AlCl_3$ at 160°C to afford two isomeric products[23]. The two isomers**6a** and **6b** were separated by column chromatography.

In our laboratory, for the past several years, we have been working on development of pyrazole, isaxazole and thiazole based heterocyclic molecules. In continuation of our program, aimed at developing pharmaceutically active agents, and we wanted to construct different heterocyclic moieties at C-8 position of coumarin nuclei, for this reason we have chosen isomer **6a** and this compound was again acetylated in Ac_2O/Pyridine yielded **7**.Then compound **7** was subjected to rearrangement reaction according to the Baker-Venkatraman Rearrangement procedure. The resulted 1,3-diketone **8** has been treated with*ortho*- diamines**a-k**[4-hydro/4-fluoro/4-chloro/4-bromo/4-methyl/4-methoxy/4-nitro/4,5-dichloro/4,5-dimethyl 1,2-diaminobenzene, 2,3-diamino pyridine, 6-bromo-2,3-diamino pyridine] in ethanol at appropriate temperature to offered corresponding 1,5-benzodiazepine derivatives (**9a-k**). All the reactions were monitored by TLC and the products were purified then analyzed by the FT-IR, ^{1}H-NMR and mass spectral data.



Scheme 1. Step wise synthesis for 4,7-dihydroxy-8-(4-methyl-1h-benzo[b][1,4] diazepin-2-yl)-3-phenyl-chromen-2-one analogues.

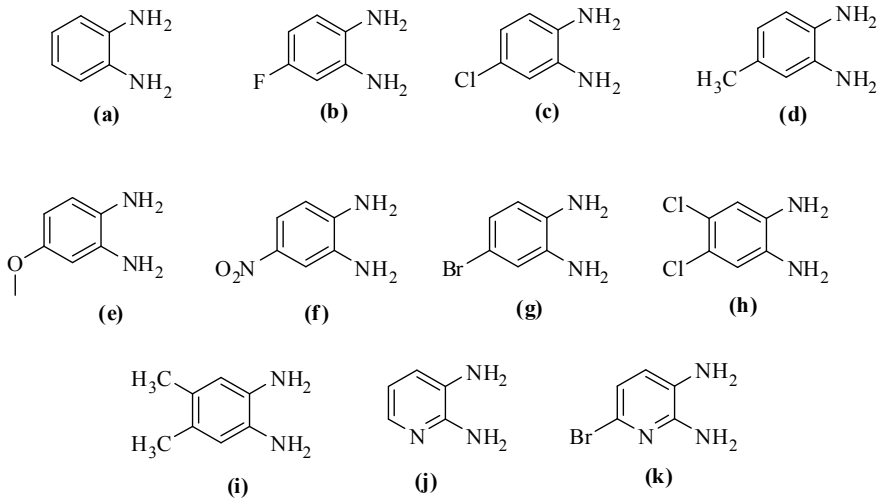


Fig. 1 Structures of*ortho*-diamines (**a-k**) in scheme 1.

EXPERIMENTAL SECTION

Thin Layer Chromatography (TLC) was performed on E.Merk AL Silica gel 60 F_{254} plates and visualized under UV light. The infrared (IR) spectra were determined in a Perkin-Elmer Fourier transform (FDIR spectrum). ^{1}HNMR spectra were recorded on Varian EM-360 (400MHz mercury plus) spectrometer in DMSO-d_6 or $CDCl_3$ and calibrated using solvent signals [7.25($CDCl^3$) and 2.50(DMSO-d_6)]. All chemical shifts recorded in δ (ppm) using TMS as an internal standard. The mass spectra were recorded on Agilent ion trap MS. Spectrometer at energy of ionizing electron is equal to 70ev.

1. Preparation of 1-(2,4-Dihydroxy-phenyl)-2-phenyl-ethanone (1):

Phenyl acetic acid (1.35g, 9.99mmol) was added to a solution of resorcinol (1.0g, 9.09mmol) in BF_3-Et_2O (10mL) at room temperature and this mixture was heated at 85°C for 4h. The reaction mixture was allowing to room temperature and quenched with saturated sodium acetate (25mL) solution and extracted with ethyl acetate (3 X 50mL). The combined organic layers successively washed with water, brine solution and dried over anhydrous $Na_2SO_4$and evaporated under vacuum. The crude product was purified with column chromatography using 60-120 silica mesh and the pure product elute at 20% ethyl acetate in pet ether to afford 1.8g (86.9%) of 1-(2,4-Dihydroxy-phenyl)-2-phenyl-ethanone**1** as light brown colour solid.^{1}H NMR (400MHz, $CDCl_3$):δ (ppm): 12.64 (s, 1H), 7.76 (d, 1H), 7.36-7.28 (m, 5H), 6.38 (d, 2H), 5.38 (br, 1H), 4.23 (s, 2H); m/z: 229 (M+1);IR (KBr) cm^{-1}:3550 (-OH,br), 1680 (C=O), 1450 (CH=CH).

2. Preparation of 1-(2-hydroxy-4-methoxy-phenyl)-2-phenyl-ethanone (2):

Potassium carbonate (725mg, 5.25mmol) and Methyl iodide (0.28mL, 4.38mmol) was added to a solution of 1-(2,4-Dihydroxy-phenyl)-2-phenyl-ethanone (1.0g, 4.38mmol) in acetone at room temperature. This solution was heated at 65°C for 2h, the mixture cool to room temperature, filtered, and the volatiles were evaporated by rotary diluted in ethyl acetate (50mL) and successively washed with water, brine solution to afford 900mg (84.9%) of 1-(2-hydroxy-4-methoxy-phenyl)-2-phenyl-ethanone **2**as off white colour solid. ^{1}H NMR (400MHz, $CDCl_3$):δ (ppm): 12.64 (s, 1H), 7.76 (d, 1H), 7.36-7.28 (m, 5H), 6.38 (d, 2H), 4.23 (s, 2H), 3.98 (s, 3H): m/z: 243 (M+1);IR (KBr) νcm^{-1}: 3550 (-OH, br), 2933 (CH str. Of CH_3), 1692 (C=O), 1450 (CH=CH).

3. Preparation of 4-hydroxy-7-methoxy-3-phenyl-chromen-2-one (3):

Sodium (380mg, 16.52mmol) was added slowly to a solution of 1-(2-hydroxy-4-methoxy-phenyl)-2-phenyl-ethanone (1.0g, 4.13mmol) in diethylcarbonate (5mL, 5vol) at 0°C over period of 10min. The mixture was wormed to room temperature and stirred for overnight. The reaction mixture quenched with sufficient methanol, diluted with ether and extracted with water (2 X 50mL) and washed with diethyl ether (2 X 25mL), The water layer acidified with 2N HCl and extracted with ethyl acetate (3 X 50mL) the combined organic layers dried over Na_2SO_4 and concentrated under reduced pressure to afford 650mg (59%) of 4-hydroxy-7-methoxy-3-phenyl-chromen-2-one**3** as brown colour solid.^{1}H NMR (400 MHz, $CDCl_3$: δ (ppm): 7.80 (d, 1H), 7.56-7.43 (m, 5H), 6.96 (d, 1H), 6.69 (s, 1H), 3.98 (s, 3H); m/z: 269 (M+1);IR (KBr)ν cm^{-1}: 3450 (-OH, br), 1725(C=O of lactone ring), 1450 (CH=CH), 1156 (C-O-C).

4. Preparation of 4,7-dihydroxy-3-phenyl-chromen-2-one (4):

A suspension of 7-methoxy-3-phenyl-chroman-2,4-dione (2.0g, 7.46mmol), Hydroiodic acid (2vol, 4mL) in acetic acid (10vol, 20mL) and acetic anhydride (5vol, 10mL) was heated to 100°C for 45min. The reaction mixture allows to room temperature, volatiles were evaporated, diluted with ethyl acetate (50mL) and washed with water (25mL) and brine (25mL) solution. The organic layer was evaporated by rotary. The crude compound was recrystalized from aq.ethanol to afford 1.2g of (63.4%) 4, 7-dihydroxy-3-phenyl-chromen-2-one**4** as pale yellow colour solid. ^{1}H-NMR, (400 MHz, DMSO-d_6): δ (ppm) 11.06 (s, 1H), 10.52 (s, 1H), 7.86 (d, 1H), 7.42-7.31 (m, 5H), 6.80 (d, 1H), 6.72 (d, 1H); m/z: 255(M+1).

5. Preparation of acetic acid 4-acetoxy-2-oxo-3-phenyl-2H-chromen-7-yl ester (5):

Acetic anhydride (1.8mL, 17.71mmol) was added to a solution of 4,7-dihydroxy-3-phenyl-chromen-2-one (1.5g, 5.90mmol) in dry pyridine at 0°C. The reaction mixture was wormed to room temperature and stirred for 4h. To this reaction mixture ice cold water added, solid precipitated out. The solid was filtered and washed with water, dried to afford 1.8g (94.7%) ofacetic acid 4-acetoxy-2-oxo-3-phenyl-2H-chromen-7-yl ester **5** as white solid^{1}H NMR (400MHz, DMSO-d_6):δ (ppm): 8.08 (d, 1H), 7.52-7.36 (m, 5H), 7.28(d, 2H), 2.34 (s, 3H), 2.32 (s, 3H); m/z: 339 (M+1).

6. Preparation of 8-acetyl-4,7-dihydroxy-3-phenyl-chromen-2-one (6a):

A mixture of $AlCl_3$ (1.57g, 11.83mmol) and acetic acid -4-acetoxy-2-oxo-phenyl-2H-chromen-7-yl ester (1.0g, 2.95mmol) was heated at 160°C for 1h. The black residue was allowing cooling to room temperature, added 2N HCl (10mL) and heated to 100°C for 2h. The reaction mixture allows to room temperature and filtered the mass and the filtrate was extracted with ethyl acetate (3 X 30mL). The combined organic layers washed with water and brine solution. The crude compound was purified by column chromatography using 60-120 silica mesh, the pure product was eluted at 25% of ethyl acetate in pet ether to afford 400mg (45.7%) of 8-acetyl-4, 7-dihydroxy-3-phenyl-chromen-2-one**6a**as brown colour solid.^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.97 (d, 1H), 7.78 (d, 1H), 7.51-7.41 (m, 5H), 2.35 (s, 3H); m/z: 295 (M-1); IR (KBr)ν cm^{-1}: 3580 (-OH, br), 2933 (CH str. Of CH_3), 1725 (C=O of lactone ring), 1692 (C=O), 1450 (CH=CH).

7. **Synthesis of acetic acid 7-acetoxy-8-acetyl-2-oxo-3-phenyl-2H-chromen-4-yl ester (7):**

Acetic anhydride (0.95mL, 10.13mmol) was added to a solution of 8-acetyl-4,7-dihydroxy-3-phenyl-chromen-2-one (1.0g, 3.37mmol) in dry pyridine at 0°C. The reaction mixture was wormed to room temperature and stirred for 4h. To this mixture ice cold water was added, the solid precipitated out. The solid was filtered and dried to afford 1.0g (78.1%) ofacetic acid7-acetoxy-8-acetyl-2-oxo-3-phenyl-2H-chromen-4-yl ester **7**as light brown colour solid.^{1}H-NMR (400MHz, $CDCl_3$):δ 7.76 (s, 1H), 7.26-7.18 (m, 5H), 6.88 (d, 1H), 2.36 (s, 3H), 2.16 (s, 6H); m/z: 381 (M+1).

8. Synthesis of acetic acid 7-hydroxy-2-oxo-8-(3-oxo-butytyl)-3-phenyl-2H-chromen-4-yl ester (8):

A suspension of Sodium hydride (105mg, 2.63mmol) in dry THF (5mL) was added to a solution of acetic acid 7-acetoxy-8-acetyl-2-oxo-3-phenyl-2H-chromen-4-yl ester (500mg, 1.315mmol) in dry THF (10mL) at 0°C. The reaction mixture heated to 65°C for 2h, The mixture was allowed to room temperature and quenched with saturated NH_4Cl solution and extracted with ethyl acetate (3 X 25mL), the combined organic layers dried over anhydrous Na_2SO_4, concentrated under reduced pressure to afford 380mg (76%) of acetic acid 7-hydroxy-2-oxo-8-(3-oxo-butytyl)-3-phenyl-2H-chromen-4-yl ester **8**as a light brown colour solid.^{1}H-NMR(400MHz, DMSO-d_6):δ 8.04(d, 1H), 7.68-7.28 (m, 5H), 7.03 (s, 1H), 3.83 (s, 2H), 2.32 (s, 3H), 2.18 (s, 3H); m/z: 379 (M-1); IR (KBr) νcm^{-1}: 3580 (-OH, br), 2933 (CH str. Of CH_3), 1740 (C=O of ester), 1725 (C=O of lactone ring),1692 (C=O), 1450 (CH=CH).

9. General preparation methods for synthesis of compound 9a-k:

ortho-diamines (**a-k**) in ethanol was added to a solution of acetic acid 7-hydroxy-2-oxo-8-(3-oxo-butytyl)-3-phenyl-2H-chromen-4-yl ester (100mg, 0.263mmol) in ethanol (5mL) at room temperature and this mixture was heated at 80°C for 1.5h to 2h, after cooling the reaction mixture to room temperature, evaporated the volatiles, diluted with water and extracted with chloroform(3 X 10mL). The combined organic layers dried over Na_2SO_4 and concentrated to afford corresponding compound **9a-k.**

a. 4,7-Dihydroxy-8-(4-methyl-1H-benzo[b][1,4]diazepin-2-yl)-3-phenyl-chromen-2-one (9a):

Yield (45 %); ^{1}H NMR (400MHz, $CDCl_3$):δ 13.1(s,1H),12.2(s,1H); 7.12-7.2(m, 4H), 7.76 (d, 1H), 7.36-7.28 (m, 5H), 6.88 (d, 1H), 2.48 (s, 3H), 1.26 (s, 2H); IR (KBr)ν cm^{-1}: 3520 (-OH, br), 2933 (CH str. Of CH_3), 1720 (C=O of lactone ring), 1602 (C=N), 1410 (CH=CH).

b. 8-(8-Fluoro-4-methyl-1H-benzo[b][1,4]diazepin-2-yl)-4,7-dihydroxy-3-phenyl-chromen-2-one (9b):

Yield (52 %);[1]H NMR (400MHz, $CDCl_3$): δ13.0(s,1H),12.2(s,1H), 7.12-7.2(m, 3H), 8.12 (d, 1H), 7.36-7.18 (m, 5H), 6.98 (d, 2H), 5.93 (s, 1H), 2.32 (s, 3H); IR (KBr)ν cm^{-1}: 3522 (-OH, br), 2987 (CH str. Of CH_3), 1723 (C=O of lactone ring), 1602 (C=N), 1410 (CH=CH), 1156 (C-O-C).

c. 8-(8-Chloro-4-methyl-1H-benzo[b][1,4]diazepin-2-yl)-4,7-dihydroxy-3-phenyl-chromen-2-one (9c):

Yield (53%); [1]H NMR (400MHz, $CDCl_3$):δ 13.0(s,1H),12.1(s,1H), 7.12-7.2(m, 4H), 7.69-7.65 (m, 5H); 6.98 (d, 2H); 5.96 (s, 1H); 2.34 (s, 3H); IR (KBr)ν cm^{-1}: 3520 (-OH, br), 2933 (CH str. Of CH_3), 1720 (C=O of lactone ring), 1602 (C=N), 1410 (CH=CH).

d. 8-(4,8-Dimethyl-1H-benzo[b][1,4]diazepin-2-yl)-4,7-dihydroxy-3-phenyl-chromen-2-one (9d):

[1]H NMR (400MHz, $CDCl_3$):δδ 13.1(s,1H),12.2(s,1H)7.12-7.2(m,3H), 7.56 (d, 1H); 7.36-7.28 (m, 5H): 6.98 (d, 1H); 2.28 (s, 3H); 1.66 (s, 2H) 1.12 (s, 3H) IR (KBr)ν cm^{-1}: 3520 (-OH, br), 2933 (CH str. Of CH_3), 1720 (C=O of lactone ring), 1602 (C=N), 1410 (CH=CH).

e. 4,7-Dihydroxy-8-(8-methoxy-4-methyl-1H-benzo[b][1,4]diazepin-2-yl)-3-phenyl-chromen-2-one(9e):

Yield (45 %); [1]H NMR (400MHz, DMSO-d_6):δ 13.1(s,1H),12.2(s,1H), 7.12-7.2(m, 3H), 7.58 (d, 1H), 7.38-7.30 (m, 5H), 6.98 (d, 1H), 3.1 (s, 3H), 1.66 (s, 2H), 1.10 (s, 3H); IR (KBr)ν cm^{-1}: 3520 (-OH, br), 2988 (CH str. Of CH_3), 1722 (C=O of lactone ring), 1602 (C=N), 1410 (CH=CH).

f. 4,7-Dihydroxy-8-(4-methyl-8-nitro-1H-benzo[b][1,4]diazepin-2-yl)-3-phenyl-chromen-2-one (9f):

[1]H NMR (400MHz, DMSO-d_6):δ 13.1(s,1H),12.2(s,1H) 7.12-7.6(,3H), 7.58 (d, 1H), 7.38-7.30 (m, 5H), 6.98 (d, 1H), 2.2(s, 3H),1.66 (s, 2H); IR (KBr)ν cm^{-1}: 3520 (-OH, br), 2988 (CH str. Of CH_3), 1722 (C=O of lactone ring), 1602 (C=N), 1410 (CH=CH).

g. 8-(8-bromo-4-methyl-1H-benzo[b][1,4]diazepin-2-yl)-4,7-dihydroxy-3-phenyl-chromen-2-one (9g):

Yield (59 %); [1]H NMR (400 MHz, $CDCl_3$):δ 13.1 (s, 1H), 12.2 (s, 1H), 7.12-7.2 (m, 3H), 7.89 (d, 1H), 7.72-7.65 (m, 5H), 6.98 (d, 2H), 5.96 (s, 1H), 2.32 (s, 3H); IR (KBr)ν cm^{-1}: 3420 (-OH, br), 2987 (CH str. Of CH_3), 1720 (C=O of lactone ring), 1602 (C=N), 1410 (CH=CH).

h. 8-(7,8-Dichloro-4-methyl-1H-benzo[b][1,4]diazepin-2-yl)-4,7-dihydroxy-3-phenyl-chromen-2-one (9h):

[1]H NMR (400 MHz, DMSO-d_6):δ 13.1(s,1H),12.2(s,1H), 7.2 (2H), 7.1 (s, 2H), 7.38-7.30 (m, 5H), 6.98 (d, 1H), 2.28 (s, 3H),1.66 (s, 2H); IR (KBr)ν cm^{-1}: 3520 (-OH, br), 2988 (CH str. Of CH_3), 1722 (C=O of lactone ring), 1602 (C=N), 1410 (CH=CH).

i. 4,7-Dihydroxy-8-(2-methyl-5H-pyrido[3,4-b][1,4]diazepin-4-yl)-3-phenyl-chromen-2-one(9j):

[1]H NMR (400 MHz, DMSO-d_6):δ 13.1 (s, 1H), 12.2 (s, 1H), 7.82 (d. 1H), 7,4 (d,1H),7.2 (dd, 1H), 7.58 (d, 1H), 7.38-7.30 (m, 5H), 6.98 (d, 1H), 2.28 (s, 3H),1.66 (s, 2H); IR (KBr)ν cm^{-1}: 3520 (-OH, br), 2988 (CH str. Of CH_3), 1722 (C=O of lactone ring), 1602 (C=N), 1410 (CH=CH).

j. 8-(7-Bromo-2-methyl-5H-pyrido[3,4-b][1,4]diazepin-4-yl)-4,7-dihydroxy-3-phenyl-chromen-2-one (9k):

[1]H NMR (400MHz, DMSO-d_6):δ13.1(s,1H),12.2(s,1H),7.1-7.2(d, 2H), 7.38-7.30 (m, 5H), 6.98 (d, 1H), 2.28 (s, 3H), 2.2(s, 3H),1.66 (s, 2H); IR (KBr)ν cm^{-1}: 3520 (-OH, br), 2988 (CH str. Of CH_3), 1722 (C=O of lactone ring), 1602 (C=N), 1410 (CH=CH).

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PHYTOCHEMICALS FROM ANTICANCER ACTIVE JATROPHA HEYNEI N.P. BALAKR

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ABSTRACT

Phytochemical investigation of underground tubers and leaf parts of an indigenous (to Southern India) and a rare herb *Jatopha heynei*, resulted in the isolation of three compounds, 6,8-dihydroxy-7-methoxy-2H-chromen-2-one **1**, Daucosterol **2** and a flavonoid glycoside Vitexin **3**. The structures of the compounds were established on the basis of spectrosocpy data. Tubers hexane extract showed potential anticancer activity, in *in vitro* mode, against both the cell lines MCF-7 and DU-145 (IC_{50} values11.024 and 8.016 µg/mL) and significant anti-fungal activity against *C. Albicans* and *A. niger*.

KEYWORDS

Jatropha heynei, 6,8-dihydroxy-7-methoxy-2H-chromen-2-one, Daucosterol, Vitexin and anti-cancer activity.

INTRODUCTION

Jatropha heynei belongs to family: Euphorbiaceae, found in southern India, growing in a rich but well drained soil. The caudexes can grow to twelve centimetres or more, the whole plant up to 90 cm or more. *J. heynei* occurs mainly in forests of Ranga Reddy district in A.P, India. The plant is an endemic to this region and also has attributed with some medicinal values.

MATERIALS AND METHODS

1. Collection of plant material

J. heynei was collected from Chengicherla forest, Hyderabad, Andhra Pradesh and was identified by taxonomist, Prof. V.S. Raju of Kakatiya University, Warangal, Andhra Pradesh. A voucher specimen was deposited at the CIMAP-Research Centre, Hyderabad, India under the accession number CP-JH/2012.

2. Extraction and isolation

The leaves and tubers were collected separately, dried and powdered (leaves 3 kg and Tubers 3.5 kg). These powdered materials were extracted with hexane, acetone and methanol successively using hot percolation method.

Acetone extract (25 g) of tubers was purified by column chromatography by using silica gel (100-200 mesh) as a stationary phase. Initially the column was eluted with hexane, chloroform and gradually increased up to 20% methanol in chloroform. The 5% methanol in chloroform fractions gave compound **1** (30 mg) in pure form. Similarly fractions collected in10% methanol in chloroform resulted in compound **2** (40 mg) in pure form.

Likewise leaf methanol extract was defatted with hexane then purified by column chromatography by using silica gel (100-200 mesh) as a stationary phase. The column was eluted gradually with hexane, chloroform and later with ethyl acetate. The ethyl acetate fractions gave compound **3** as yellow colored amorphous powder (60 mg) in pure form.

3. Anticancer Activity

a)**Cell culture:**

Human breast cancer cell line (MCF-7) and human prostate cancer cell lines (DU-145) were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA) the cell lines were grown in DMEM medium supplemented with 10% FBS, 0.3% sodium bicarbonate, 10 mL/L antibiotic antimycotic solution (10,000 U/ml penicillin, 10 mg/L streptomycin and 25µg/mL amphotericin B), 1mL/L of 4mM L-glutamine and 1mL/L of 100 mM sodium pyruvate culture was maintained in CO_2 incubator at 37°c with a 90% humidified atmosphere and 5% CO_2

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b) Preparation of samples for MTT assay

Test compounds, extracts and isolated compounds, were taken in 10 mg/ml of DMSO and various dilutions were made with sterile PBS (1X) to get desired concentrations. All formulations were filtered with 0.22 µm sterile filter and 20 minutes of UV eradication before adding to the 96 well plates containing cells**.**

c)Cytotoxicity evaluation (MTT assay)

Cytotoxicity of formulations was assessed using MTT assay to determine the cell viability according to a reported method3. The assay is based on the reduction of MTT by the mitochondrial dehydrogenase of viable cells into purple formazan crystals which gets dissolved in DMSO and read at 570 nm. Briefly, 1x104 exponentially growing cells were seeded into each 96 well plate (counted by Trypan blue exclusion dye method) allowed to grow till 60-70% confluence then compounds(name of the compounds if applicable) were added to the culture medium with the final concentrations ranging from of 10, 25, 50 and 100 µg/mL and along with controls (negative without compound) and positive (Doxorubicin)) incubated for 24 hours CO_2 incubator at 37°C with a 90% humidified atmosphere and 5% CO_2. Then the media of the wells were replaced with 90 µL of fresh serum free media and 10 µL of MTT (5mg/mL of PBS), plates were incubated at 37°C for 2h, there after the above media was discarded allow to dry for 30 minutes. Add 100µl of DMSO in each well at 37°C for 5min. The purple formazan crystals were dissolved and immediately read absorbance at 570nm was measured using Spectra Max plus 384 UV-Visible plate reader (Molecular Devices, Sunnyvale, CA, USA). IC_{50} values were determined by probit analysis software package of MS-excel. % Cell viability (from control) versus concentration.

4. Anti-fungal activity

The plant extracts n-hexane, ethyl acetate and compound 1-5 were assayed for antifungal activity against *Aspergillus niger* (M9687) and *Candida albicans* (M7253). These microbes were grown on PDA plate (39 g/L) at 28 °C and maintained with periodic sub-culturing at 4°C and the control is fluconazole. Different concentrations (100 and 150 µg/mL in DMSO) of test solutions/control were tested.

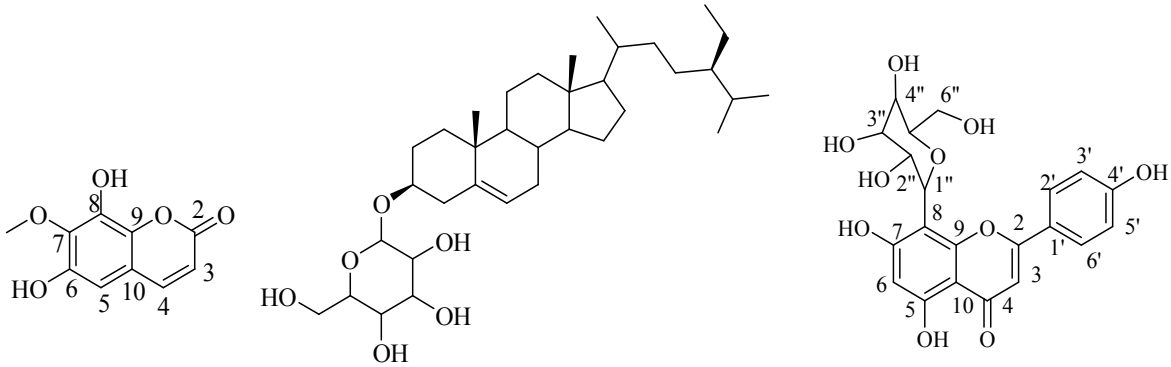
The extracts and compounds (Table 2) were screened for antifungal activity by agar well diffusion method with sterile cork borer of size 6.0 mm.12 The cultures of 48 hours old grown on PDA were used for inoculation of fungal strain on

PDA plates. An aliquot (0.02 mL) of inoculum was introduced to molten PDA and poured in to a petri dish by pour plate technique and later appropriate wells were made on agar plate by using cork borer. In this method, samples including reference standard of 0.05 mL each were introduced and an incubation period of 24-48 h at 28°C was maintained to observe antifungal activity. Antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the plant extracts. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in duplicates.

RESULTS AND DISCUSSION

1. Chemistry

The structure of the isolated compound **1** was elucidated on the basis of IR, $^{1}H/^{13}C$/2D-NMR, Mass spectra and the previously published reports as 6,8-dihydroxy-7-methoxy-2H-chromen-2-one(fig. 1)[1,2]. ^{13}C NMR spectra coupled with DEPT-135 showed a total of 10 carbons in the compound and out of which 1 CH_3, 3CH and remaining 6 quaternary carbons. All the resonance assignments were agreeing with the compound **1** as 6,8-dihydroxy-7-methoxy-2H-chromen-2-one. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 7.86 (2H, d, *J*=12), 6.19 (2H,d, *J*=12), 6.76 (s), 9.58 (s), 3.79 (s); ^{13}C NMR (300 MHz, DMSO-d_6) δ: 161.5, 112.1, 146.23, 112.6, 145.98, 140.20, 56.92, 133.65, 140.2, 110.5. The molecular ion peak appeared at m/z 208 in ESI-MS spectrum agrees with the molecular formula $C_{10}H_8O_5$ for compound **1**. Similarly compound **2** obtained as white powder (melting point 285 °C and uncorrected) was identified as steroidal glycoside, Daucosterol basing on spectroscopy. The presence of molecular ion peak at m/z 576 in ESI-MS spectrum confirms the structure assigned to compound **2**. Compounds **3** was obtained as a yellow powder, (mp 264 °C and uncorrected). It gave molisch test positive and neutral ferric chloride test positive indicating a phenolic glycoside in **3**. It showed a molecular ion peak at m/z: 433 $[M+H]^+$ in the ESI-MS spectrometry. The ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of **3** displayed a one-proton singlet at δ 13.12 indicative of 5-OH of a flavonoid moiety. The glucosidic anomeric proton appeared at δ 4.68 (1H, d, J = 9.9Hz, H-1"). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 182.9 (C-4), 164.8 (C-2), 163.5 (C-7), 161.9 (C-4'), 160.4 (C-5), 156.0 (C-9), 129.7 (C-2', 6'), 122.4 (C-1'), 116.7 (C-3', 5'), 104.6 (C-8), 104.0 (C-10), 102.4 (C-3), 98.1 (C-6), 82.5 (C-5"), 79.2 (C-3"), 74.1 (C-1"), 71.7 (C-2"), 71.4 (C-4"), 62.1 (C-6"). Thus, basing on all the spectroscopy, compound 3 was identified as vitexin [4,5] with molecular formula $C_{21}H_{20}O_{10}$.



1 Coumarin **2 Daucosterol** **3 Vitexin**

Figure 1
Structures of Isolated Compounds

2. Biological activity

a. Anticancer activity:

In vitro anticancer activity was performed on tubers hexane, acetone and methanol extracts and leaf methanol extract and isolated compound 2 and 3 respectively (Table 1). The data from table 1 infers that tubers hexane extract (IC_{50} values11.024 and 8.016 ug/ml) was significantly active when compared with others against human cancer cell line MCF-7 and DU-145. Hexane extract showed even anticancer activity better than the standard doxorubicine (IC_{50} values 13.70 ug/ml) against DU-145 cell lines. Compound 2 was better cytotoxic to MCF-7 than compound 3.

Table 2
Invitro anticancer activity of *J. heynei* and compound 2 and 3

Compound	IC50 in µg/ml	
	MCF-7	**DU-145**
Tubers Hexane	11.024±0.021	8.016±0.014
Tubers Acetone	65.130±0.022	>100
Tubers Methanol	>100	>100
Compound-2	30.277±0.012	>100
Leaf Methanol	90.189±0.045	38.196±0.031
Compound-3	43.617±0.009	>100
Doxorubicin	1.856±0.003	13.707±0.02

b. Studies on antifungal activity

Antifungal activity of all the extracts of tubers and leaves and isolated compounds 2 and 3 were assayed against the growth of *C. Albicans* and *A. niger* (Table 2). The data reveals that tubers hexane extract showed significant reduction in the growth of *C. albicans* and *A. niger* when compared to the reference compound flucanozole. The other extracts and compounds did not show any activity.

Table 2
Antifungal activity (zone of inhibition in mm) on the growth of *A. niger* and *C. albicans*

z	Zone of inhibition at different concentrations (mm)			
	C. albicans		***A. niger***	
	100 (µg/ml)	150 (µg/ml)	100 (µg/ml)	150 (µg/ml)
Tubers Hexane	30	35	27	40
Tubers Acetone	-	-	-	-
Tubers Methanol	-	-	-	-
Leaf Methanol	-	-	-	-
Compound-2	-	-	-	-
Compound-3	-	-	-	-
Fluconazole	22	25	13	17

CONCLUSION

The chromatographic separation of different leaves and tubers extracts of *J. heynei* resulted in the isolation of three pure compounds 6,8-dihydroxy-7-methoxy-2H-chromen-2-one **1**, Daucosterol **2** and Vitexin **3** respectively. Tubers hexane extract showed potential anticancer activity, in *in vitro* mode, against both the cell lines MCF-7 and DU-145 (IC_{50} 11.024 and 8.016 µg/ml) and significant anti-fungal activity against *C. Albicans* and *A. niger*.

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DEVELOPMENT OF NEW MOLECULAR ENTITIES AS POTENT ANTIMYCOBACTERIAL AGENTS: SYNTHESIS OF SUBSTITUTED QUINAZOLINES

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ABSTRACT

As part of a study to optimize the quinazolines antibacterial against *M. tuberculosis*, we have prepared several analogs **2a-f-6a-f** to examine specific structure-activity relationships. Thus synthesized compounds were evaluated for their in vitro antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhosa* and then were subsequently screened for antitubercular activity against *Mycobacterium tuberculosis* H37 Rv strain by MABA assay method. The results demonstrate that the activity against mycobacteria was related more too antibacterial activity than to changes in the lipophilicity of the compounds.

KEY WORDS

Antimycobacterial; Quinazoline; Thiadiazole; Oxadiazole; Thiazolidinone.

INTRODUCTION

Mycobacteria are ubiquitous organisms that are becoming increasingly important pathogens. The resurgence of reported cases of tuberculosis, along with the recent emergence of multidrug-resistant strains of *Mycobacterium tuberculosis*, has refocused attention on this disease[1]. Further the association of tuberculosis and HIV infection is so dramatic that, in some cases, nearly two-thirds of the patients diagnosed with TB are also HIV-1 seropositive[2]. Furthermore, numerous studies have shown that TB is a cofactor in the progression of HIV infection[3]. The reemergence of TB infection is further complicated by an increase in cases which are resistant to conventional drug therapy[4]. Clearly, the development of alternative chemotherapeutics for *Mycobacterium tuberculosis* infection is urgently needed.

Earlier, we found thiazolidinones, thiadiazoles and oxadiazoles to be potent in vitro inhibitors of *M. tuberculosis* H37R, and the toxicity of few compounds appeared to be lower in animals compared to that of the primary

antitubercular drug. These observations at our in-house antitubercular programme[6-10] gave the impetus to synthesize new analogues of quinazoline derivatives and study their possible in vitro antitubercular and antibacterial activities.

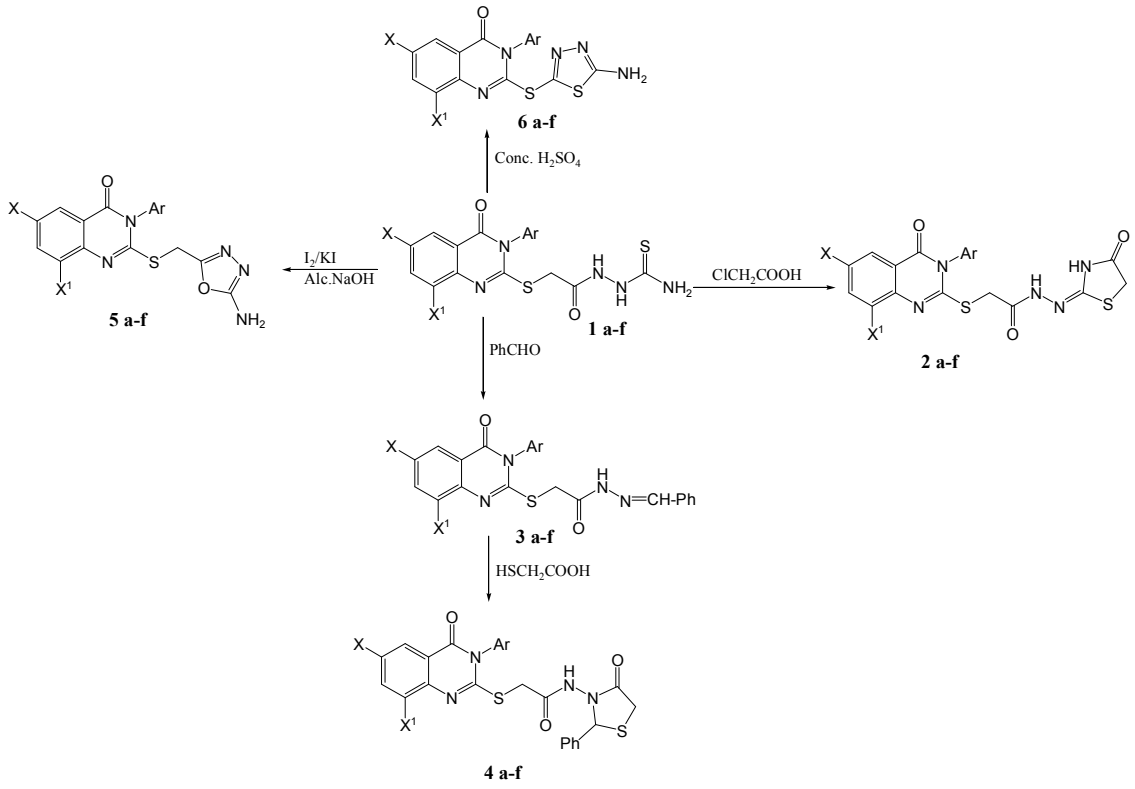
RESULTS AND DISCUSSION

CHEMISTRY

Target compounds were prepared along the reaction sequence in **Scheme 1**. For this purpose, different 1-(2-(6,8-disubstituted-4-oxo-3-aryl-3,4-dihydroquinazolin-2-ylthio) acetyl)thiosemicarbazide (**1a-f**) were prepared by the reaction of (6,8-substituted-4-oxo-3-aryl-3,4-dihydro-quinazolin-2-ylsulfanyl)acetic acid ethyl ester[11] and thiosemicarbazide in dry benzene. Thus obtained 1-(2-(6,8-disubstituted-4-oxo-3-aryl-3,4-dihydroquinazolin-2-ylthio)acetyl)thiosemicarbazide (**1a-f**) were subjected to cyclization reactions under different conditions to obtain novel scaffolds. In the very first modification, **1a-f** was condensed with chloroacetic acid by heating under reflux in ethanol over anhydrous sodium acetate for 3 h to yield (6,8-disubstituted-4-oxo-3-aryl-3,4-dihydro-quinazolin-2-yl sulfanyl)-acetic acid (4-oxo-thiazolidin-2-ylidene)-hydrazide **2a-f**.

The second chemical transformation was achieved by treating **1a-f** with aromatic aldehydes to give Schiff bases (6,8-diubstitutedo-4-oxo-3-aryl-3,4-dihydro-quinazolin-2-yl sulfanyl)acetic acid benzylidene-hydrazide **3a-f**, which on treatment with mercaptoacetic acid produced thiazolidinone derivatives 2-(6,8-disubstituted-4-oxo-3-aryl-3,4-dihydro-quinazolin-2-ylsulfanyl)-N-(4-oxo-2-phenyl-thiazolidin-3-yl)-acetamide **4a-f**. A mixture of **1a-f** and, alcoholic sodium hydroxide has been allowed to undergo a reaction with potassium iodide by heating under reflux for 6-7 h, followed by acidification, generated 2-(5-amino-[1,3,4]-oxadiazol-2-ylmethylsulfanyl)-6,8-disubstituted-3-aryl-3H-quinazolin-4-one **5a-f**. The final structural modification was accomplished when **1a-f** was stirred with sulfuric acid for 12 h, to produce 2-(5-amino-[1,3,4]thiadiazol-2-ylmethylsulfanyl)-6,8-disubstituted-3-phenyl-3H-quinazolin-4-one **6a-f**.

Scheme-1



Substitution	X	X^1	Ar
a	Br	H	$-C_6H_5$
b	Br	Br	$-C_6H_5$
c	I	H	$-C_6H_5$
d	Br	H	$-2\text{-}OH\text{-}C_6H_4$
e	Br	Br	$-2\text{-}OH\text{-}C_6H_4$
f	I	H	$-2\text{-}OH\text{-}C_6H_4$

ANTITUBERCULAR ACTIVITY

The above-synthesized products were screened against *M. tuberculosis* using Microplate Alamar Blue Assay (MABA) assay[12] on High Throughput Screening (HTS) machine at 25 µg/mL and lower concentrations using *M. tuberculosis* H37Ra as a surrogate for the virulent H37Rv strain. The results are shown in table 1. The results of MABA have been found comparable to standard BACTEC 460 system based assay.

The standard antitubercular drugs *Rifamycin*, *Isoniazid*, *p-aminosalicylic acid*, *Ethambutol* and *Ethionamide* (MIC range 0.3-3 µg/mL) were taken as positive controls. We have also done cytotoxicity

analysis of the above-synthesized compounds, using neutral red uptake by using Vero-C-1008 cell line at various concentrations (6.25 µg/mL to 50 µg/mL), none of them were found toxic. Hence the activities of the above-synthesized compounds were not due to cytotoxicity of the compounds.

Table 1.
Percent Inhibition at 25µg/mL concentration.

Compd	Activity in %	Compd	Activity in %	Compd	Activity in %
1a	68	**3a**	63	**5a**	64
1b	97	**3b**	96	**5b**	90
1c	64	**3c**	66	**5c**	64
1d	65	**3d**	64	**5d**	70
1e	92	**3e***	97	**5e**	92
1f	64	**3f**	64	**5f**	70
2a	92	**4a**	70	**6a**	68
2b	70	**4b**	94	**6b***	97
2c	71	**4c**	72	**6c**	68
2d	70	**4d**	72	**6d**	64
2e	90	**4e**	96	**6e***	96
2f	70	**4f**	72	**6f**	64

*Compounds showed 94, 96 and 94% inhibition at 12.5 µg/mL concentration.

During the preliminary screening six compounds **1a**–**f** were tested (Table 1) at 25 µg/mL concentration for their antimycobacterial activity, one of the compounds **1b** exhibited 97 % inhibition at this concentration while other compounds exhibited less than 96 % inhibition at the same concentration.

Thus we have considered **1b** and **1e** since they have shown inhibition higher than 90%. However, in spite of this consideration the structural modifications are performed on the entire series 1a-f.as lead molecule and subsequent structural modifications were carried out. As a first step towards lead optimization, the amino group was cyclised to the corresponding thiazolidinone **2a-f** however; all of these modifications resulted in a substantial decrease in activity.

Compounds **3b** and **3e** have shown 96 and 97 % inhibition at 25 µg/mL (Table 1) which was obtained by simple condensation with aromatic aldehydes. The increase in the activity may be attributed to the CH=N, which is reported to increase the bioavailability of a compound. Thus looking at the activity, it was decided to perform structural modification on the schiff base. In order to optimize the CH=N component, six thiazolidinone compounds **4a-f** were synthesized and investigated, which revealed promising activity (94 and 96 for **4b** and **4e** respectively) at 25 µg/mL, and loss of activity at 12.5 µg/mL dose.

A further modification of compounds **1a-f** produced compounds **5a-f**. The results of the antimycobacterial activity revealed their inactiveness. Surprisingly a similar modification of 1**a-f** to 6**a-f** shown drastic change in results as **6b** and **6e** were found to be active at 25 as well as at 12.5 µg/mL dose. The mechanism behind this may be a part of our next article.

Although we have not been able to substantially enhance the activity of these compounds in the present study, the data presented here are encouraging and deserve further investigation.

ANTIMICROBIAL ACTIVITY

The compounds listed in the tables were screened for the antimicrobial activity against different microorganisms under the following conditions.

Method: Well diffusion method,[13] **Medium**: The nutrient agar medium.
Solvent: Chloroform. **Concentrations**: 50μM and 100 μM.
Condition: 24 hours at 24-28 °C, **Standard**: The antibiotic *Gentamycin.* The nutrient agar medium, 20 mL was poured into the sterile petri dishes. To the solidified plates, wells were made using a sterile cork borer 10 mm in diameter. The 24 hour subcultured bacteria was inoculated in the petri-plates, with a sterile cotton swab dipped in the nutrient broth medium. After inoculating, the compounds were dissolved separately with the chloroform solvent and poured into the wells with varying concentrations ranging from 50 and 100 μM using a micropipette. The plates were left over for 24 hours at 24-28 °C. The antibiotic *Gentamycin* was used as a standard for comparative study.
The percentage of inhibition was calculated by the formula:
% Inhibition = Diameter of the inhibition zone X 100

From these data, it has been found that all the compounds tested showed broad spectrum of inhibitory properties. From the antibacterial screening it was observed that all the compounds exhibited activity against all the organisms employed. Looking at the structure activity relationship, good inhibition in bacteria was observed for most of the compounds but the compounds **1b**, **1e**, **2b**, **2e**, **3b**, **6b** and **6e** show excellent activity, whereas **3e**, **4b**, **4e**, **5b**, and **5e** show moderate activity and others showed little activity.

Table 2.
Antibacterial activity of the synthesized compounds (% of inhibition)

Comp	Organisms[a]				Comp	Organisms[a]			
	Sa	Pa	Ec	St		Sa	Pa	Ec	St
1a	17	16	17	14	**4a**	21	20	23	21
1b	32	34	34	32	**4b**	27	28	30	28
1c	18	19	16	17	**4c**	16	14	18	20
1d	17	18	20	24	**4d**	20	24	18	16
1e	31	33	34	34	**4e**	26	28	30	28
1f	18	16	18	14	**4f**	18	14	16	20
2a	24	20	21	23	**5a**	20	14	17	19
2b	31	33	35	33	**5b**	28	30	30	27
2c	20	21	18	24	**5c**	15	17	16	20
2d	18	14	16	21	**5d**	20	21	24	23
2e	32	34	36	34	**5e**	27	28	29	27
2f	24	20	20	21	**5f**	18	19	17	20
3a	18	16	20	24	**6a**	20	14	16	14
3b	30	34	34	34	**6b**	32	34	34	36
3c	23	24	21	20	**6c**	18	14	17	20
3d	18	14	16	14	**6d**	20	24	18	17
3e	28	29	29	28	**6e**	32	34	36	36
3f	17	18	14	16	**6f**	16	14	20	21
Gentamycin	31	33	34	32	*Gentamycin*	31	33	34	32

[a]Organisams: Sa- *Staphylococcus aureus,* Ec- *Escherichia coli,* Pa-*Pseudomonas aeruginosa,* St- *Salmonella typhosa*

EXPERIMENTAL SECTION

The melting points were recorded on a electrothermal apparatus and are uncorrected. ^{1}H NMR spectra on a Bruker Avance 300 MHz instrument using $CDCl_3$ as a solvent (chemical shifts in δ ppm) using TMS as internal standard; mass spectra on a Finning LCQ mass spectrometer. Elemental analysis was performed on a Heracus CHN-Rapid Analyser. The purity of the compounds was checked on silica gel coated Al plates (Merck).

Preparation of 2-2-[(6-Bromo-4-oxo-3-phenyl-3,4-dihydro-2-quinazolinyl)sulfanyl]acetyl-1-hydrazinecarbothioamide(1a), 2-2-[(6,8-dibromo-4-oxo-3-phenyl-3,4-dihydro-2-quinazo linyl)sulfanyl]acetyl-1-hydrazinecarbothioamide (1b), 2-2-[(6-Iodo-4-oxo-3-phenyl-3,4-di hydro-2-quinazolinyl)sulfanyl]acetyl-1-hydrazinecarbothioamide (1c), 2-2-[(6-Bromo-4-oxo-3-(4-hydroxy-phenyl)-3,4-dihydro-2-quinazolinyl)sulfanyl] acetyl-1-hydrazine carbo thioamide (1d), 2-2-[(6,8-dibromo-4-oxo-3-(4-hydroxy-phenyl)-3,4-dihydro-2-quinazo linyl)sulfanyl]acetyl-1-hydrazinecarbothioamide (1e), 2-2-[(6-Iodo-4-oxo-3-(4-hydroxy-phenyl)-3,4-dihydro-2-quinazolinyl)sulfanyl]acetyl-1-hydrazinecarbo-thioamide (1f). The above mentioned compounds were prepared according to the literature[11].

1. (6-Bromo-4-oxo-3-phenyl-3,4-dihydro-quinazolin-2-ylsulfanyl)-acetic acid (4-oxo-thiazolidin-2-ylidene)-hydrazide (2a):

A mixture of compound **1a** (1 mmol), chloroacetic acid (1.5 mmol) and ethanol (40 mL) over anhydrous sodium acetate, were taken into a reaction flask and heated under reflux for 3 h. The volume of the mixture was reduced to half by distilling-off excess alcohol. The concentrate was diluted with cold water and cooled further to 0 °C. The solid thus resulted was filtered washed with small portions of cold water and dried; which was then recrystallized.

Recrystallization solvent- Ethanol; Yield 68 %; buff white powder; mp 172-177 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.76 (s, 4H, CH_2-CO), 7.21-7.91 (m, 8H, ArH), 8.07 (b, 2H, NH); MS (%) 504 (M+, 100); $C_{19}H_{14}BrN_5O_3S_2$ requires: C, 45.24; H, 2.80; N, 13.89; found: C, 45.47; H, 2.98; N, 13.65.

Other compounds in this series were prepared in similar way.

2. (6,8-Dibromo-4-oxo-3-phenyl-3,4-dihydro-quinazolin-2-ylsulfanyl)-acetic acid-(4-oxo-thiazolidin-2-ylidene)-hydrazide (2b):

Recrystallization solvent- Ethylacetate; Yield 70 %; buff white powder; mp 206-211 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.71 (s, 4H, CH_2-CO), 7.31-7.85 (m, 7H, ArH), 8.27 (b, 2H, NH); MS (%) 583 (M+, 100); $C_{19}H_{13}Br_2N_5O_3S_2$ requires: C, 39.12; H, 2.25; N, 12.01; found: C, 39.34; H, 2.57; N, 12.26.

3. (6-Iodo-4-oxo-3-phenyl-3,4-dihydro-quinazolin-2-ylsulfanyl)-acetic acid (4-oxo-thiazolidin-2-ylidene)-hydrazide (2c):

Recrystallization solvent- Ethylacetate; Yield 58 %; buff white powder; mp 241-246 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.63 (s, 4H, CH_2-CO), 7.17-7.86 (m, 8H, ArH), 8.14 (b, 2H, NH); MS (%) 551 (M+, 100); $C_{19}H_{14}IN_5O_3S_2$ requires: C, 41.39; H, 2.56; N, 12.70; found: C, 41.62; H, 2.71; N, 12.89.

4. (6-Bromo-4-oxo-3-(4-hydroxy-phenyl)-3,4-dihydro-quinazolin-2-ylsulfanyl)-acetic acid (4-oxo-thiazolidin-2-ylidene)-hydrazide (2d):

Recrystallization solvent- Ethylacetate; Yield 59 %; buff powder; mp 213-218 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.69 (s, 4H, CH_2-CO), 4.94 (s, 1H, OH), 7.24-7.81 (m, 7H, ArH), 8.43 (b, 2H, NH); MS (%) 520 (M+, 100); $C_{19}H_{14}BrN_5O_4S_2$ requires: C, 43.85; H, 2.71; N, 13.46; found: C, 43.61; H, 2.46; N, 13.68.

5. (6,8-Dibromo-4-oxo-3-(4-hydroxy-phenyl)-3,4-dihydro-quinazolin-2-ylsulfanyl)-acetic acid (4-oxo-thiazolidin-2-ylidene)-hydrazide (2e):

Recrystallization solvent- Ethylacetate; Yield 51 %; buff powder; mp 204-209 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.58 (s, 4H, CH_2-CO), 4.82 (s, 1H, OH), 7.14-7.86 (m, 6H, ArH), 8.57 (b, 2H, NH); MS (%) 599 (M+, 100); $C_{19}H_{13}Br_2N_5O_4S_2$ requires: C, 38.08; H, 2.19; N, 11.69; found: C, 38.23; H, 2.38; N, 11.84.

6. (6-Iodo-4-oxo-3-(4-hydroxy-phenyl)-3,4-dihydro-quinazolin-2-ylsulfanyl)-acetic acid (4-oxo-thiazolidin-2-ylidene)-hydrazide (2f):

Recrystallization solvent- Ethylacetate; Yield 53 %; buff powder; mp 186-191 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.26 (s, 4H, CH_2-CO), 4.76 (s, 1H, OH), 7.18-7.84 (m, 7H, ArH), 8.67 (b, 2H, NH); MS (%) 567 (M+, 100); $C_{19}H_{14}IN_5O_4S_2$ requires: C, 40.22; H, 2.49; N, 12.34; found: C, 40.52; H, 2.73; N, 12.57.

7. (6-Bromo-4-oxo-3-phenyl-3,4-dihydro-quinazolin-2-ylsulfanyl)-acetic acid benzylidene-hydrazide (3a):

A mixture of **1a** (1 mmol), benzaldehyde (1 mmol), ethanol (25 mL) and a few drops of glacial acetic acid was taken in a clean dry reaction flask, the reaction mixture was then heated on a water-bath for 4 h and then cooled and kept in ice-chest, over night. It was triturated with cold water and the product was filtered, washed thoroughly with ice-cold water, dried and then recrystallized.

Recrystallization solvent- Ethanol; Yield 70 %; pale yellow powder; mp 187-192 ° C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.76 (s, 2H, CH_2-CO), 7.12-7.98 (m, 13H, ArH), 8.34 (s, 1H, CH=N), 9.5 (b, 1H, NH); MS (%) 494 (M+, 100); $C_{23}H_{17}BrN_4O_2S$ requires: C, 55.99; H, 3.47; N, 11.36; found: C, 56.16; H, 3.64; N, 11.53. Other compounds in this series were prepared in similar way.

8. (6,8-Dibromo-4-oxo-3-phenyl-3,4-dihydro-quinazolin-2-ylsulfanyl)-acetic acid benzy lidene-hydrazide (3b):

Recrystallization solvent- Ethylacetate; Yield 61 %; pale yellow powder; mp 208-213 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.84 (s, 2H, CH_2-CO), 7.28-7.85 (m, 12H, ArH), 8.64 (s, 1H, CH=N), 9.46 (b, 1H, NH); MS (%) 572 (M+, 100); $C_{23}H_{16}Br_2N_4O_2S$ requires: C, 48.27; H, 2.82; N, 9.79; found: C, 48.46; H, 2.94; N, 9.88.

9. (6-Iodo-4-oxo-3-phenyl-3,4-dihydro-quinazolin-2-ylsulfanyl)-acetic acid benzylidene-hydrazide (3c):

Recrystallization solvent- Ethanol; Yield 62 %; buff white powder; mp 252-257 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.59 (s, 2H, CH_2-CO), 7.20-7.82 (m, 13H, ArH), 8.41 (s, 1H, CH=N), 9.34 (b, 1H, NH); MS (%) 540 (M+, 100); $C_{23}H_{17}IN_4O_2S$ requires: C, 51.12; H, 3.17; N, 10.37; found: C, 51.43; H, 3.36; N, 10.54.

10. [6-Bromo-3-(4-hydroxy-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-ylsulfanyl]-acetic acid benzylidene-hydrazide (3d):

Recrystallization solvent- Ethanol; Yield 56 %; pale yellow powder; mp 218-223 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.88 (s, 2H, CH_2-CO), 5.41 (s, 1H, OH), 7.27-7.75 (m, 12H, ArH), 8.56 (s, 1H, CH=N), 9.24 (b, 1H, NH); MS (%) 509 (M+, 100); $C_{23}H_{17}BrN_4O_3S$ requires: C, 54.23; H, 3.36; N, 11.00; found: C, 54.41; H, 3.54; N, 11.24

11. [6,8-Dibromo-3-(4-hydroxy-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-ylsulfanyl]-acetic acid benzylidene-hydrazide (3e):

Recrystallization solvent- Ethanol; Yield 58 %; pale yellow powder; mp 215-219 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.68 (s, 2H, CH_2-CO), 5.25 (s, 1H, OH), 7.34-8.13 (m, 11H, ArH), 8.62 (s, 1H, CH=N), 9.47 (b, 1H, NH); MS (%) 588 (M+, 100); $C_{23}H_{16}Br_2N_4O_2S$ requires: C, 46.96; H, 2.74; N, 9.52; found: C, 47.23; H, 2.95; N, 9.73.

12. [6-Iodo-3-(4-hydroxy-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-ylsulfanyl]-acetic acid benzylidene-hydrazide (3f):

Recrystallization solvent- Ethanol; Yield 65 %; buff white powder; mp 198-203 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.83 (s, 2H, CH_2-CO), 5.18 (s, 1H, OH), 7.24-7.86 (m, 12H, ArH), 8.61 (s, 1H, CH=N), 9.74

(b, 1H, NH); MS (%) 556 (M+, 100); $C_{23}H_{17}IN_4O_3S$ requires: C, 49.65; H, 3.08; N, 10.07; found: C, 49.82; H, 3.26; N, 10.32.

13. 2-(6-Bromo-4-oxo-3-phenyl-3,4-dihydro-quinazolin-2-ylsulfanyl)-*N*-(4-oxo-2-phenyl-thiazolidin-3-yl)-acetamide (4a):

A mixture of **3a** (1 mmol), mercaptoacetic acid (1 mmol), benzene (20 mL) and anhydrous zinc chloride (0.5 g) were taken in a clean dry reaction flask; the reaction mixture was then heated on a water-bath for 7-8 h. The reaction mixture was left overnight and the solid thus separated was filtered, washed with cold water, dried and then recrystallized.

Recrystallization solvent- Ethylacetate:1,4-dioxane; Yield 70 %; buff white; mp 195-200 ° C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.79 (s, 4H, CH_2-CO), 7.09-8.13 (m, 13H, ArH), 9.68 (b, 1H, NH); MS (%) 567 (M+, 100); $C_{25}H_{19}BrN_4O_3S_2$ requires: C, 52.91; H, 3.37; N, 9.87; found: C, 53.21; H, 3.64; N, 10.07

Other compounds in this series were prepared in similar way.

14. 2-(6,8-Dibromo-4-oxo-3-phenyl-3,4-dihydro-quinazolin-2-ylsulfanyl)-*N*-(4-oxo-2-phenyl-thiazolidin-3-yl)-acetamide (4b)"

Recrystallization solvent- Ethylacetate:1,4-dioxane; Yield 70 %; Beige powder; mp 213-218 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.64 (s, 4H, CH_2-CO), 7.16-8.05 (m, 12H, ArH), 9.71 (b, 1H, NH); MS (%) 646 (M+, 100); $C_{25}H_{18}Br_2N_4O_3S_2$ requires: C, 46.45; H, 2.81; N, 8.67; found: C, 46.68; H, 2.96; N, 8.83.

15. 2-(6-Iodo-4-oxo-3-phenyl-3,4-dihydro-quinazolin-2-ylsulfanyl)-*N*-(4-oxo-2-phenyl-thiazolidin-3-yl)-acetamide (4c):

Recrystallization solvent- Ethylacetate:1,4-dioxane; Yield 73 %; Beige powder; mp 261-266 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.82 (s, 4H, CH_2-CO), 7.26-8.21 (m, 13H, ArH), 9.61 (b, 1H, NH); MS (%) 614 (M+, 100); $C_{25}H_{19}IN_4O_3S_2$ requires: C, 48.87; H, 3.12; N, 9.12; found: C, 48.92; H, 3.20; N, 9.24.

16. 2-(6-Bromo-4-oxo-3-(4-hydroxy-phenyl)-3,4-dihydro-quinazolin-2-ylsulfanyl)-*N*-(4-oxo-2-phenyl-thiazolidin-3-yl)-acetamide (4d):

Recrystallization solvent- Ethylacetate:1,4-dioxane; Yield 70 %; Beige powder; mp 228-233 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.82 (s, 4H, CH_2-CO), 5.09 (s, 1H, OH), 7.11-8.24 (m, 12H, ArH), 9.70 (b, 1H, NH); MS (%) 583 (M+, 100); $C_{25}H_{19}BrN_4O_4S_2$ requires: C, 51.46; H, 3.28; N, 9.60; found: C, 51.65; H, 3.47; N, 9.73.

17. 2-(6,8-Dibromo-4-oxo-3-(4-hydroxy-phenyl)-3,4-dihydro-quinazolin-2-ylsulfanyl)-*N*-(4-oxo-2-phenyl-thiazolidin-3-yl)-acetamide (4e):

Recrystallization solvent- Ethylacetate:1,4-dioxane; Yield 72 %; Beige powder; mp 248-253 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.66 (s, 4H, CH_2-CO), 5.25 (s, 1H, OH), 7.13-8.19 (m, 11H, ArH), 9.71 (b, 1H, NH); MS (%) 662 (M+, 100); $C_{25}H_{18}Br_2N_4O_4S_2$ requires: C, 45.33; H, 2.74; N, 8.46; found: C, 45.46; H, 2.89; N, 8.61.

18. 2-(6-Iodo-4-oxo-3-(4-hydroxy-phenyl)-3,4-dihydro-quinazolin-2-ylsulfanyl)-*N*-(4-oxo-2-phenyl-thiazolidin-3-yl)-acetamide (4f):

Recrystallization solvent- Ethylacetate:1,4-dioxane; Yield 77 %; Beige powder; mp 231-236 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.84 (s, 4H, CH_2-CO), 5.17 (s, 1H, OH), 7.26-8.11 (m, 12H, ArH), 9.72 (b, 1H, NH); MS (%) 630 (M+, 100); $C_{25}H_{19}IN_4O_4S_2$ requires: C, 47.63; H, 3.04; N, 8.89; found: C, 47.78; H, 3.13; N, 8.97.

19. 2-(5-Amino-[1,3,4]oxadiazol-2-ylmethylsulfanyl)-6-bromo-3-phenyl-3*H*-quinazolin-4-one (5a):

A mixture of **1a** (1 mmol) and alcoholic sodium hydroxide (25 mL/2.0 g) has been allowed to undergo a reaction with potassium iodide (3.6 g) by heating under reflux for 6-7 h. The product obtained on acidification of the reaction mixture with hydrochloric acid (12 mL) has been purified by recrystallization.

Recrystallization solvent- Ethanol; Yield 64 %; Cream microcrystal; mp 162-167 ° C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.89 (s, 2H, NH_2), 4.26 (s, 2H, CH_2-S), 7.12-7.94 (m, 8H, ArH); MS (%) 431 (M+, 100); $C_{17}H_{12}BrN_5O_2S$ requires: C, 47.45; H, 2.81; N, 16.28; found: C, 47.56; H, 2.97; N, 16.39.

Other compounds in this series were prepared in similar way.

20. 2-(5-Amino-[1,3,4]oxadiazol-2-ylmethylsulfanyl)-6,8-dibromo-3-phenyl-3*H*-quinazolin-4-one (5b):

Recrystallization solvent- Ethanol; Yield 54 %; Cream microcrystal; mp 203-208 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.91 (s, 2H, NH_2), 4.22 (s, 2H, CH_2-S), 7.19-7.84 (m, 7H, ArH); MS (%) 509 (M+, 100); $C_{17}H_{11}Br_2N_5O_2S$ requires: C, 40.10; H, 2.18; N, 13.75; found: C, 40.38; H, 2.40; N, 13.88.

21. 2-(5-Amino-[1,3,4]oxadiazol-2-ylmethylsulfanyl)-6-iodo-3-phenyl-3*H*-quinazolin-4-one (5c):

Recrystallization solvent- IPA; Yield 58 %; Cream microcrystal; mp 229-234 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.93 (s, 2H, NH_2), 4.30 (s, 2H, CH_2-S), 7.08-7.86 (m, 8H, ArH); MS (%) 477(M+, 100); $C_{17}H_{12}IN_5O_2S$ requires: C, 42.78; H, 2.53; N, 14.67; found: C, 42.94; H, 2.73; N, 14.45.

22. 2-(5-Amino-[1,3,4]oxadiazol-2-ylmethylsulfanyl)-6-bromo-3-(4-hydroxy-phenyl)-3*H*-quinazolin-4-one (5d):

Recrystallization solvent- IPA; Yield 53 %; Cream microcrystal; mp 215-221 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.98 (s, 2H, NH_2), 4.21 (s, 2H, CH_2-S), 5.20 (s, 1H, OH), 7.22-7.91 (m, 7H, ArH); MS (%) 446 (M+, 100); $C_{17}H_{12}BrN_5O_3S$ requires: C, 45.75; H, 2.71; N, 15.69; found: C, 45.36; H, 2.58; N, 15.49.

23. 2-(5-Amino-[1,3,4]oxadiazol-2-ylmethylsulfanyl)-6,8-dibromo-3-(4-hydroxy-phenyl)-3*H*-quinazolin-4-one (5e):

Recrystallization solvent- Ethanol; Yield 61 %; Cream microcrystal; mp 213-218 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.03 (s, 2H, NH_2), 4.31 (s, 2H, CH_2-S), 5.24 (s, 1H, OH), 7.24-8.14 (m, 6H, ArH); MS (%) 525 (M+, 100); $C_{17}H_{11}Br_2N_5O_3S$ requires: C, 38.88; H, 2.11; N, 13.34; found: C, 38.65; H, 2.34; N, 13.18.

24. 2-(5-Amino-[1,3,4]oxadiazol-2-ylmethylsulfanyl)-6-iodo-3-(4-hydroxy-phenyl)-3*H*-quinazolin-4-one (5f):

Recrystallization solvent- Ethanol; Yield 55 %; Cream microcrystal; mp 221-216 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.71 (s, 2H, NH_2), 4.26 (s, 2H, CH_2-S), 5.17 (s, 1H, OH), 7.18-7.83 (m, 7H, ArH); MS (%) 493 (M+, 100); $C_{17}H_{12}IN_5O_3S$ requires: C, 41.39; H, 2.45; N, 14.20; found: C, 41.56; H, 2.74; N, 14.37.

25. 2-(5-Amino-[1,3,4]thiadiazol-2-ylsulfanyl)-6-bromo-3-phenyl-3*H*-quinazolin-4-one (6a):

Compound **1a** (1 mmol) was dissolved in sulfuric acid (12.5 mL) with cooling and stirred at ambient temperature for 20-24 h. The crude product obtained after filtration was washed with portions of chilled water (3 x 25 mL) and purified by recrystallization.

Recrystallization solvent- Ethylacetate:benzene; Yield 77 %; Brown powder; mp 182-187 ° C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.08 (s, 2H, NH_2), 7.13-7.75 (m, 8H, ArH); MS (%) 433 (M+, 100); $C_{16}H_{10}BrN_5OS_2$ requires: C, 44.45; H, 2.33; N, 16.20; found: C, 44.62; H, 2.54; N, 16.34.

Other compounds in this series were prepared in similar way.

26. 2-(5-Amino-[1,3,4]thiadiazol-2-ylmethylsulfanyl)-6,8-dibromo-3-phenyl-3*H*-quinazolin-4-one (6b):

Recrystallization solvent- Ethylacetate:benzene; Yield 63 %; Brown powder; mp 203-208 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.11 (s, 2H, NH_2), 7.19-7.81 (m, 7H, ArH); MS (%) 511 (M+, 100); $C_{16}H_9Br_2N_5OS_2$ requires: C, 37.59; H, 1.77; N, 13.70; found: C, 37.72; H, 1.84; N, 13.91.

27. 2-(5-Amino-[1,3,4]thiadiazol-2-ylmethylsulfanyl)-6-iodo-3-phenyl-3*H*-quinazolin-4-one (6c):

Recrystallization solvent- Ethylacetate:benzene; Yield 60 %; Brown powder; mp 229-234 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.13 (s, 2H, NH_2), 7.04-7.93(m, 8H, ArH); MS (%) 479 (M+, 100); $C_{16}H_{10}IN_5OS_2$ requires: C, 40.09; H, 2.10; N, 14.61; found: C, 40.16; H, 2.34; N, 14.74.

28. 2-(5-Amino-[1,3,4]thiadiazol-2-ylmethylsulfanyl)-6-bromo-3-(4-hydroxy-phenyl)-3*H*-quinazolin-4-one (6d):

Recrystallization solvent- Ethylacetate:benzene; Yield 63 %; Brown powder; mp 192-197 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.12 (s, 2H, NH_2), 5.24 (s, 1H, OH), 7.21-7.84 (m, 7H, ArH); MS (%) 448 (M+, 100); $C_{16}H_{10}BrN_5O_2S_2$ requires: C, 42.87; H, 2.25; N, 15.62; found: C, 42.93; H, 2.37; N, 15.78.

29. 2-(5-Amino-[1,3,4]thiadiazol-2-ylmethylsulfanyl)-6,8-dibromo-3-(4-hydroxy-phenyl)-3*H*-quinazolin-4-one (6e):

Recrystallization solvent- Ethylacetate:benzene; Yield 68 %; Brown powder; mp 194-199 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.17 (s, 2H, NH_2), 5.23 (s, 1H, OH), 7.18-7.64 (m, 6H, ArH); MS (%) 527 (M+, 100); $C_{16}H_9Br_2N_5O_2S_2$ requires: C, 36.45; H, 1.72; N, 13.28; found: C, 36.61; H, 1.87; N, 13.36.

30. 2-(5-Amino-[1,3,4]thiadiazol-2-ylmethylsulfanyl)-6-iodo-3-(4-hydroxy-phenyl)-3H-quinazolin-4-one (6f):

Recrystallization solvent- Ethylacetate:benzene; Yield 62 %; Brown powder; mp 187-192 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.20 (s, 2H, NH_2), 5.17 (s, 1H, OH), 7.24-7.86 (m, 7H, ArH); MS (%) 495 (M+, 100); $C_{16}H_{10}IN_5O_2S_2$ requires: C, 38.80; H, 2.03; N, 14.14; found: C, 38.94; H, 2.25; N, 14.41.

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SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF 2-(3-FLUORO-PHENYL)-1-[1-(SUBSTITUTED-PHENYL)-1H-[1, 2, 3]-TRIAZOL-4-YL-METHYL)-1H-BENZO [D] IMIDAZOLE DERIVATIVES

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ABSTRACT

Herein, we report the synthesis of some novel 2-(3-fluoro-phenyl)-1-[1-(substituted-phenyl)-1H-[1,2,3]-triazol-4-yl-methyl)-1*H*-benzo[*d*]imidazole derivatives prepared by the alkylation of 2-(3-fluoro-phenyl)-1H-benz[*d*]imidazole with substituted 4-bromomethyl-1-(substituted-phenyl)-1H-[1,2,3]-triazole. The key step is the synthesis of 2-(3-fluoro-phenyl)-1H Benz [*d*] imidazole by the condensation of *o*-phenylenediamine with 3-fluoro benzaldehyde in toluene or xylene without using any catalysts. The synthesized compounds were evaluated initially for their antibacterial activity. One among three most potent derivatives is under further evaluation, showing better activity compared to rifampin.

KEYWORDS:

Antimycobacterial activity, Benzimidazole, *o*-phenylenediamine, [1,2,3]-triazole, Alkylation.

INTRODUCTION

After nitrogen, fluorine occupies the position of second favorite hetero-element in life science-oriented research. Over 10% of newly registered pharmaceutical drugs and some 40% of newly registered agrochemicals contain one or more fluorine atoms[1]. Fluorine containing benzimidazoles, which are showing biological activity, are well documented in the literature. Some of these are like Astemizole (antiallergic, anti-histaminic), Lansoprazole (anti-ulcerative), Flubendazole (Anthelmintic), Droperidol (anti-psychotic) etc[2]. Benzimidazole-containing structure stems are the recent pharmacophore of interest, because they display significant activity against several viruses such as HIV, herpes (HSV-1), RNA_3 influenza and human cytomegalovirus (HCMV)[3-4].

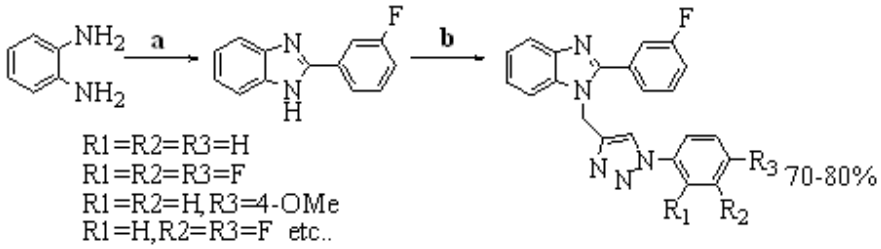
In addition, benzimidazoles have been used as topoisomerase inhibitors, selective neuropeptide YY1 receptor antagonist, 5-HT_3 antagonists in isolated pig ileum, antitumor agents, smooth muscle cell proliferation inhibitors, treatment for intestinal cystitis, as factor Xa inhibitors derivatives[5], antiviral, anti-ulcer, antihypertension, antiulcer[6], antibacterial and antifungal[7], human and veterinary anthelmintic[8], cardiotonic[9] and in

diverse areas of chemistry like ligands for asymmetric catalysis[10].

In light of the affinity, they display towards a variety of enzymes and protein receptors, medicinal chemists would certainly classify them as "privileged sub-structures" for drug design[11]. In recent years, some solid-phase synthetic methods are explored in the synthesis of benzimidazole derivatives[13]. Recently, the condensation of **(6)** with **(7)** in the presence of transition metal triflate salts such as $Sc(OTf)_3$ or $Yb(OTf)_3$ were reported[14]. In addition to this, literature precedence reveals that the compound was also synthesized by the condensation of **(6)** with **(7)** in the presence of oxidizing agents like cupric acetate (Weidenhagen procedure) [15], mercuric oxide[16], chloronil[17], lead tetraacetate[18], manganese oxide[19], nitrobenzene[20], polyphosphoric acid[21], (bromodimethyl) sulphonium bromide[22], L-proline[23], $TiCl_4$ [24] have been used. Further, N-heterocyclic compounds are broadly distributed in nature including amino acids, purines, pyrimidines and many natural products. Triazoles, like many other five membered heterocyclic compounds are used very often in the pharmaceutical and medicinal applications. Several methods have been described for the synthesis of [1,2,3]-triazoles recently and especially the copper catalyzed addition of organo-azide to terminal alkynes has become a useful and widely applicable method for the synthesis of [1,2,3]-triazoles.[25] N-heterocyclic species having [1,2,3]-triazole ring system exhibits numerous example of activities in the literature including anti-HIV activity,[26] antimicrobial activity against gram positive bacteria,[27] inhibition of histidine biosynthesis ,[28] ß-selective adrenergic receptor activity agonist,[29] bacterial and medicinal fungicides of second generation,[30] anti-inflammatory agents.[31] In addition to this,[1,2,3]-triazoles have found broad applications in agrochemicals as fungicides and plant growth regulator as well as industrial applications in dyes, corrosion inhibition (of copper and copper alloys) and photostabilizers.[32]

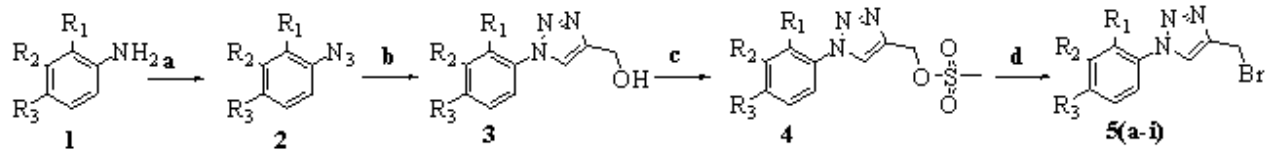
Owing to the immense importance and versatile bioactivities exhibited by benzimidazoles and [1,2,3]-triazole derivatives, it was thought worthwhile to generate a library of compounds with clubbed imidazo-triazoles. In continuation of our in-house library, we now disclose the synthesis and antimycobacterial activity of 2-(3-fluoro-phenyl)-1-[1-(substituted-phenyl)-1H-[1,2,3]-triazol-4-yl-methyl)-1*H*-benzo[*d*]imidazole derivatives.

Scheme 1



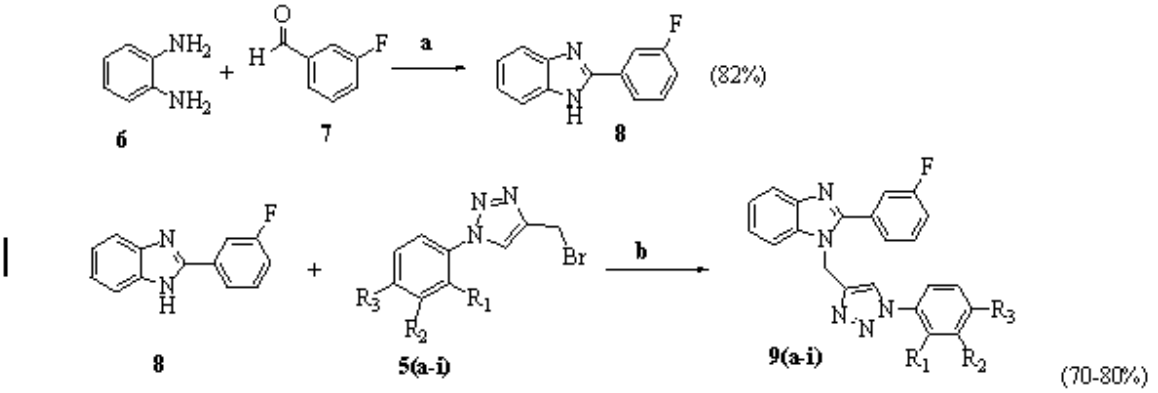
a: 3-Fluoro benzaldehyde, Toluene, 110^{O}C; **b**: NaH, Substituted [1,2,3]Triazole, DMF, RT

Scheme 2



Reagents & conditions: **a**: $NaNO_2$,HCl,H_2O,NaN_3; **b** :Propargyl alcohol,CuI,Acetonitrile;**c**:Mesyl chloride,Triethyl amine,dichloromethane;**d** :LiBr, Acetone

Scheme 3



Reagents and conditions: a: Toluene, 110^{O}C, 30-60 min.; b: NaH, 4-bromomethyl-1-phenyl-1H-[1,2,3]-triazole DMF, RT

RESULTS AND DISCUSSION

Chemistry

The synthesis of 4-bromomethyl-1-(substituted-phenyl)-1H-[1,2,3]-triazole is shown in *scheme 1*.The first step involves the diazotization of substituted aromatic amines **1** with sodium nitrite in hydrochloric acid and the resulting diazonium salt was reacted with sodium azide to give the azidobenzenes **2**[33]. Azido benzenes **2** was then treated with propargyl alcohol in the presence of copper iodide at room temperature to give (1- substituted-phenyl-1H-[1,2,3]triazo-4-yl)-methanol **3**[34], which was then converted into mesylate compound by using mesyl chloride and triethyl amine at room temperature **4**[35]. In the final step, the mesylate compound **4** was treated with lithium bromide in acetone gives 4-(bromomethyl)-1- substituted-phenyl-1H-[1,2,3]-triazole (**5a-i)**.

Condensation of *o*-phenylenediamine **6** with 3-fluoro benzaldehyde **7** in toluene or xylene at 110^{o}C for 1 hr by aerial oxidation, furnished the 2-(3-fluoro-phenyl)-1H benz [*d*]imidazole **8.** The reaction was carried out without using oxidizing agents or catalysts. The subsequent reaction of 3-fluoro benzimidazole **8** with substituted 4-(bromo methyl)-1- phenyl-1H-1,2,3- triazole (**5a-i**) using NaH as a base proceeded at room temperature to furnish 2-(3-fluoro-phenyl)-1-[1-(substituted-phenyl)-1H-[1,2,3]-triazol-4-ylmethyl)-1*H*-benzo[*d*]imidazole derivatives (**9a-i**).

BIOLOGICAL ACTIVITY: ANTIBACTERIAL ACTIVITY TEST

The synthesized [1,2,3]-triazole derivatives were evaluated for the antibacterial activity by the agar well -diffusion method (Bore method). One Gram positive strain (*Bacillus subtilis*) and one Gram negative strain (*Escherichia coli*) has been used. The test was performed according to the agar well- diffusion method (Bore method) adopted with some modifications for the synthesised compounds using Gentamycin as a reference standard. In this method, the wells were punched in the agar plates using a sterile stainless stell borer (6 mm diameter). The bacterial strains were maintained on the agar medium and the impregnated disk incubated to the incubator at $37^{O}C$ for 24 hrs. Dimethyl sulfoxide was used as a control. After 24 hrs, the sterile disks were impregnated with different compounds dissolved in dimethyl sulfoxide having 100 ul concentration of each compound for the three different concentrations (0.25%, 0.5% and 1.0%). To ensure that, the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO. The dilution medium for the positive controls was sterile distilled water. The impregnated disk incubated at $37^{o}C$ for 24 hrs for bacterial inhibition. The zone inhibition caused by the various compounds on the micro-organisms were examined and the respective diameters were measured in mm. The results of the preliminary screening test are listed in table 1.

Table 1
Antibacterial Activity of Compounds: (9a-9i)

Compounds	*Bacillis subtilis*			*Escherichia coli.*		
	Concentration used			Concentration used		
	0.25	0.50	1.0	0.25	0.5	1.0
Gentamycin	-	+++	NA	-	+++	-
3-F benzimidazole	-	++	NA	-	-	-
9a	+	+++	NA	-	-	-
9b	+	+++	NA	-	-	-
9c	+	+++	NA	-	-	-
9d	-	+++	NA	-	-	-
9e	+	+++	NA	-	-	-
9f	-	+++	NA	-	-	-
9g	+	+++	NA	-	-	-
9h	-	+++	NA	-	-	-
9i	-	+++	NA	-	-	-

Highly active = +++ (inhibition zone >12 mm), Moderately active = ++ (inhibition zone 9-12 mm), Slightly active = + (inhibition zone 6-9 mm), Inactive = - (inhibition zone < 6 mm), NA= Not analyzed

EXPERIMENTAL SECTION

Melting points were recorded by Veggo programmable (microprocessor based) melting point apparatus and are uncorrected. The TLC analysis was carried out using precoated silica gel plates and visualization was done by exposure to iodine and UV lamp. IR spectra were recorded using a FTIR bruker Vector 22 Spectrophotometer. ^{1}H NMR spectra recorded on Varian 400 MHz spectrometer. Elemental

analyses were performed on Elementor Vario instrument. EI-MS spectra recorded on micromass-quatro–II. All the raw materials, reagents and solvents used were of commercial grade only.

General experimental procedure for the preparation of 4-(bromomethyl)-1- (2,3,4-trifluorophenyl)-1H- [1,2,3]-triazole (5a-5i)

To a Solution of 2,3,4-trifluorobenzenamine **1a** (2.0g, 13.6 mmol) dissolved in 50 ml HCl:H_2O (1:1) was cooled at -5°C by ice-salt mixture. Then a solution of sodium nitrite (1.87g, 27.2 mmol) dissolved in water (15 mL) was added slowly at -5°C. After completion of addition, the reaction mixture was stirred at -5 °C for 60 min. Then the reaction mixture was neutralized with sodium acetate (22.3g, 272 mmol). Following this, a solution of NaN_3 (1.77g, 27.2 mmol) in water (15 mL) was added slowly over the period of 30 min by maintaining the temp at -5°C to 0°C. After stirring for 30 min, the solution was allowed to warm at room temperature. Extracted with ethyl acetate (100mL x 2), dried the organic layer over sodium sulphate and evaporated to yield 1-azido 2,3,4-trifluoro benzene **2a** as an oily product (1.8 g).

In the second stage, 1-azido 2,3,4-trifluoro benzene **2a** (1.8 g, 10.4mmol) was dissolved in acetonitrile (25 mL). Propargyl alcohol (1.16 g, 20.8 mmol) and copper iodide (0.39 g, 5.2 mmol) were added to the above reaction mixture. The reaction mixture was stirred at room temperature for 8-10 h, a solid material separated out, was filtered, suck dried. [1-(2,3,4-trifluorophenyl)-1H-1,2,3-triazol-4-yl] methanol **3a** (1.89 g) was obtained as off white solid.

In the penultimate stage, to a solution of [1-(2,3,4-trifluorophenyl)-1*H*-1,2,3-triazol-4-yl]-methanol (1.85 g, 8.07 mmol) dissolved in dichloromethane (20 mL) was added triethyl amine (1.22 g, 12.1 mmol) and mesyl chloride (1.1 g, 9.7 mmol) at room temperature. The reaction mixture stirred for 30 min and completion of reaction was monitored by TLC. Concentrate under vacuum and charged water, a solid material separates out, was filtered, suck dried. [1-(2,3,4-trifluorophenyl)-4-{methylsulphonyl}-methyl]-1*H*-1,2,3-triazole 4**a** (1.9 g) was obtained as off white solid.

In the final stage, to a solution of [1-(2,3,4-trifluorophenyl)-4-{methylsulphonyl}-methyl] -1*H*-1,2,3-triazole (1.9 g, 6.19 mmol) in acetone, charged lithium bromide (1.07 g,12.37 mmol) and then refluxed for 1-2 h. After completion of reaction (monitored on TLC), distilled out acetone completely invacuo. Charged water, a solid material separates out, was filtered, suck dried. 4-(bromomethyl)-1-(2,3,4-trifluorophenyl)-1H-[1,2,3]-triazole (1.5gm) **5a** was obtained as buff colored solid. Similarly, series of 4-(bromomethyl)-1-substituted-phenyl-1H-[1,2,3]-triazole **5a-5i** was synthesized by using the respective amine of **1a-1i.**

General experimental procedure for the synthesis of 2-(3-fluoro-phenyl)-1H benz[*d*]imidazole (8). (1.0 gm batch)

A mixture of 3-fluoro benzaldehyde (**7**) (9.25 mmol) and *o*-phenylenediamine (**6**) (9.25 mmol) in toluene (5mL) was refluxed for 1 h. The reaction mixture was then cooled 5 °C gradually, stirred for 15-20 min. The pale yellow solid material formed was filtered through buchner funnel and washed with ice-cold toluene. The obtained solid **8** was triturated with diethyl ether and filtered. The synthesized compound was characterized by MS, NMR. Yield- 73%; MS: m/z 213.2 (M^+); ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 7.4 (m, 2H, ArH), 7.21 (m, 2H, ArH), 7.12-7.16 (m, 2H, ArH), 5.65 (s, 1H, -CH_2),

General experimental procedure for the synthesis of 2-(3-fluoro-phenyl)-1-[1-(substituted-phenyl)-1H-[1,2,3]-triazol-4-yl-methyl)-1*H*-benzo[*d*] imidazole 9 (a-i)

To a suspension of sodium hydride (0.94 mmol) in dimethyl formamide was added 2-(3-fluorophenyl)-1H-benz[*d*] imidazole (**8**) (0.47 mmol) at room temperature and stirred the reaction mixture for 30 min. To this reaction mixture, substituted 4-bromomethyl-1H-[1,2,3]-triazole (**5a-i**) (0.52 mmol) was then added at room temperature and stirred for 30 min. After completion, the reaction mixture was quenched with ice water. The solid that separates was filtered and dried at RT (**9a-i**).

1. Synthesis of 2-(3-fluoro-phenyl)-1[1-(2,3,4-trifluoro-phenyl)-1H-[1,2,3]-triazol-4-yl-methyl]-1H-benz [*d*]imidazole (9a):

The compound was obtained using 4-bromomethyl-1-(2,3,4-trifluorol-phenyl)-4H-[1,2,3]-triazole (**5a)** as a buff colour crystalline solid **9a** . Yield 74%; mp: 178-180 ^{o}C; IR (KBr): 3064, 1591, 1521, 1046, 748 cm^{-1}.; ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 7.86 (s, 1H, CH-triazole ring), 7.82 (d, 1H, ArH), 7.67 (m, 3H, ArH), 7.51 (m, 2H, ArH), 7.30 (dd, 2H, ArH), 7.26 (m, 1H, ArH), 7.15 (dd, 1H, ArH,), 5.65 (s, 2H, CH2); MS: m/z 424.2 (M^+); Anal. Calcd. for $C_{22}H_{13}F_4N_5$: C, 62.41; H, 3.10; N, 16.54; found C, 62.43; H, 3.18; N, 16.66.

2. Synthesis of 1[1-(3,4-difluoro-phenyl)-1H-[1,2,3]-triazol-4-ylmethyl]-2-(3-fluoro-phenyl)-1H- benz [*d*] imidazole (9b):

The compound was obtained using 4-bromomethyl-1-(3,4-difluoro-phenyl)-4H-[1,2,3]-triazole (**5b)** as a faint brownish crystalline solid **9b.** Yield 75%; mp: 162-164 ^{o}C; IR (KBr): 3063, 1619, 1589, 1451, 1275, 892 cm^{-1}.; ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 7.86 (s, 1H, CH-triazole ring), 7.7 (m, 1H, ArH), 7.62(m, 3H, ArH), 7.51 (dd, 2H, ArH), 7.39 (m, 3H, ArH), 7.30 (dd, 2H, ArH), 5.66 (s, 2H, CH_2); MS: *m/z* 406.4 (M^+); Anal. Calcd. for: $C_{22}H_{14}F_3N_5$: C, 65.18; H, 3.48; N, 17.28; found C, 65.28; H, 3.57; N, 17.45.

3. Synthesis of 2-(3-fluoro-phenyl)-1-[1-(3-fluoro-phenyl)-1H-[1,2,3]-triazol-4- ylmethyl]-1H- benz [*d*] imidazole (9c):

The compound was obtained using 4-bromomethyl-1-(3-fluoro-phenyl)-4H-[1,2,3]-triazole (**5c)** as a brownish colour crystalline solid **9c.** Yield 79%; mp: 174-176 ^{o}C; IR (KBr): 3042, 2360, 1585, 1450, 1264, 874 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.95 (s, 1H, CH-triazole ring), 7.54 (m, 2H, ArH), 7.67 (m, 2H, ArH), 7.57 (dd, 2H, ArH), 7.40 (t, 2H, ArH), 7.29 (t, 1H, ArH), 7.19 (dd, 3H, ArH), 5.69(s, 2H, CH_2); MS: *m/z* 388.3 (M^+); Anal. Calcd. for: $C_{22}H_{15}F_2N_5$: C, 68.21; H, 3.90; N, 18.08; found C, 68.50; H, 3.95; N, 17.99.

4. Synthesis of 2-(3-fluoro-phenyl)-1[1-(2-methoxy-phenyl)-1H-[1,2,3]-triazol-4-yl methyl]-1H- benz [*d*] imidazole (9d):

The compound was obtained using 4-bromomethyl-1-(2-methoxy-phenyl)-4H-[1,2,3]-triazole **5d** as a off white crystalline solid **9d.** Yield 78%; mp: 160-162 ^{o}C; IR (KBr): 3042, 1614, 1590, 1449, 1220, 797 cm^{-1}.; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.2 (s, 1H, CH-triazole ring), 8.0 (m, 2H, ArH), 7.8 (m, 2H, ArH), 7.5 (t, 2H, ArH), 7.42 (m, 4H, ArH), 7.2 (dd, 2H, ArH), 5.69 (s, 2H, CH_2), 3.8(S, 3H,-OCH_3); MS: *m/z* 400.2 (M^+); Anal. Calcd. for: $C_{23}H_{18}FN_5O$: C, 69.16; H, 4.54; N, 17.53; found C, 69.22; H, 4.56; N, 17.54.

5. Synthesis of 2-(3-fluoro-phenyl)-1[1-(4-methoxy-phenyl)-1H-[1,2,3]triazol-4-yl methyl]-1H- benz [*d*] imidazole (9e):

The compound was **o**btained using 4-bromomethyl-1-(4-methoxy-phenyl)-4H-[1,2,3]-triazole **5e** as a buff coloured crystalline solid **9e.** Yield 71%; mp: 98-100 ^{o}C; IR (KBr): 3050, 1620, 1582, 1384, 1197, 797, 744 cm^{-1}.; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.9 (s, 1H, CH-triazole ring), 7.75 (m, 2H, ArH), 7.68 (dd, 2H, ArH), 7.52 (m, 3H, ArH), 7.40 (dd, 2H, ArH), 7.26 (d, 2H, ArH), 7.2 (d, 1H, ArH), 5.62 (s, 2H, CH2), 3.8(S, 3H, -OCH_3); MS: *m/z* 400.3 (M^+); Anal. Calcd. for: $C_{23}H_{18}FN_5O$: C, 69.16; H, 4.54; N, 17.53; found C, 69.39; H, 4.61; N 17.68.

6. Synthesis of 1[1-(2-fluoro- 4-methyl-phenyl)-1H-[1,2,3]-triazol-4-ylmethyl]-2-(3-fluoro-phenyl)-1H-benz [*d*] imidazole (9f):

The compound was obtained using 4-bromomethyl-1-(2-fluoro-4-methyl-phenyl)-4H-[1,2,3]-triazole **5f** as a pale yellow crystalline solid **9f** . Yield 72%; mp: 154-156 ^{o}C; IR (KBr): 3060, 1619, 1518, 1469, 1284, 1010, 780 cm^{-1}.; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.91 (s, 1H, CH-triazole ring), 7.80 (d, 1H), 7.72 (t, 2H), 7.55 (dd, 2H), 7.40 (m, 3H), 7.32 (m, 2H), 7.2 (d, 1H), 5.65 (s, 2H, CH_2), 2.4 (S, 3H, CH_3); MS: *m/z* 402.3 $(M)^+$; Anal. Calcd. for: $C_{23}H_{17}F_2N_5$: C, 68.82; H, 4.27; N, 17.45; found C, 68.79; H, 4.13; N, 17.22.

7. Synthesis of 1[1-(2,4-difluoro-phenyl)-1H-[1,2,3]-triazol-4-ylmethyl]-2-(3-fluoro-phenyl)-1H-benz[*d*]imidazole (9g):

The compound was obtained using 4-bromomethyl-1-(2,4-difluoro-phenyl)-4H-[1,2,3]-triazole (**5g)** (0.142 g, 0.52 mmol) as a buff coloured crystalline solid **9g** . Yield 71%; mp: 112-114 ^{o}C; IR (KBr): 3066, 1618, 1520, 1393, 1146, 795 cm^{-1}.; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.95 (s, 1H, CH- triazole ring), 7.85 (m, 2H, ArH), 7.7 (t, 2H, ArH), 7.5 (m, 2H, ArH), 7.40 (dd, 2H, ArH), 7.32 (m, 2H, ArH), 7.12 (dd, 1H, ArH), 5.62 (s, 2H, CH2); MS: *m/z* 406.0 $(M)^+$; Anal. Calcd. for: $C_{22}H_{14}F_3N_5$: C, 65.18; H, 3.48; N, 17.28; found C, 65.22; H, 3.49; N, 17.31.

8. Synthesis of 2-(3-fluoro-phenyl)-1[1-phenyl-1H-[1,2,3]-triazol-4-ylmethyl]-1H- benz[*d*]imidazole (9h):

The compound was obtained using 4-bromomethyl-1-(1-phenyl)-4H-[1,2,3]-triazole (**5h)** (0.123 g, 0.52 mmol) as a off white crystalline solid **9h.** Yield 74%; mp: 132-134 ^{o}C; IR (KBr): 3052, 1622, 1516, 1378, 1042, 1127, 795 cm^{-1}.; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.95 (s, 1H, CH-triazole ring), 7.6-7.8 (m, 6H, ArH), 7.52 (m, 2H, ArH), 7.47 (t, 2H, ArH), 7.30 (m, 2H, ArH), 6.93 (d, 1H, ArH), 5.62 (s, 2H, CH_2); MS: *m/z* 370.2 $(M)^+$; Anal. Calcd. for: $C_{22}H_{16}FN_5$: C, 71.53; H, 4.37; N, 18.96; found C, 71.41; H, 4.45; N, 18.99.

9. Synthesis of 2-(3-fluoro-phenyl)-1[1-(4-trifluoromethyl-phenyl)-1H-[1,2,3]- triazol-4yl-methyl]-1H-benz[*d*]imidazole (9i):

The compound was obtained using 4-bromomethyl-1-(4-trifluoromethyl-phenyl)-4H-[1,2,3]- triazole (**5i)** as a pale yellow crystalline solid **9i.** Yield 74%; mp: 182-184 ^{o}C; IR (KBr): 3066, 1618, 1520, 1393, 1146, 795 cm^{-1}.; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.95 (s, 1H, CH-triazole ring), 7.9 (dd, 1H, ArH), 7.78-7.84 (m, 2H, ArH), 7.70 (m, 2H, ArH), 7.59 (m, 2H, ArH), 7.55 (m, 2H, ArH), 7.42 (m, 2H, ArH), 7.28 (dd, 1H, ArH), 5.40 (s, 2H, CH2); MS: *m/z* 438.2 $(M)^+$; Anal. Calcd. for $C_{23}H_{15}F_4N_5$: C, 63.16; H, 3.46; N, 16.01; found C, 63.22; H, 3.472; N, 16.11.

CONCLUSION

We have developed an efficient methodology for the synthesis of a 2-(3-fluoro-phenyl)-1-[1-(substituted-phenyl)-1H-[1,2,3]-triazol-4-yl-methyl)-1*H*-benzo[*d*]imidazole derivatives and maximum number of compounds were found to be highly active against gram positive bacteria (*B. subtilis*). However, none of them showed any promising activity against gram negative bacteria (*E. coli*). The significant gram positive (*B. subtilis*) activity of the compounds may be due to the presence of [1,2,3]-triazole ring attached to the benzimidazole, as observed in the preliminary biological screening.

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Pd (OAc)$_2$/1,10-phenanthroline catalyzed one-pot synthesis of 5-Substituted-1H-tetrazoles from the aryl halides via 1,3-Dipolar cycloaddition

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ABSTRACT

This paper presents an efficient method for the cyanation of aryl halides using palladium acetate (Pd (OAc) $_2$) as a catalyst and 1, 10-phenanthroline as a nitrogen ligand and the formed benzonitriles were converted to 5-substituted-1*H*-tetrazoles under one-pot operation. A variety of aryl halides underwent cyanation with $K_4[Fe(CN)_6]$, followed by addition of NaN_3 gave the products in good yields. Broad availability of substrates and the high tolerance of palladium catalysts against a variety of functional groups is the advantage of this method. Therefore, this route has become a useful tool for the synthesis of 5-substituted-1*H*-tetrazoles.

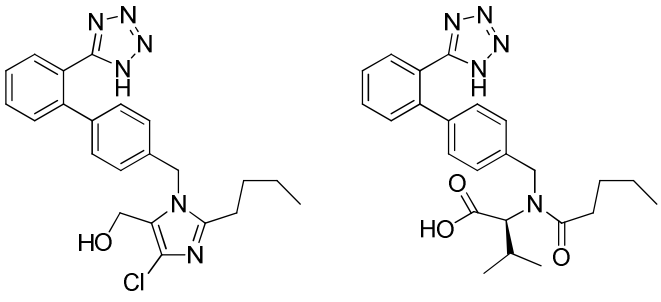
KEYWORDS

Palladium acetate, sodium azide, 1, 10-phenanthroline, 1H-tetrazoles

INTRODUCTION

Tetrazoles are a class of heterocycles with a wide range of applications that are receiving considerable attention.[1] This functional group has a role in coordination chemistry[2] as well as in various materials science applications, including photography and specialty explosives.[3] Moreover, extensive work has been carried out in the field of **(Figure-1)**.

medicinal chemistry.[4] Tetrazoles are frequently used as metabolically stable surrogates for carboxylic acids, as the tetrazoles generally offer a more favourable pharmacokinetic profile.[5] Driven in particular by the widespread incorporation of the tetrazole functionality into angiotensin II antagonist structures such as losartan and valsartan



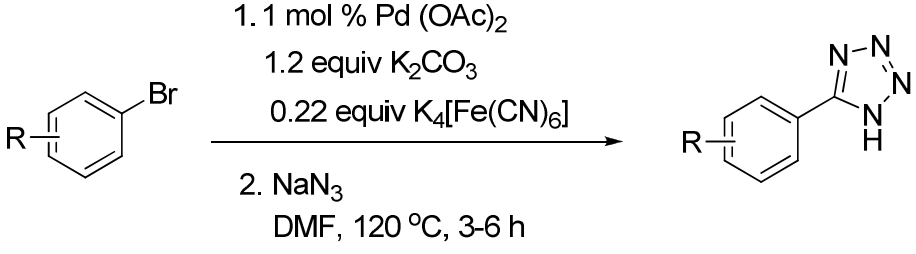
Losartan Valsartan

Figure-1

Benzonitriles are of considerable interest for organic chemistry as an integral part of dyes, herbicides, agrochemicals, pharmaceuticals, and natural products. The nitrile group also serves as an important intermediate structure for a multitude of possible transformations into other functional groups such as tetrazoles. These benzonitriles can be prepared in numerous ways.[6] Most often they are synthesized by the Rosenmund von Braun reaction[7] from aryl halides or diazotization of anilines and subsequent Sandmeyer reaction[8] on a laboratory as well as on an industrial scale. A drawback of the Rosenmund-von Braun and the Sandmeyer reactions is the use of stoichiometric amounts of copper (I) cyanide as cyanating agent, which leads to equimolar amounts of heavy metal waste. A useful alternative for the preparation of substituted benzonitriles is the transition-metal-catalyzed cyanation of aryl-X compounds (X = Cl, Br, I, OTf etc.) with cheap and readily available cyanation agents like sodium or potassium cyanide.

Potassium ferrocyanide(II) (K4[Fe(CN)6]) recently rediscovered[9] as a cyanide source by Beller, is particularly intriguing because all six CN are available for reaction and it is inexpensive, easily handled, and nontoxic.[10]

In general, the most direct and versatile method of the synthesis of 5-substituted-1*H*-tetrazoles is [2+3] the cycloaddition between nitriles and azides. A plethora of synthetic protocols and variations on this general theme have been reported in the literature during the past few years.[11] In the majority of cases, sodium azide (NaN_3) has been used as an inorganic azide source in combination with an ammonium halide as the additive employing dipolar aprotic solvents. In some instances, the use of Brønsted or Lewis acids, or stoichiometric amounts of Zn (II) salts have been reported as suitable additives to afford the desired azide–nitrile addition process.[12] In the present work, we report the one-pot synthesis of 5-substituted-1*H*-tetrazoles from aryl halides via 2+3 cycloaddition of benzonitriles with sodium azide in the presence of palladium as catalyst and 1,10-Phenanthroline as a ligand, where the benzonitriles were generated insitu from the aryl halides using $K_4[Fe(CN)_6]$ as shown in **Scheme 1.**



Scheme 1

MATERIAL AND METHODS

All chemicals were purchased from Sigma-Aldrich and S.D. Fine Chemicals, Pvt. Ltd. India and used as received. ACME silica gel (100–200 mesh) was used for column chromatography and TLC was performed on Merck precoated silica gel 60 F254 plates. All the other chemicals and solvents were obtained from commercial sources and purified using standard methods. The IR spectra of all compounds were recorded on a PerkinElmer, Spectrum GX FTIR spectrometer using KBr pellet method. The IR values are reported in reciprocal centimeters (cm–1). The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer. Chemical shifts (d) are reported in ppm, using TMS as an internal standard in $CDCl_3$ + DMSO-d_6, ESI mass spectra were recorded on a Finnigan LCQ Advantagemax spectrometer. EI mass spectra were recorded on a GC-MS QP2010 Plus (Shimadzu).

Typical experimental procedure for the one-pot synthesis of 5-substituted-1*H*-tetrazoles

A mixture of aryl halide (1 mmol), $K_4[Fe(CN)_6]$ (0.22 equiv), K_2CO_3 (1.2 mmol), palladium acetate (1 mol %) and 1,10-phenanthroline (4 mol %) in DMF (3 mL) was stirred at 120 ^{o}C, and the reaction was monitored by TLC. After the formation of benzonitriles by complete consumption of bromoarenes, NaN_3 (2 equiv) was added and the reaction mixture was stirred at the same temperature. After completion of the reaction (as monitored by TLC), the reaction mixture was treated with ethyl acetate (30 mL) and 5 N HCl (20 mL). The resultant organic layer was separated and the aqueous layer was again extracted with ethyl acetate (20 mL). The combined organic layers were washed with water, concentrated and the crude product was purified by column chromatography on silica gel using (hexane–ethyl acetate) to afford pure product. All products were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopic techniques.

5-Phenyl-1H-tetrazole (Table 1, entry 1):

IR (neat): 3489, 3128, 2919, 1852, 1606, 1562, 1461, 1407, 1161, 988, 725, 684 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 7.46 - 7.57 (m, 3H), 8.02 – 8.11 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6): δ. ESI MS (m/z): 146 (M^+): Anal. Calcd for $C_7H_6N_4$: C, 57.53; H, 4.14; N, 38.34. Found: C, 57.32; H, 4.08; N, 38.24 %.

5-(2-Naphthyl) -1H-tetrazole (Table 1, entry 2)

IR (neat): 3422, 2924, 2855, 1821, 1505, 1376, 1047, 766 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 7.49 – 7.67 (m, 3H), 7.82 – 8.09 (m, 2H), 8.65 – 8.82 (m, 1H); ESI MS (m/z): 197 (M^+): Anal. Calcd for $C_{11}H_8N_4$: C, 67.34; H, 4.11; N, 28.55. Found: C, 67.30; H, 4.02; N, 28.32 %.

5-(4-Methoxyphenyl) -1H-tetrazole (Table 1, entry 3)

IR (neat): 3445, 3078, 2972, 2854, 1613, 1503, 1443, 1263, 1024, 834, 747, 609, 519 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO- d_6): δ 3.80 (s,3H) 7.00 (d, 2H, J = 8.7 Hz), 8.00(d, 2H, J = 8.7 Hz); ESI MS (m/z): 177 (M^+): Anal. Calcd for $C_8H_8N_40$: C, 54.54; H, 4.58; N, 31.80. Found: C, 54.38; H, 4.14; N, 31.48 %.

5-(3-Methoxyphenyl)-1H-tetrazole (Table 1, entry 4)

IR (neat):3446, 3065, 2921, 1862, 1590, 1488, 1246, 1018, 745, 680 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO- d_6): δ 3.88 (s, 3H,), 7.01 (dd, 1H J =1.9, 7.5 Hz), 7.40 (t, 1H, J = 8.1 Hz), 7.59 – 7.68 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO- d_6): δ 54.7, 111.5, 116.4, 118.7, 124.8,129.6, 155.1, 159.2: ESI MS (m/z): 177 (M^+): Anal. Calcd for $C_8H_8N_40$: C, 54.54; H, 4.58; N, 31.80. Found: C, 54.40; H, 4.20; N, 31.68 %.

5-(4-Tolyl) -1H-tetrazole (Table 1, entry 5)

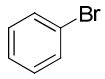
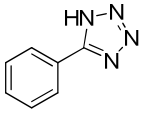
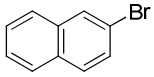
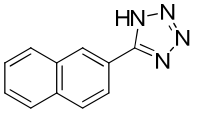
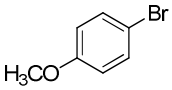
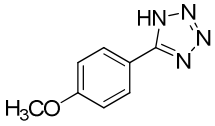
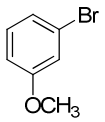
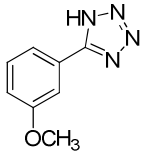
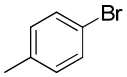
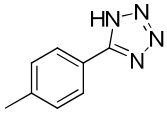
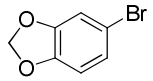
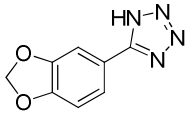
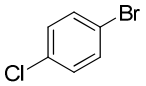
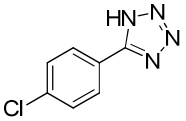
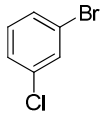
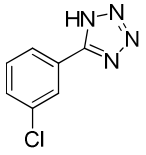
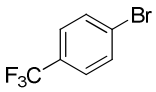
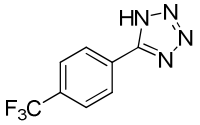
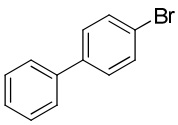
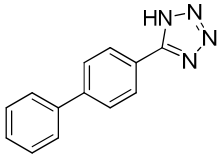
IR (neat): 3446, 3045, 2920, 2851, 1878, 1614, 1501, 1433, 1161, 1052, 988, 821, 740, 503 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO- d_6): δ 2.44 (s, 3H), 7.31 (d, 2H, J = 7.9 Hz), 7.94 (d, 2H, J = 7.9 Hz): ESI MS (m/z): 161 (M^+): Anal. Calcd for $C_8H_8N_4$: C, 59.99; H, 5.03; N, 34.98. Found: C, 59.78; H, 4.98; N, 34.88 %.

5-(Benzo[1,3]dioxol-5yl) -1H-tetrazole (Table 1, entry 6)
IR (neat): 3448, 3065, 2895, 1852, 1576, 1459, 1254, 1043, 925, 818, 745 cm^{-1} ^1H NMR (300 MHz, $CDCl_3$ + DMSO- d_6): δ 6.07 (s, 2H), 6.92 (d, 1H, *J* = 8.1 Hz), 7.54(dd, 1H, *J* = 1.5Hz), 7.66 (dd, 1H, *J* = 8.1, 1.5Hz). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO- d_6): δ 100.9, 106.5, 108.0, 117.3, 121.0, 147.5, 149.0; ESI MS (m/z): 191 (M^+): Anal. Calcd for $C_8H_6N_40_2$: C, 50.53; H, 3.18; N, 29.46. Found: C, 50.45; H, 3.10; N, 29.42 %.
5-(4-Chlorophenyl)-1H-tetrazole (Table 1, entry 7)
IR (neat): 3448, 3068, 2920, 1913, 1606, 1565, 1484, 1431, 1053, 986, 831, 739, 504 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$ + DMSO- d_6): δ 7.49 (d, 2H, *J* = 8.7 Hz), 8.06 (d, 2H, *J* = 8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$ +DMSO- d_6): δ 122.6, 127.6, 128.4, 135.7, 154.7; ESI MS (m/z): 181 (M^+): Anal. Calcd for $C_7H_5ClN_4$: C, 46.55; H, 2.79; N, 31.02. Found: C, 46.48; H, 2.68; N, 29.95 %
5-(3-Chlorophenyl)-1H-tetrazole (Table 1, entry 8)
IR (neat): 3454, 2916, 1977, 1560, 1471, 1091, 772, 541 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$ + DMSO- d_6): δ 7.41-7.56 (m, 2Hz), 7.95 - 8.06 (m, 1H), 8.10 (s, 1H); ESI MS (m/z): 181 (M^+): Anal. Calcd for $C_7H_5ClN_4$: C, 46.55; H, 2.79; N, 31.02. Found: C, 46.50; H, 2.70; N, 29.98 %.
5-(4-(Trifluoromethyl)phenyl)-1H-tetrazole (Table 1, entry 9)
IR (neat): 3448, 3073, 2926, 1904, 1574, 1441, 1330, 1131, 1064, 985, 845, 697 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$ + DMSO- d_6): δ 7.44 (dd, 1H, *J* = 7.7, 7.9 Hz), 7.64 (d, 1H, *J* = 7.9 Hz). ESI MS (m/z): 215 (M^+): Anal. Calcd for $C_8H_5F_3N_4$: C, 44.87; H, 2.35; N, 26.16. Found: C, 44.80; H, 2.28; N, 26.06 %.
5-(4-(Phenyl)-phenyl) -1H-tetrazole (Table 1, entry 10)
IR (neat): 3014, 2857, 1657, 1563, 1435, 1277, 1156, 1054, 926, 865, 696, 657 cm^{-1}.^1H NMR (300 MHz, $CDCl_3$ + DMSO- d_6): δ 7.52 (dd, 2H *J* = 7.4 Hz), 7.63 (t, 1H, *J* = 7.4 Hz), 7.78 (dd, 2H, *J* = 7.2, 8.3 Hz), 7.91(d, 2H, *J* = 8.3 Hz), 8.24 (d, 2H, *J* = 8.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO- d_6): δ 126.2, 127.6, 129.0, 129.7, 132.0, 136.1, 138.4, 194.7; ESI MS (m/z): 223 (M^+): Anal. Calcd for $C_{13}H_{10}N_4$: C, 70.26; H, 4.54; N, 25.21. Found: C, 70.22; H, 4.46; N, 25.19 %.

RESULTS AND DISCUSSIONS

Initially, the cyanation of bromobenzene was examined with $K_4[Fe(CN)_6]$ as a model system the efficiency of different bases in combination with various solvents were tested in the presence of 2 mol % of $Pd(OAc)_2$, 4 mol % of 1,10-phenanthroline at 120 ^oC. The solvent has a pronounced effect in these reactions, the solvents tested includes ,DMSO, DMF, DMA, toluene and out of which DMF has been proven to the best solvent. Among the various bases screened such as K_3PO_4, Na_2CO_3, K_2CO_3, KO*t*-Bu, NaOH, Cs_2CO_3, the relatively weak and inexpensive gave the best result. After completion of the reaction, without isolation of the intermediate benzonitriles, was treated with NaN_3 to furnish 5-substituted-1*H*-tetrazoles via [2+3] cycloaddition.
Under the optimized reaction conditions, we chose a variety of structurally divergent aryl halides to convert in to benzonitriles followed by 5-substituted 1*H*-tetrazoles to understand the scope and the generality of the $Pd(OAc)_2$/1,10-phenanthroline catalyzed cyanation and [2+3] cycloaddition. As exemplified in **Table 1**, the reaction proceeds smoothly to completion and the products were obtained in good yields. Unsubstituted bromoarenes such as bromobenzene and 2-bromo naphthalene reacted well to give the desired products in good yield (Table 1, entries 1 and 2). Encouraged with these results both electron withdrawing and electron donating groups substituted bromoarenes were tested to expand the scope of reaction. Among the different bromoarenes screened *p*-methyl bromobenzene is more reactive and gave the product in good yield when compared to methoxy and dioxole substituted bromoarenes (Table 1, entries 3-6).

Table 1: One-pot synthesis of 5-substituted-1*H*-tetrazoles from aryl halides via cyanation followed by [2+3] cycloaddition with sodium azide using palladium acetate as a catalyst, 1,10-phenanthroline as a ligand.[a]

Entry	Substrate	Product	Time (h)	Yield (%)
1			4	90
2			4	87
3			6	80
4			6	80
5			6	85
6			6	75
7			4	80
8			4	80
9			3	90
10			4	82

[a]Reaction conditions: aryl halide (1 mmol), $K_4[Fe(CN)_6]$ (0.22 equiv), K_2CO_3 (1.2 mmol), NaN_3 (2 mmol), $Pd(OAc)_2$ (1 mol %), 1,10-phenanthroline (4 mol %), DMF (3 mL) stirred at 120 ^oC.
Apart from these chloro, trifluoromethyl substituted bromoarenes were undergoing reaction smoothly to give products in excellent yields (Table 1, entries 7-9). Furthermore, 4-phenyl substituted bromobenzene also underwent both Cyanation and cycloaddition reactions well to give product in good yield (Table 1, entry10).

CONCLUSIONS

In conclusion, we have developed an efficient route for the one-pot synthesis of 5-substituted-1*H*-tetrazoles from aryl halides through palladium catalyzed Cyanation followed by [2+] cycloaddition. This conceptually new approach provides a straightforward and efficient access to 5-substituted-1*H*-tetrazoles. In addition, the reaction can be carried out without isolating the nitrle intermediate and should prove to be useful for generating multivalent structures.

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Essentials of Process Chemistry for Synthetic Chemists- A Need

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Ever expanding Pharmaceutical and Chemical Industries require proportionately a large number of Manufacturing Chemists and R&D Chemists. Since no industry can afford to recruit multiple numbers of graduates in Chemical Engineering, it looks towards graduates in Chemistry preferably, those who can readily take up the assigned work. Then, the question comes, which kind of Chemistry graduates are almost ready made? Immediately, the answer is, Chemistry graduates with knowledge in Process Chemistry and such graduates can become most successful manufacturing chemists and as well as research chemists in the Pharmaceutical/Chemical industry and attached departments of R&D. Such Chemistry graduates, definitely have a bright chance in the Pharmaceutical/Chemical industry and readily employable. Then, the second question comes which kind of Chemist will be suitable to acquire the knowledge of Process Chemistry?

The answer is very simple. The Chemistry Graduates with a strong background in theoretical principles and "good hands" at bench-work (in a chemical laboratory) are quite suitable to acquire some knowledge of Process Chemistry. A Synthetic Organic Chemist, more specifically must have the knowledge of the following to be a process chemist/manufacturing chemist:

Unit Processes: Oxidation, reduction, hydrogenation, hydrolysis, hydration, dehydration, halogenations, nitrification or nitration, sulfonation, amination, ammoniation, alkaline fusion, alkylation, dealkylation, esterfication, saponification, polymerisation, polycondensation and catalysis. Similarly knowledge in:

Unit Operations: Agitation (mixing), heat flow & fluid flow, distillation, filtration, drying, evaporation, crystallization, leaching, extraction, absorption, adsorption, condensation, vaporization, separation, sedimentation and crushing.

'*Process Chemistry'* is essentially based on chemical principles dealing with chemical reactions but with the intermingled and well-co-ordinated applications of Chemical Engineering.

Any Chemical Process, having established fully well the most suitable and favourable conditions at laboratory level (milligram to gram) and having optimized and standardised the experimental procedure, is fit to go to the Pilot Plant level (kilograms). And here from the process chemistry comes into existence. Having established optimum conditions for the process to go smoothly, taking all the pros and cons into account, a green signal will be given to transfer the process along with its detailed data sheet and standardised procedure specifying quantity and quality of reactants, reagents, solvents and catalysts, order of their introduction into reaction vessel, temperature, pressure, time etc.,

It is important to provide a detailed work-up procedure, since a given reaction under given conditions may yield different quantities of the product, simply because of the variation in work-up of the reaction mixture. The process should not use too high or two low temperatures and pressure.

The expertise of chemists is essential in choosing most appropriate starting materials, reagents, solvents and catalysts. They should be relatively cheaper but at the same time should have greater efficiency, safety and easy to handle. Particularly, the solvents and catalysts should be highly recoverable and recyclable for economy of the process.

Having established the process positively at '*Pilot Plant'*, it is quite ready to go to the manufacturing plant. Here with the true process chemistry comes into operation, fully.

A chemical process hither to conducted in reaction flasks, reaction vessels or reaction kettles, now requires '**Reactors.**'

Chemical Reactors are designed to contain chemical reactions. It is the site of conversion of raw materials into products. The design of a chemical reactor where bulk drugs will be synthesized on a commercial scale would depend on multiple aspects of chemical engineering. Since it is a very vital step in the overall design

of a process, designers ensure that the reaction proceeds with the highest efficiency towards the designed product output producing highest yield of product in the most cost efficient way.

Reactors are designed based on features like mode of operation or types of phases present or the geometry of reactors.

They are thus called:

- Batch or Continuous Reactors depending upon the mode of operation
- Homogenous or Heterogeneous reactors depending upon the phases present

The reactors may also be classified as:

- Stirred Tank reactors, or
- Tubular Reactors, or
- Packed Bed Reactors, or
- Fluidized Bed Reactors,

depending upon the flow pattern and manner in which the phases make contact with each other.

Batch Process: A process in which all the reactants are added together at the beginning or the process and products removed at termination of reaction is called a 'Batch Process'. In this process, all the reagents are added at the commencement and no addition or withdrawal is made while the reaction is proceeding or progressing. Batch processes are suitable for small production and for processes where a range of different products or grades is to be produced in the same equipment.

Continuous Process: A process in which the reactants are fed to the reactor and the products or by-products are withdrawn in between while the reaction is in progress. Continuous production will normally lead to lower production costs as compared to batch production. Continuous Reactors are usually preferred for large scale production.

Semi Batch Process: A process that do not fit into either of the above but it combines both. This is operated with both continuous and batch and inputs and outputs can be in between the progress of the reaction. A semi batch process can also be one which is interrupted periodically for some specific purpose for example, regeneration of catalyst or for removal of a gas which may have to be done, continuously.

Catalytic Processes: Most of the chemical reactions either proceed in the presence of a catalyst, or to increase their yield in the presence of catalyst. Hence, the behaviour of the catalyst is also as consideration, particularly in high temperature reactions.

Homogenous Reactions: Homogeneous reactions are those in which the reactants, products and any catalyst used for one continuous phase; for example gaseous or liquid. Homogeneous gas phase reactions will always are operated continuously. Tubular (Pipeline) reactors are normally used for homogeneous gas phase reactions. Homogenous liquid phase reactors may be batch or continuous. Batch reactions of single or miscible liquids are almost invariably done in stirred or pump around tanks. The agitation is needed to mix multiple feeds at the stand and to enhance heat exchange or heating media during the process

Heterogeneous Reactions: In a heterogeneous reaction, two or more phases exist and the overloading problems in the reactor design are to promote mass transfer between the phases.

Liquid-Liquid reactions industrial importances are numerous. Mechanically agitated tanks are favoured because the interfacial area can be made large as much as 100 times that of spray towers. When agitation is sufficient to produce a homogeneous dispersion and the rate varies with further increase of agitation, mass transfer rates are likely to be significant.

Liquid-Solid reactions: The solid may be a reactant or catalyst – continuous processing are in commercial use. Most solid catalysts employ fixed beds, although fluidized beds have the merit of nearly uniform temperature and can be distinguished for continuous regeneration, they cost more and more difficult to operate, and suffer from back mixing.

Liquid-Solid – Gas reactions: In reactions involving gas liquid and solid phases, the solid phase is generally a porous catalyst. It may be a fixed bed or it may be suspended in fluid mixture. In general, the reaction occurs either in the liquid phase or at the liquid/solid interface.

Solid – Solid reactions: Many reactions of solids are industrially feasible only at elevated temperatures which are often obtained by contact with combustion gases, particularly when the reaction is done on a large scale, usually, thermal and mass transfer resistances are major factors in the performance of solid reactions.

Gas-Solid reactions: In some reactions, the solid either takes part in the reaction or act as a catalyst. For example, finely divided Nickel (Ni) is used in the preparation of Nickel carbonyl.

Gas- Liquid reactions: In certain process, Liquid may either take part in the reaction or act as a catalyst, for example, in the manufacture of Adipic acid etc.

Reactor Geometry & Reactor Designs:

Stirred Tank Reactors: Consist of a tank fitted with a mechanical agitator and a cooling jacket or coils. They are operated as batch reactors or continuous reactors. Several reactors may be used in series, it can be considered as a basic chemical reactor.

Tubular reactors: used generally for gaseous reactions for some liquid phase reactions, when a high heat transfer is required small diameter tubes are used. Several tubes may be arranged in parallel, connected to a many fold. For high temperature reactions the tubes may be arranged in a furnace.

Packed Bed Reactors: There are two types, those in which the solid is a catalyst. In chemical industries mainly the industrial packed catalytic reactors are used. The diameter ranges from few centimetres, used for gas-gas and gas-liquid reactions.

Fluidized Bed Reactors: It is a combination of the two most common, packed and stirred tank, continuous flow reactors. It is very important to chemical industry because of its excellent heat and masses transfer characteristics. The essential feature of a fluidised bed reactor is that the solids are held in suspension by the upward flow of the reacting fluid. This promotes high mass and heat transfer rates and good mixing.

Fundamentals of Reactor Design:

The design of a chemical reactor deal with multiple aspects of chemical engineering; chemical reactions, chemical energetics and equations/laws of thermodynamics play an important role in the selection and design of chemical reactions

Chemical Reactions:

Brief representation of the chemical change in terms of symbols and formulae of the reactants and products is called a chemical equation

A chemical equation in which the number of atoms to each element is equal on the reactant side and product side is called a balanced chemical equation

A chemical equation, therefore, must fulfil the following conditions:

a) It should represent a true chemical change
b) It should be balanced
c) It should be molecular

A Chemical equation has both qualitative and quantitative significance.

Qualitatively a chemical equation tells the names of the various reactants and products

Quantitatively it expresses

i) the relative number of molecules of the reactants taking part in the reaction to produce the number of product molecules produced
ii) the relative number of moles of reactants and products
iii) the relative volumes of gaseous reactants and products

The chemical equation can be made more informative by incorporating the following:

a) physical states of reactants and products can be indicated by using the abbreviation e.g. (s) as solids, (l) as liquids, (g) as gases and (aq) as aqueous solution
b) In order to indicate the strength of acid or base, dil as dilute or conc, as concentration is written between the formula of acid or base
c) The reaction conditions such as presence of catalyst, temperatures, pressure etc. may be written above the arrow between the reactants and products
d) Heat change taking place during the reaction may be expressed in any one of the two ways: *$\Delta H = -X$ k.cal/mole (for exothermic reaction)

*ΔH= + X k.cal/mole (for endothermic reaction)

Enthalpy

* ΔH = change in enthalpy, that is heat evolved or absorbed in a reaction at constant temperature and pressure

Chemical equations give the quantitative relationship between the reactants and the products. This quantitative information can be utilized to carry out variety of calculations which are required many times, to assess the economic viability of the chemical process. Calculations based on the quantitative relationship between the reactants and the products are also referred to as "*Stoichiometry*"

Chemical energetics: chemical reactions are always associated with energy changes quite often, the energy change accompanying a chemical reaction is more significant than the reaction itself. The branch of science which deals with the energy changes associated with chemical reactions is called "Chemical Energetics". The energy changes may not always appear as heat energy, but also as electric energy, work energy, or radiant energy, as well. These energy changes are possible because during chemical reaction certain bonds are cleaved and certain bonds are formed. Energy is consumed during cleavage while energy is released during the formation of a new bond.

Thermodynamics: Since the bond energy varies from one bond to another, the chemical reactions are always accompanied by absorption or release of energy. Most of the time the energy is in the form of heat. Therefore, it becomes imperative that some concepts of thermodynamics may be stood. Thermodynamics literally means the conversion of heat into work and vice versa: because of them, refers to heat and dynamics refers to movement. Thermodynamics may therefore be defined as the branch of science which deals with the quantitative relationship between heat and other forms of energies. When it is applied to chemical reactions then it is referred to as "Chemical Thermodynamics"

Thermodynamics is primarily based upon three fundamental generalizations, popular as **Laws of Thermodynamics** they are:

First law of Thermodynamics: This deals with the equivalence of different forms of energies

Second law of Thermodynamics which deals with the direction of chemical change

Third law of Thermodynamics which helps to evaluate the thermodynamic parameter like "***Entropy***"

Therefore, the design of an Industrial chemical reactor must satisfy the following requirements:

1. **The chemical factors:** the kinetics of the reactions, the design must provide sufficient residence time for the desired reaction to proceed to the required degree of conversion
2. **The mass transfer factors** with heterogeneous reactions, the reaction rate may be controlled by the rates of diffusion of the reacting species, rather than the chemical kinetics
3. **The heat transfer factors:** the removal or addition of the heat of the reaction
4. **The safety factors**: the confinement of hazardous reactants and products and the control of the reaction and the process conditions
5. **Economic factors:** minimization of cost involved to purchase and operate; normal operating expenses include: energy input, energy removal, raw material costs, labour etc energy changes can come in the form of heating or cooling, pumping, agitation etc

A general procedure for reactor design:

- The kinetic and thermodynamic data on the desired reaction is initially collected, values will be needed for the rate of reaction over a range of operating conditions, for example, pressure, and

temperature and flow rate and catalyst concentration. This data may be normally obtained from either laboratory or pilot plant studies

- Data on physical properties is required for the design of the reactor
- The rate controlling mechanism which has a predominant role is then identified,for example: kinetic , mass or heat transfer
- A suitable reactor type is then chosen based on experience with similar studies or from the laboratory and pilot plant work
- Selection of optimal reaction conditions is initially made in order to obtain the desired yield
- The size of the reactor is decided and its performance is estimated
- Materials for the construction of the reactor is / are selected
- A preliminary mechanical design for the reactor including the vessel design, heat transfer, surfaces etc. is made
- The design is optimized and validated
- An approximate cost of the proposed and validated design is then calculated in choosing the reactor conditions, and optimizing the design, the interaction of the reactor design with the other process operations must not be overlooked, the degree of conversion of the raw materials in the reactor will determine the size and the cost of any equipment needed to separate and recycle unreacted materials. In these circumstances, the reactor and associated equipment must be optimized as a unit.

''For any manufacturing process selection of most appropriate and suitable chemical reactor is a must''

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SEMIEMPIRICAL STUDY OF 1,2-BIS(3-THIENYL)PERFLOUROCYCLOPENTENE DERIVATIVES

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ABSTRACT

Light-induced reversible behavior of 1,2–bis(3-thienyl)perfluorocyclopentenes containing various substituents on thiophene rings is examined theoretically using semi empirical PM3 (parametric method). A Linear relationship between experimentally observed λ_{max} values with that of theoretical data states that a low end semi empirical computational parametric method PM3 is sufficient to plan for the synthesis of new photochromic molecules.

KEYWORDS

1,2–bis(3-thienyl)perfluorocyclopentenes; Photochromism; heats of formation; PM3; NDDO

INTRODUCTION

During the past several decades, there have been important achievements in the synthesis of new families of organic photo chromic molecules[1]. The photochromic molecules have received greater attention not only because of their medicinal importance[2,3,4] but also due to their various potential applications as molecular level switches[5,6] optical memory display devices[7], photochromic fluorescent proteins[8,9]. Among these molecules, diarylethenes with heterocyclic aryl rings are regarded as the best candidates for switching devices because of their excellent thermal stability, remarkable fatigue resistance, ready detectability of both forms, no thermal inter conversions of the isomers in a large temperature range, and nondestructive read-out procedures and high coloration sensitivity[10]. This is exploited as a tool to synthesize fused aromatic and heterocyclic ring systems. These diaryl/diheteroarylethene derivatives can undergo UV light-induced photocyclization in solution and undergo cycloreversion upon irradiation with visible light.

The photo chromic process of diarylethenes is based on a reversible transformation between the hexatriene structure (open form) and cyclohexadiene structure the closed ring isomer as shown in Fig. 1. The open form has two conformations, anti parallel and parallel conformations, which exchange even at room temperature[11,12]. In general, upon irradiation with ultraviolet light, the open anti parallel diarylethene conformer undergoes photocyclization reaction to a closed ring isomer, if the distance between two reactive carbon atoms on the aryl moiety is within 0.42 nm[13]. While the the closed-ring isomer absorbs in the visible region the open form does not. Upon irradiation with visible light the colored closed-ring isomer returns to the initial colorless open form[14,15].

As theoretical calculations can give an insight into experimental results quantum-chemical methods have been increasingly used now-a-days, to guide

the synthesis of novel perfluorocyclopentenes. Generally highly sophisticated computations are performed to predict the properties of photochromic compounds. In this paper, we present a simple way to predict and plan the synthesis of a photochromic molecule.

COMPUTATIONAL METHODOLOGY

Semi-empirical methods belonging to NDDO (neglect of double differential overlap) approximation have been used to study perfluorocyclopentene derivatives. All the molecules were built using Hyperchem and their geometry optimization is performed. Molecular orbital calculations of perfluorocyclopentenes are computed using the reasonably accurate parametric model PM3 method[16-19]. Minimum-energy values and heats of formations of the optimized geometries have been computed. The molecular orbital energy diagrams are generated using Argus Lab Chemical Modeling Software 2004. The highest occupied molecular orbital HOMO, and the lowest unoccupied molecular orbital LUMO and (HOMO-LUMO) gap energies were also deduced for the stable structures. Electronic transition energies of the molecules are calculated from absorption maximum. Theoretically obtained data has been compared with experimental results[20-22].

RESULTS AND DISCUSSION

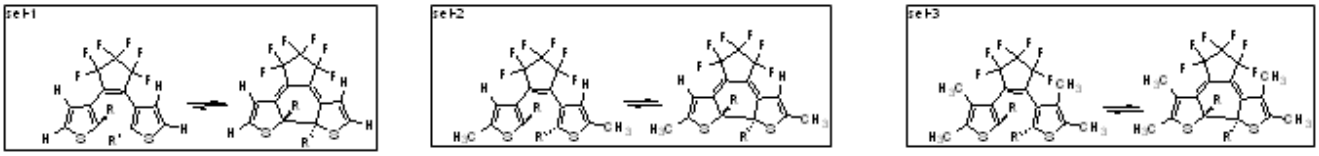


Figure 1
1,2-bis(2-unsubstituted/substituted-3-thienyl)perflourocyclopentene [1] (set-1),
1,2-bis(2-unsubstituted/substituted-5-methyl-3-thienyl)perflourocyclopentene [2] (set-2) and
1,2-bis(2-unsubstituted/substituted-4,5-dimethyl,3-thienyl)perflourocyclopentene [3] (set-3)

The molecules are grouped as three different sets viz., 1,2-bis(2-unsubstituted/substituted-3-thienyl)perflourocyclopentene [1] (set-1), 1,2-bis(2-unsubstituted/substituted-5-methyl-3-thienyl)perflourocyclopentene [2] (set-2) and 1,2-bis(2-unsubstituted/substituted-4,5-dimethyl,3-thienyl)perflourocyclopentene [3] (set-3) for clarity (Fig.1). The binding energies, dipole moments (μ), heats of formation ΔH_f, and log P values of the optimized geometries are displayed in Table Ia, Table Ib and Table Ic. The HOMO, LUMO and (HOMO-LUMO) gap energies were also calculated for the stable structures. The effects of the acceptor strength on the electronic properties, including the HOMO level, LUMO level, band gap and the maximal absorption wavelength (λ_{abs}) were also studied.

The series of study provides a basis for a comprehensive understanding of the effects of the electron donating and withdrawing groups on the geometric and electronic properties. The low negative heats of formation values (~ 260 kcal/mole) indicate that all the molecules are quite stable. The heats of formation values ΔH_f are comparable with that of ionic compounds. It is observed from Table I that open forms have relatively high heats of formation when compared to closed ring compounds. Derivatives with electron donating substituents have reasonably high values of ΔH_f than electron withdrawing substituents

.

Table 1a
The binding energies, dipole moments (μ), heats of formation ΔH_f, and log P values of [1] (set1).

Open form [1] (set 1)							Closed form [1](set 1)						
S.No	[1]-A	Binding energy kcal/mole	heat of formation kcal/mole	Wavelength (nm)	dipole moment (D)	LogP	S.No	[1] -B	Binding energy kcal/mole	heat of formation kcal/mole	Wavelength (nm)	dipole moment (D)	LogP
1	1A-2-H	-2985.91	-205.59	282.5	3.666	1.29	1	1B-2-H	-2989	-208.7	549.11	2.23	1.95
2	2A-2-ME	-3550.57	-220.061	242.94	3.546	1.34	2	2B-2-ME	-3539.63	-209.12	555.39	2.24	2.1
3	3A-2-ipr	-4667.35	-236.46	262.9	3.513	3.14	3	3B-2-ipr	-4644.29	-213.25	557.7	2.39	3.85
4	4A-2-OH	-3178.94	-279.502	287.21	5.416	2.62	4	4B-2-OH	-3169.02	-269.58	577.46	1.84	2.75
5	5A-2-OCH3	-3715.34	-265.71	295.8	5.635	2.69	5	5B-2-OCH3	-3701.9	-252.27	575.27	2.82	3.31
6	6A-2-Cl	-2942.43	-208.33	282.23	3.154	3.57	6	6B-2-Cl	-2931.78	-197.68	558.7	2.23	4.24
7	7A-2-NH2	-3314.77	-204.246	303.39	2.907	1.24	7	7B-2-NH2	-3300.47	-189.97	539.45	3.28	2.06
8	8A-2-CN	-3368.7	-124.804	296.64	3.526	0.92	8	8B-2-CN	-3355.25	-111.35	515.74	1.62	1.67
9	9A-2-NO2	-3345.37	-205.019	284.12	4.3	3.04	9	9B-2-NO2	-3309.26	-168.91	517.7	1.34	3.86
10	10A-2-Ph	-5396.44	-148.617	263.83	3.434	4.21	10	10B-2-Ph	-5369.87	-122.05	540.7	3.15	4.97

Table 1b
The binding energies, dipole moments (μ), heats of formation ΔH_f, and log P values of [2] (set 2).

Open form [2] (set 2)							Closed form [2] (set 2)						
S.No	[2]	Binding energy kcal/mole	heat of formation kcal/mole	Wavelength (nm)	dipole moment (D)	LogP	S.No	[2]	Binding energy kcal/mole	heat of ormation kcal/mole	Wavelength (nm)	dipole moment (D)	LogP
11	1A-2-H	-3550.44	-219.93	267.11	3.87	1.34	**11**	1B-2-H	-3550.44	-219.93	551.77	3.23	2.00
12	2A-2-ME	-4115.90	-235.20	267.30	3.78	1.40	12	2B-2-ME	-4100.89	-220.19	559.62	3.21	2.16
13	3A-2-ipr	-5232.60	-251.52	264.78	3.73	3.14	13	3B-2-ipr	-5204.75	-223.68	587.45	3.37	3.85
14	4A-2-OH	-3743.97	-296.34	300.59	5.60	2.68	14	4B-2-OH	-3732.02	-282.39	528.05	3.81	2.81
15	5A-2-OCH3	-4280.27	-280.45	302.22	5.78	2.74	15	5B-2-OCH3	-4263.34	-263.52	585.55	2.32	3.36
16	6A-2-Cl	-3507.79	-223.50	285.69	3.74	3.63	16	6B-2-Cl	-3498.45	-214.17	578.90	2.78	4.30
17	7A-2-NH2	-3879.26	-218.55	310.64	2.67	1.29	17	7B-2-NH2	-3858.59	-197.88	551.22	3.59	2.11
18	8A-2-CN	-3934.38	-140.29	291.60	3.73	0.97	18	8B-2-CN	-3916.45	-122.37	519.78	2.39	1.73
19	9A-2-NO2	-3911.47	-220.93	287.27	4.39	3.10	19	9B-2-NO2	-3876.16	-185.62	561.57	1.93	3.92
20	10A-2-Ph	-5961.51	-163.51	267.12	3.64	4.27	20	10B-2-Ph	-5936.17	-138.16	523.96	3.58	5.03

Table 1C
The binding energies, dipole moments (μ), heats of formation ΔH_f, and log P values of [3] (set3).

Open form [3] (set 3)							Closed form [3] (set 3)						
S.No	[3]	Binding energy kcal/mole	heat of formation kcal/mole	Wavelength (nm)	dipole moment (D)	LogP	S.No	[3]	Binding energy kcal/mole	heat of formation kcal/mole	Wavelength (nm)	dipole moment (D)	LogP
21	1A-2-H	-4199.04	-238.34	266.76	3.72	1.65	21	1B-2-H	-4110.72	-230.02	564.73	3.01	2.31
22	2A-2-ME	-4683.22	-252.33	288.90	3.38	1.71	22	2B-2-ME	-4660.67	-229.78	569.22	3.00	2.47
23	3A-2-ipr	-5801.28	-270.02	287.66	3.36	3.45	23	3B-2-ipr	-5763.50	-232.24	598.25	3.18	4.21
24	4A-2-OH	-4310.55	-310.73	297.93	5.22	2.99	24	4B-2-OH	-4291.80	-291.98	576.05	3.56	3.11

25	5A-2-OCH3	-4847.18	-297.18	295.97	2.48	3.05	25	5B-2-OCH3	-4822.71	-272.71	592.96	2.32	3.67
26	6A-2-Cl	-4075.86	-241.38	303.06	4.41	3.93	26	6B-2-Cl	-4052.92	-218.44	593.05	2.56	4.60
27	7A-2-NH2	-4445.96	-235.05	322.26	1.65	1.60	27	7B-2-NH2	-4421.42	-210.51	553.19	3.84	2.42
28	8A-2-CN	-4499.43	-155.15	303.13	4.27	1.28	28	8B-2-CN	-4476.46	-132.19	567.11	2.33	2.04
29	9A-2-NO2	-6525.85	-177.65	285.58	4.78	4.58	29	9B-2-NO2	-6494.61	-146.42	589.36	3.24	5.33
30	10A-2-Ph	-4475.83	-235.10	297.36	3.33	3.41	30	10B-2-Ph	-4434.79	-194.06	537.20	2.97	4.22

Perfluorocyclopentene derivatives in open form are highly oriented when compared to closed ones due to cyclisation. Also, an electronegative atom in the substituent plays an important role in deciding the dipole moment of these derivatives. This is reflected as decrease in dipole moment μ values of the derivatives in the order –OH, -OCH3, -NO2, -CN, -Cl. Positive Log P value state that these molecules can have biological activity. Also Lipinski rule of thumb emphasizes likeness of the molecule as drug.

The difference in energies ΔE and wavelengths Δλ for open to closed forms of the derivatives are listed in Table IIa, Table IIb and Table IIc. The photoabsorption of all perfluorocyclopentene derivatives fall in the visible region between a maximum value of 2.57 eV and a minimum of 1.73 eV. In addition, the electron–donating substituents of the bis (3-thienyl) diarylethenes increase the absorption maxima of the closed ring isomers and the electron – withdrawing substituents decrease the cycloreversion.

Table IIa

List of wavelength λ and energy E and difference in energies ΔE and wavelengths Δλ for the open to closed forms of [1] (set1).

	Table II a SET 1- [1]						
S.No	[1]	λ (nm) (open)	λ (nm)closed)	Δλ(nm)	E(ev) open	E(eV) closed	ΔE(eV)
1	R=H	282.50	549.11	266.61	4.38	2.25	2.13
2	R=CH3	265.00	555.39	290.39	4.67	2.23	2.44
3	R=IPR	303.39	539.45	236.06	4.36	2.22	2.14
4	R=NH2	303.39	539.45	236.06	4.08	2.29	1.89
5	R=OH	287.21	557.70	270.49	4.30	2.10	2.20
6	R=OCH3	295.80	575.35	279.45	4.19	2.15	2.04
7	R=CL	309.06	552.11	243.05	4.01	2.25	1.76
8	R=CN	296.64	515.74	219.10	4.18	2.40	1.78
9	R=NO2	284.12	517.70	233.58	4.70	2.29	2.41
10	R=Ph	263.83	540.70	276.87	4.30	2.14	2.16

Table IIb

List of wavelength λ and energy E and difference in energies ΔE and wavelengths Δλ for the open to closed forms of [2] (set2)

	Table II b SET 2 [2]						
S.No	[2]	λ (nm) (open)	λ (nm)closed)	Δλ(nm)	E(ev) open	E(eV) closed	ΔE(eV)
11	R=H	267.11	551.77	284.66	4.64	2.24	2.40
12	R=CH3	267.30	559.62	292.30	4.64	2.13	2.43
13	R=IPR	264.78	587.45	322.67	4.68	2.11	2.57
14	R=NH2	310.64	551.22	240.58	4.00	2.25	1.75
15	R=OH	300.59	523.96	223.37	4.13	2.33	1.80

16	R=OCH3	302.22	585.55	283.33	4.10	2.12	1.98
17	R=CL	285.69	578.90	293.31	3.93	2.14	1.79
18	R=CN	291.60	519.78	228.18	4.25	2.38	1.87
19	R=NO2	287.27	528.05	240.78	4.32	2.34	1.98
20	R=Ph	267.12	561.57	294.45	4.64	2.20	2.44

Table IIc

List of wavelength λ and energy E and difference in energies ΔE and wavelengths Δλ for the open to closed forms of [3] (set 3).

	Table II c	SET 3 - [3]					
S.No	[3]	λ (nm) (open)	λ (nm) closed)	Δλ(nm)	E(ev) open	E(eV) closed	ΔE(eV)
21	R=H	266.76	564.73	297.97	4.65	2.19	2.46
22	R=CH3	288.90	569.22	280.32	4.29	2.18	2.11
23	R=IPR	287.66	598.25	310.59	4.31	2.07	2.24
24	R=NH2	322.26	553.19	230.93	3.84	2.24	1.60
25	R=OH	307.36	537.20	239.84	4.03	2.30	1.73
26	R=OCH3	295.97	592.96	297.39	4.19	2.09	2.10
27	R=CL	303.06	593.05	289.99	4.08	2.09	1.99
28	R=CN	303.13	567.11	263.98	4.09	2.19	1.91
29	R=NO2	297.93	576.05	278.12	4.16	2.15	2.01
30	R=Ph	285.58	589.36	303.78	4.34	2.10	2.24

Figure 2.

a) Optimised geometry of the model compound 1,2-bis(2R,3-thienyl)perflourocyclopentene (set-1)

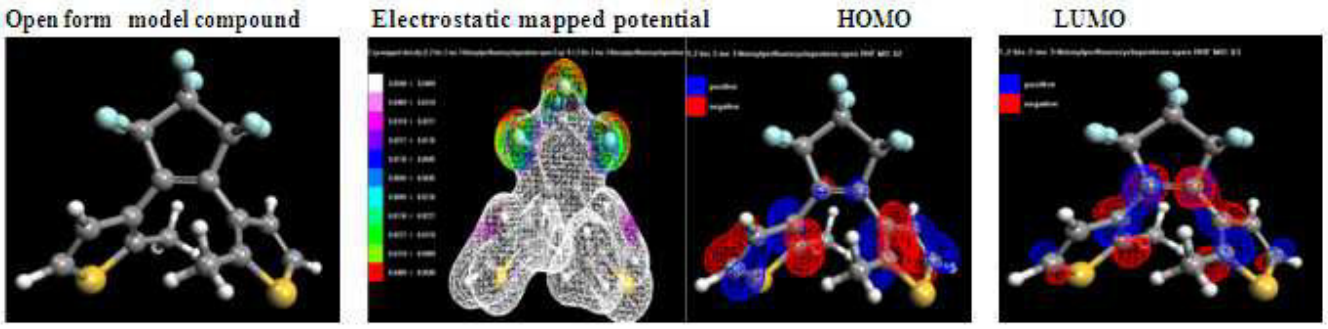
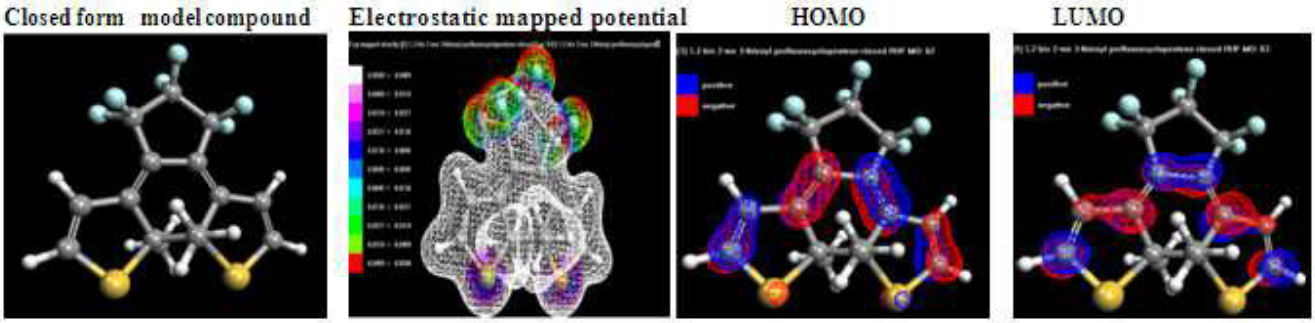


Figure 2
b) Optimised geometry of the model compound 1,2-bis(2R,3-thienyl)perflourocyclopentene (set-1)



The optimised geometry of a model compound 1,2-bis(2-methyl-3-thienyl)perflourocyclopentene of set 1 is displayed in Fig 2a, 2b along with electrostatic potential density map, molecular orbital energy diagrams of HOMO and LUMO. The electrostatic potential, mapped with electron density diagrams show a negative charge density on fluorine atoms and positive charge density on rest of the molecule. These plots reveal reactive sites on the molecules - portraying the potential donor atoms. The frontier molecular orbital (MO) contribution is very important in determining the separated HOMO, LUMO states of the model. The molecular orbital energy density plots of the model compound are shown in Fig. 2. The HOMO state density is distributed entirely over the thiophene rings, while the electron density of LUMO is mainly localized on the region away from the thiophene ring.

Table III
Comparison of PM3 calculations with that of Experimental and DFT calculations

S.No.	Perfluorocyclopentene derivative	EXP	PM3	DFT
1	1,2-bis(3-thienyl)perflourocyclopentene	549	469	475
2	1,2-bis(2-methyl,3-thienyl)perflourocyclopentene	509	554	554
3	1,2-bis(2-isopropyl,3-thienyl)perflourocyclopentene	535	588	536
6	1,2-bis(2-chloro,3-thienyl)perflourocyclopentene	539	538	605
8	1,2-bis(2-cyano,3-thienyl)perflourocyclopentene	496	515	530
12	1,2-bis(2,5-dimethyl,3-thienyl)perflourocyclopentene	503	559	509
22	1,2-bis(2,4,5-trimethyl,3-thienyl)perflourocyclopentene	529	569	548

The geometric structures were investigated earlier by the well known and more accurate density functional theory (DFT) method. The obtained absorption maxima from PM3 results for few molecules for which experimental and Density functional theory DFT results are available are listed in Table III. Comparison of wavelength with experimental data shows a definite difference of ~ 50 nm exists between PM3 and experiment results uniformly for all the molecules. They are in par with that of already existing more accurate DFT

computational calculations. So, we conclude that one can plan a new photochromic molecule using PM3 semi empirical method itself.

CONCLUSIONS

The theoretical results suggest that photo chromic characteristics of diarylethenes primarily depend on the conformations of open ring isomers, the heteroaryl moieties, the electron donor/acceptor substituents and the conjugation length of heteroaryl systems. Particularly, the heteroaryl moieties and substituents on the reactive position greatly influence the photoreactivity and the distinguishable features of diarylethenes. The energy gap between the HOMO and LUMO is in accordance with electronic transitions. Logp values dictate the biological activity of the molecule. Finally, the linear relationship between experimental and theoretical absorption maxima, comparable heats of formation and energies states that a low end computational tool like PM3 can be employed to predict, and further to design new photochromic molecules.

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A SIMPLE PROCESS FOR THE PREPARATION OF OLANZAPINE

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ABSTRACT

2-Methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5]benzodiazepine (Olanzapine) is an anti-psychotic drug with a thienobenzodiazepinyl structure and is indicated for the treatment of schizophrenia and bipolar disorder. Having superior activity over the other compounds of the same class, Olanzapine became the "target" for the industries and research institutions. In this paper, we disclosed a simple two-step process for the preparation of Olanzapine by following the reaction conditions of Suzuki coupling, particularly with the aid of palladium catalyst.

KEYWORDS

Olanzapine, anti-psychotic drug, Suzuki coupling.

INTRODUCTION

Olanzapine **1** is chemically known as 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [2,3-b][1,5]benzodiazepine (1) and can be represented by the following formula (Fig.1). Compound **1** is an anti-psychotic drug with a thienobenzodiazepinyl structure and is indicated for the treatment of schizophrenia and bipolar disorder[2a-b]. Compound 1 known to display a broad pharmacological profile and is a selective monoaminergic antagonist with high affinity binding to serotonin 5 $HT_{2A/2C}$, dopamine DI_4, muscarinic M_{1-5} and adrenergic α_1 receptors[3a-b]. Compound **1** has antagonist activity at noradrenergic alpha-receptors. The above-identified properties indicate that **1** is a potential neuroleptic with relaxant, anxiolytic, or anti-emetic properties, and is useful in treating psychotic conditions such as schizophrenia, schizophrenia-form diseases, and acute mania. At lower doses 1 can be used in the treatment of mild anxiety states. There were reports for its high level of activity in the clinical evaluation of psychiatric patients suffering from schizophrenia and it exhibits this high activity at surprisingly low dosage levels.[4] Compound 1 binds weakly to $GABA_A$, BZD and β adrenergic receptors inhibitor

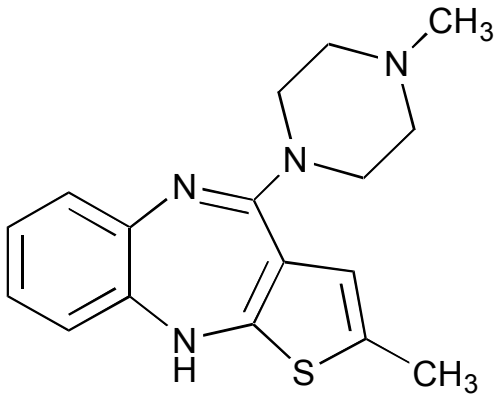


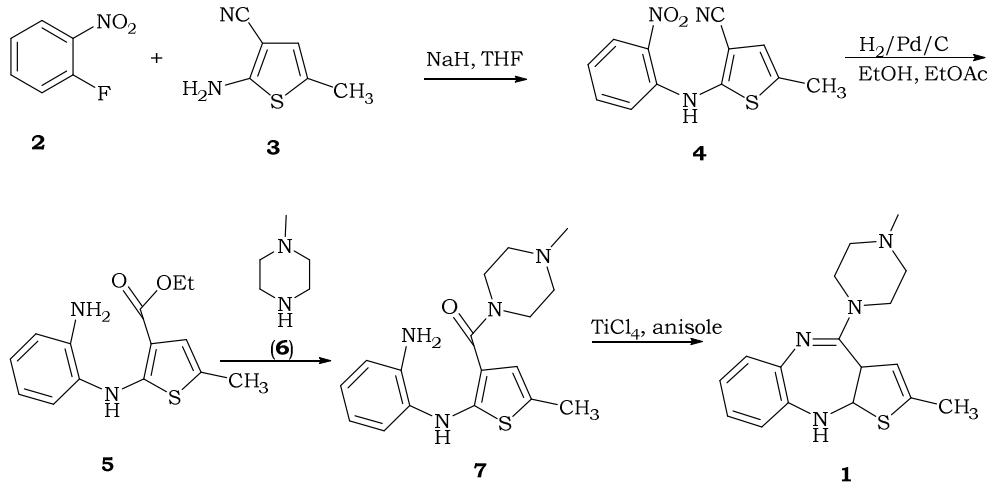
Figure. 1

Compound 1, is one of the best selling drug in the category of anti-psychotics and its sales were US 4966.6M$ for the year 2004-2005[5]. Compound 1 is covered in the patents for the treatment of headache, pain,[6] gastrointestinal disorders,[7] treating a condition resulting from the cessation and withdrawal from the use of nicotine[8], dyskinesias[9], anorexia[10], fungal dermatitis[11], excessive aggression[12], autism and mental retardation[13], insomnia[14], and migraine pain[15]. It can also be administered along with other active pharmaceutical ingredients[16a-g]. Benzodiazepine moieties are studied for their activities prior to the origin of 1[17a-c]. Out of all these moieties, compound 1 possesses superior activity over the other similar moieties.

Having superior activity over the other compounds of the same class, 1 became the "target" for the industries and research institutions. Therefore, the studies were initiated to find novel processes for the preparation of 1, to find different physical forms and its properties, pharmacological studies and other activity studies.

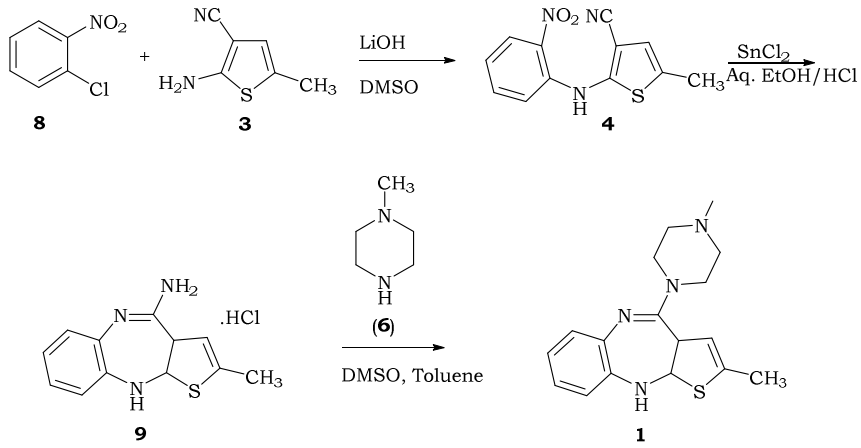
The first reported synthesis[18] of Olanzapine (**1**) by Chakrabarti *et al.,* involves the reaction of (scheme 1) 2-fluoronitrobenzene (**2**) with 2-amino-5-methylthiophene-3-carbonitrile (**3**) in the presence of sodium hydride and THF to give 2-(2-nitrianilino)-5-methyl thiophene derivative **4**. The nitrile group of thiophene derivative **4** was catalytically hydrogenated and followed by treatment with ethanol to get an amino ester **5**, which was then reacted with N-methyl piperazine (**6**) to give the amino amide **7** followed by cyclization in the presence of titanium tetrachloride to give the desired compound **1**.

Scheme 1



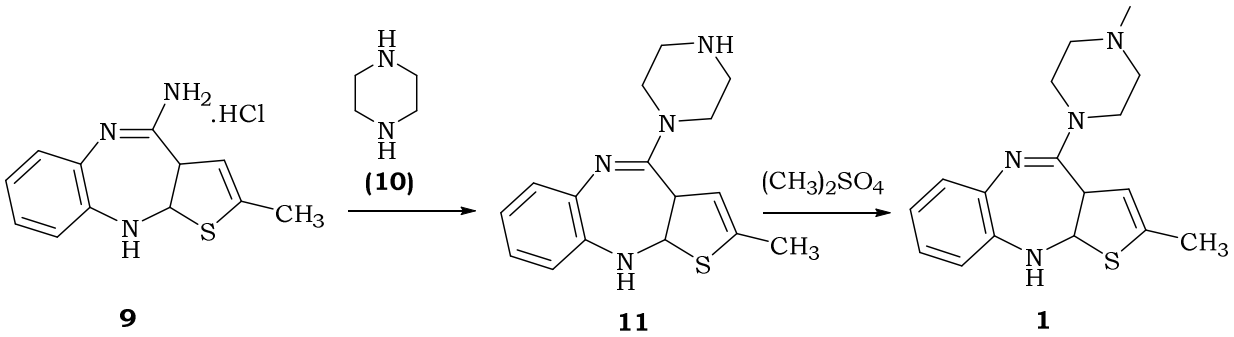
David O. Calligaro *et al.,* disclosed two methods[19] (schemes 2 & 3) for the synthesis of Olanzapine. Scheme 2 involves the reaction of 2-chloronitrobenzene (**8**) with 2-amino-5-methylthiophene-3-carbonitrile (**3**) in the presence of lithium hydroxide to give the 2-(2-nitrianilino)-5-methyl thiophene derivative **4** which is cyclized in the presence of stannous chloride to give the benzodiazepine derivative **9** in its salt form. The benzodiazepine derivative is then reacted with N-methyl piperazine (**6**) to give **1**.

Scheme 2



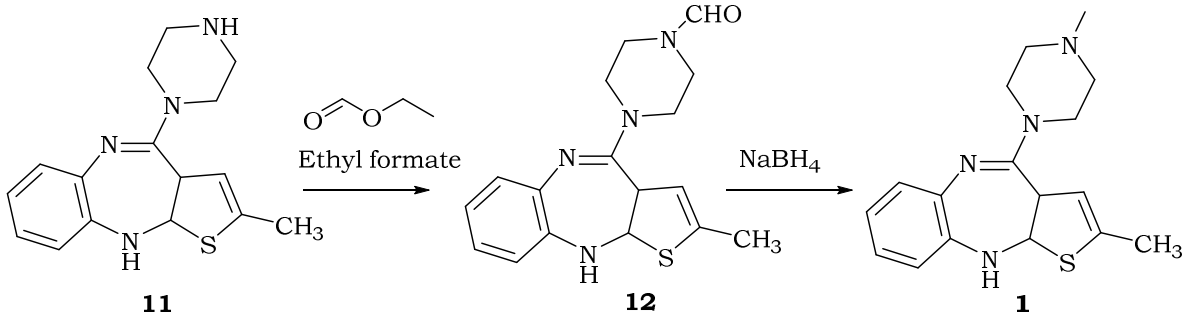
The scheme 3 discloses an alternate process for preparing compound **1** involving the reaction of benzodiazepine derivative **9** with piperazine (**10**) to result *N*-desmethyl Olanzapine (**11**) which upon methylation using dimethyl sulphate afforded **1**.

Scheme 3



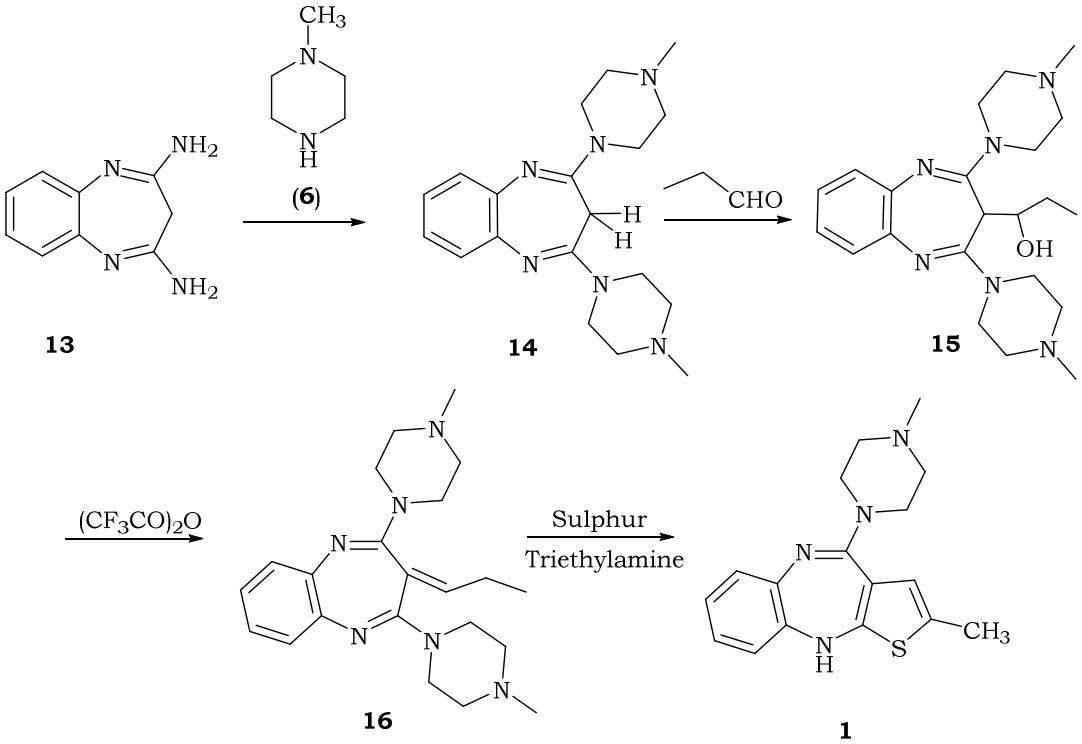
Zbigniew Majka *et al.,* describes a process[20] (scheme 4) for the preparation of Olanzapine involving the use of *N*-desmethyl Olanzapine (**11**) as the starting material wherein it is reacted with ethyl formate to give the corresponding *N*-formyl Olanzapine (**11**) which upon reduction with a group I or II metal borohydride gives Olanzapine (**1**).

Scheme 4



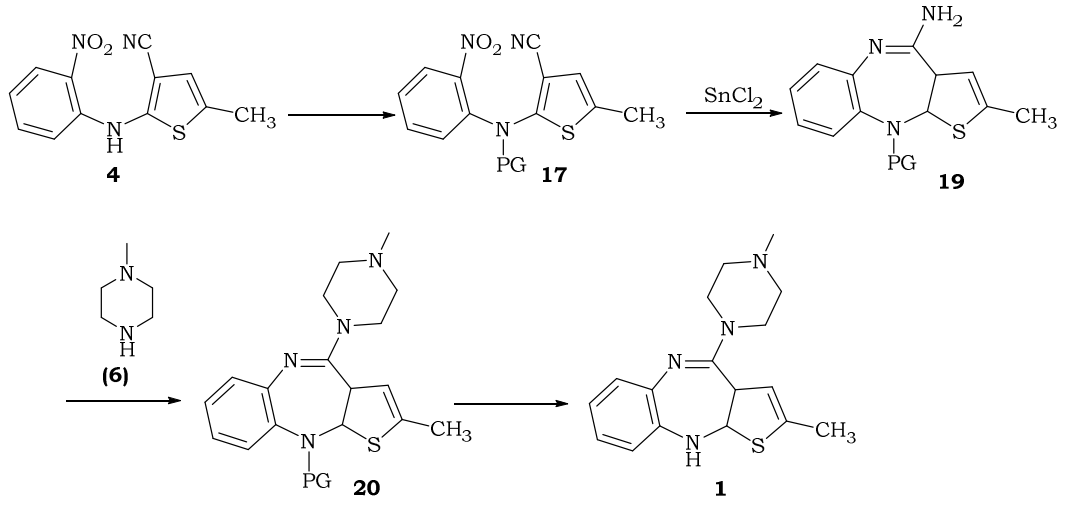
Roman Lenarsic describes a process[21] for the preparation (Scheme 5) of Olanzapine starting from the reaction of benzodiazepine-2,4-diamine (**13**) with N-methyl piperazine to give bis (methyl piperazinyl) benzodiazepine (**14**). The compound **14** upon reaction with propionaldehyde in the presence of lithium diisopropylamide resulted benzodiazepine-1-propanol (**15**), which upon reduction in the presence of trifluoroacetic anhydride yielded the corresponding propylidene derivative **16**. The propylidene derivative upon reaction with sulfur in the presence of triethylamine gave the targeted compound **1**.

Scheme 5



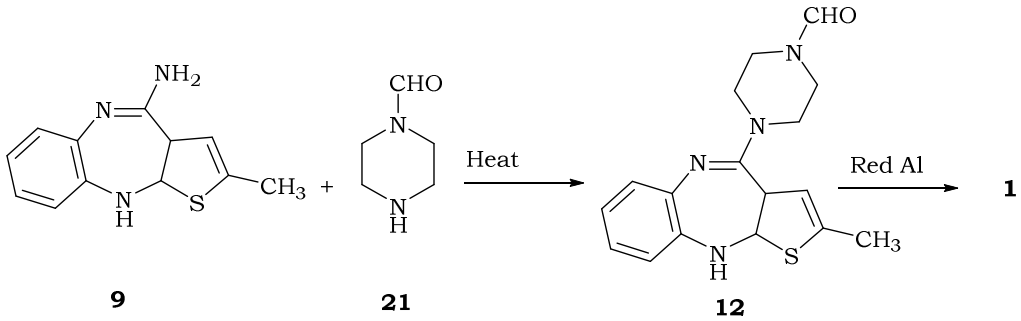
Zhengyong Wang[22] describes a multi step process for the preparation (scheme 6) of Olanzapine by protecting the amino group of 2-(2-nitrianilino)-5-methyl thiophene derivative **4** to produce the protected intermediate **17**. Cyclizing the protected intermediate **17** via reduced intermediate compound **18** with tin chloride to produce benzodiazepine derivative **19**, which was then reacted with N-methyl piperazine to give protected *N*-protected Olanzapine (**20**), finally which upon deprotection provided Olanzapine (1).

Scheme 6



Rolf Keltjens *et al*., describes[23] an improved process for the preparation (scheme 7) of Olanzapine. The process comprises reaction of compound **9** or its salt with *N*-formyl piperazine **21** to form *N*-formyl Olanzapine (**12**). The *N*-formyl Olanzapine or its salt was reduced with Red-Al to form Olanzapine (**1**) or its salt.

Scheme 7



9 21 12

Summary of reported synthetic schemes

As evident from the earlier reported synthetic schemes, Olanzapine was synthetically prepared either by cyclizing the open chain derivative to form diazepine ring during the final stage of the synthesis or it was prepared by cyclizing the open chain derivative to diazepine ring to form an intermediate compound followed by condensation of the cyclized intermediate with piperazine or its derivative to give Olanzapine. In another alternative disclosed methodology, Olanzapine was prepared by *N*-methylation of the piperazine derivative after cyclization and subsequent condensation with piperazine. In all the above known synthetic procedures for the preparation of Olanzapine, the reduction of nitro group is a common step before the cyclization of the open chain derivative to afford diazepine moiety.

Use of many different reagents for the functional group transformation results in degradation of the product which inturn requires tedious purification procedures and results in low yield of the final desired compound.

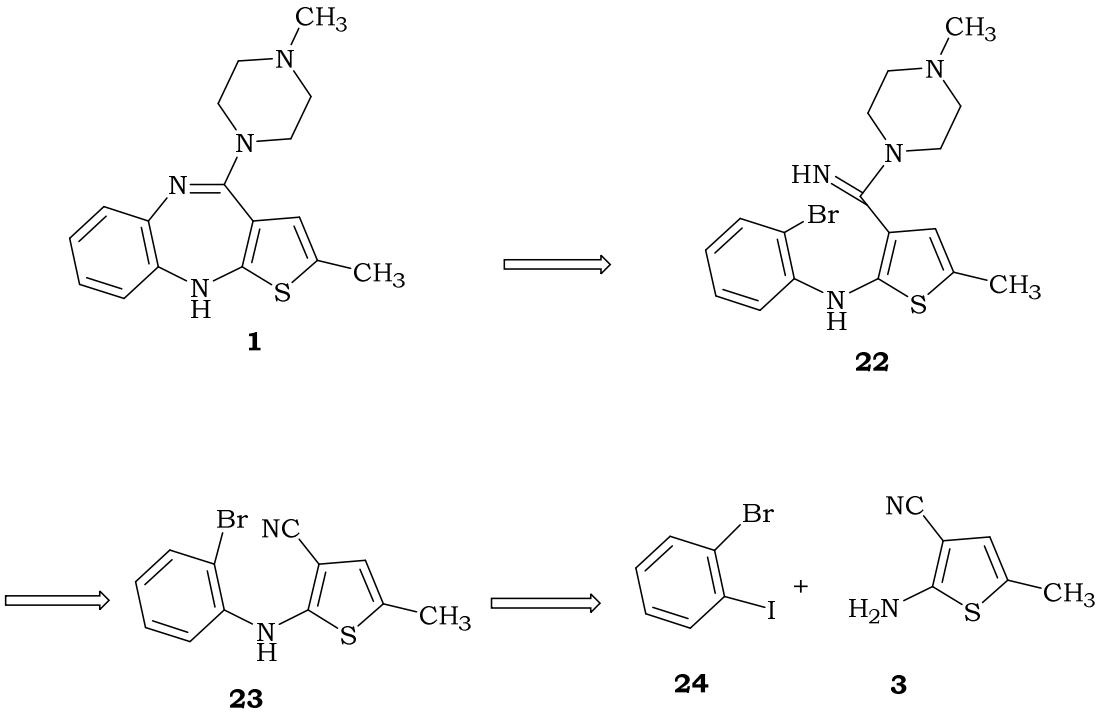
PRESENT WORK

The earlier processes for the preparation of Olanzapine mainly utilized reagents for the reduction of nitro group before the cyclization of the open chain derivative to afford diazepine derivative. The present work specifically avoids the use of nitro derivative as starting materials and corresponding reducing agents for the reduction of nitro group. The present work involves a simple two step process for the preparation of Olanzapine involving Suzuki coupling mechanism with the aid of organo metallic reagents. The resulted product was in comparison with authentic sample.

RESULTS AND DISCUSSION

1. Retro synthetic Pathway for Olanzapine:

Scheme 8

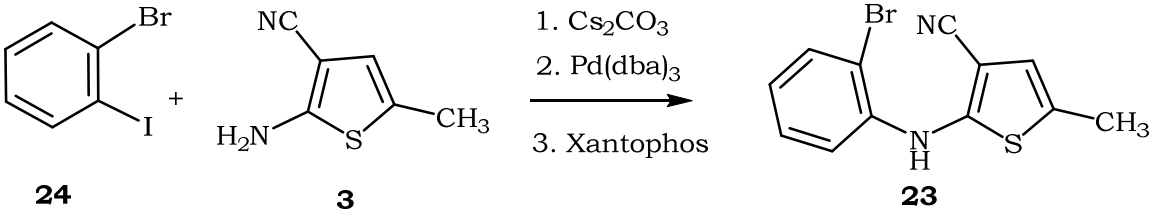


Accordingly scheme 8 has been systematically designed to get appreciable yield of Olanzapine with higher storage stability and low content of impurities. Scheme 8 described palladium catalyzed coupling of 2-amino-5-methylthiophene-3-carbonitrile (**3**) with 1-bromo-2-iodobenzene (**24**) in the presence of xantophos ligand and cesium carbonate base to give a novel intermediate compound 2-(2-bromophenylamino)-5-methylthiophene-3-carbonitrile (**23**). The compound **23** was further reacted with *N*-methyl piperazine in presence of trimethyl aluminum to afford another novel imine derivative **22**, which upon subsequent cyclization gave Olanzapine (**1**).

2. Synthesis of 2-(2-bromophenylamino)-5-methylthiophene-3-carbonitrile (23):

Palladium-catalyzed cross-coupling reactions are among the most useful synthetic methods for the reaction of halo derivatives with an amine derivates, and the application of this property has been specifically utilized for the synthesis of novel compound **23**. A suitable moles of compound **24** is coupled (scheme 8a) with **3** in the presence of tris (dibenzylidineacetone) dipalladium catalyst and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene as ligand to afford novel compound **23**. The use of base such as cesium carbonate during the coupling reaction provided the required intermediate **23** with good yield.

Scheme 8a

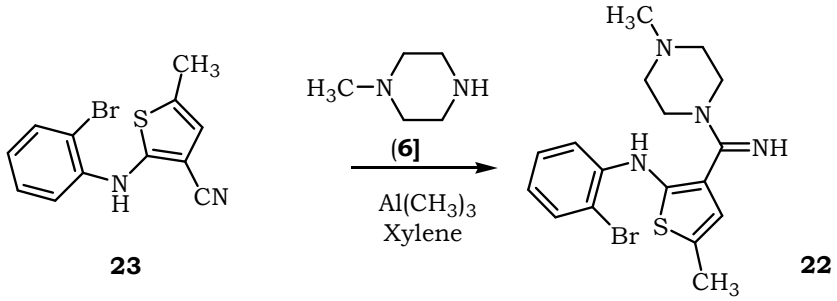


IR Spectrum of **23** exhibited a sharp signal at about 3322 cm^{-1} corresponding to NH absorption and CN function absorption at 2222 cm^{-1}. In ^{1}H-NMR spectrum, the down field region was characterized by the presence of five aromatic proton signals at δ 7.59(d, 1H, Ar-H), 7.30 (dd, 2H, Ar-H), 6.75 (m, 1H, Ar-H) and 6.63 (s, 1H, Ar-H). Sharp singlet signal appeared at δ 2.41(s, 3H, Ar-CH_3) is attributed to aromatic methyl group. The mass spectrum displayed a molecular ion peak at m/z 293 (M+1) along with the bromime isotopic abundance at m/z 295(M+1). Thus, all the spectral data (IR, ^{1}H-NMR, ^{13}C-NMR & IR) was in conformity with the assigned structure of 2-(2-bromophenylamino)-5-methylthiophene-3-carbonitrile (**23**).

3. Synthesis of *N*-(2-bromophenyl)-3-(imino(4-methylpiperazin-1-yl)methyl)-5-methyl thiophen-2-amine (22):

The preparation of another novel imine derivative **22** is achieved by reaction (scheme 8b) of compound **23** with *N*-methyl piperazine in presence of trimethyl aluminum and xylene as solvent.

Scheme 8b



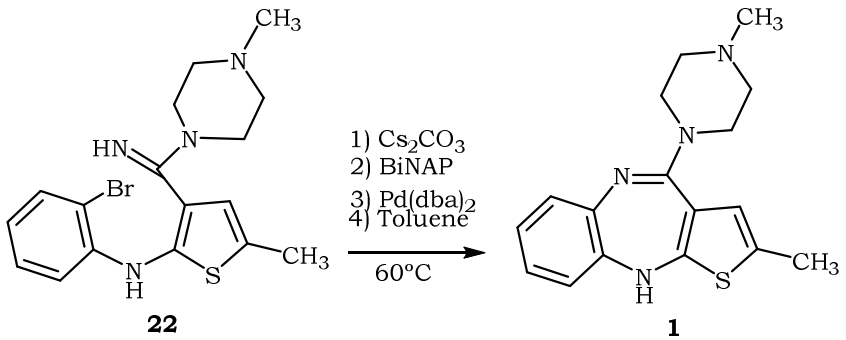
23 **22**

In the IR spectrum, the two absorption peaks corresponding to amino and amide NH appeared at 3366 and 3259 cm^{-1} respectively. The ^{1}H NMR spectrum displayed five aromatic protons at δ 7.57(dd, 1H, Ar-H), 7.24 (m, 2H, Ar-H), 6.78 (m, 1H, Ar-H) and 6.43 (s, 1H, Ar-H), piperazine protons at δ 3.38 (br, 8H, CH_2) and two methyl groups at δ 2.22 (s, 6H, $2CH_2$). The mass spectrum displayed a protonated molecular ion peak at m/z 393.0 (M^{+1}) with positive segment polarity.

4. Synthesis of Olanzapine (1):

Preparation of Olanzapine is finally achieved by palladium-catalyzed cyclization of novel imine intermediate **22** in the presence of 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BiNAP) ligand and cesium carbonate base in toluene as suitable solvent (Scheme 8c). The resulted compound was compared with the authentic sample of Olanzapine by HPLC analysis and also confirmed by its complete spectral data.

Scheme 8c



22 **1**

The UV spectrum of Olanzapine (**1**) recorded in methanol (conc=0.001% w/v) using Perkin-Elmer UV-VIS spectro photometer model Lambda 35. It exhibited three peaks with maxima at λ 202, 226 and 271 nm. The FT-IR spectrum of **1** was recorded using KBr pellet. The absorption peak observed at 3236 cm^{-1} due to N-H stretching. The ^1H NMR spectrum (Fig.5.9) recorded in $CDCl_3$ showed characteristic signals at δ 7.57 (s, 1H, NH), 6.85-6.78 (m, 3H, Ar-H), 6.68 (d, 1H, Ar-H), 6.32 (s, 1H, Ar-H), 3.32-3.20 (br, 4H, CH_2), 2.38 (br, 4H, CH_2), 2.26 (s, 3H, CH_3),), 2.21 (s, 3H, CH_3).

EXPERIMENTAL SECTION

1. Preparation of compound 23:

To a stirred solution of **3** (5.4 g, 0.039 mol), tris (dibenzylidineacetone)dipalladium (1.5 g, 0.00164 mol), cesium carbonate (36.0 g, 0.108 mol), Xantophos (1.0 g, 0.0017 mol) in 1,4-dioxane (50. 0 mL) was added a solution of **24** (10.8 g, 0.039 mol) in 10.0 mL of xylene at room temperature. After complete addition, the reaction mass was heated to 90 ^0C and further stirred for the reaction completion. After completion of the reaction water was added mixture was extracted with ethyl acetate. Organic layer was separated and washed with brine solution and dried over sodium sulfate. The mixture was concentrated under reduced pressure and the resulting residue was purified by column chromatography to yield compound (9.0 g, Yield: 78.9 %, HPLC purity 98.7%). IR (cm^{-1}): 3322 (NH), 2222 (CN); ^1H NMR ($CDCl_3$, δ ppm): 2.41(s, 3H, Ar-CH_3), 6.63 (s, 1H, Ar-H), 6.75 (m, 1H, Ar-H), 7.30 (dd, 2H, Ar-H), δ 7.59(d, 1H, Ar-H); ^{13}C NMR (200 MHz, $CDCl_3$): δ 15.3, 96.4, 112.4, 114.7, 116.0, 122.7, 123.1, 128.6, 130.0, 133.1, 139.7, 153.8. MS: *m/z* 293 (M+1); Analysis Calcd. for $C_{13}H_9BrN_2S$: C, 49.16; H, 3.09; N, 9.55. Found: C, 49.32; H, 3.39; N, 9.61.

2. Preparation of 22 from 23:

To a clear solution of **23** (2.5 g, 0.0085mol), *N*-methylpiperazine (1.70 gm, 0.0170 mol] in xylene (50.0 mL) was added trimethyl aluminium (5.5 mL, 0.0110 mol, 2.0M solution in toluene) at 90-100 ^0C with stirring. The resulting solution was heated to 110-115 ^0C and stirred for the reaction completion. After completion of the reaction, the solution was cooled to 0 ^0C and sodium potassium tartrate salt solution was added. The resulting mixture was extracted with ethyl acetate (3x50 mL) and washed with 3.0 N hydrochloric acid solution (2x10 mL). The combined aqueous layer was washed with ether and basified the aqueous layer with aqueous sodium hydroxide solution (20%). The resulting mixture was extracted with ethyl acetate (3x50 mL) and the combined organic layers were washed with water, brine and dried over sodium sulfate. The mixture was concentrated under reduced pressure to yield the targeted compound **22** as brown colored solid (2.0 g, Yield: 60.0%, HPLC purity 85.0%). IR (cm^{-1}): 3366 (NH), 3259 (NH); ^1H NMR (DMSO-d_6, δ ppm): 2.22 (s, 6H, $2CH_2$), 3.38 (br, 8H, CH_2), 6.43 (s, 1H, Ar-H), 6.78 (m, 1H, Ar-H), 7.24 (m, 2H, Ar-H), 7.57(dd, 1H, Ar-H); ^{13}C NMR (200 MHz, DMSO): δ 15.5, 46.2, 46.9, 55.0, 118.7, 119.3, 123.0, 123.8, 124.0, 128.0, 141.1,144.4, 153.8, 157.9. MS: *m/z* 393 (M+1); Analysis Calcd. for $C_{17}H_{21}BrN_4S$: C, 51.91; H, 5.38; N, 14.24. Found: C, 51.67; H, 5.49; N, 14.01.

3. Olanzapine (1):

The compound **22** (1.5 g, 0.0038 mol), cesium carbonate [1.60 g, 0.0049 mol], BINAP [0.3 g, 0.0005 mol] in toluene (10.0 mL) was added catalytical amount of tris [dibenzylidineacetone] dipalladium and stirred at room temperature for 1.0 hour. The resulting reaction was heated to 60 ^0C for 4.0 hours. After completion of the reaction the solution was cooled to room temperature, water (50 mL) was added and extracted with ethyl acetate [3x25 mL] and the combined organic layers were washed with brine and dried over sodium sulfate. The mixture was concentrated under reduced pressure to afford crude compound. The crude was purified by column chromatograph and followed by recrystallisation from methylene chloride yielded 0.5 g of compound as light yellow colored solid (Yield: 45.0%, HPLC Purity: 99.2%). IR (cm^{-1}): 3236 (NH); ^1H NMR (DMSO-d_6, δ ppm): 2.21 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.38 (br, 4H, CH_2), 3.32-3.20 (br, 4H, CH_2), 6.32

(s, 1H, Ar-H), 6.68 (d, 1H, Ar-H), 6.85-6.78 (m, 3H, Ar-H), 7.57 (s, 1H, NH); ^{13}C NMR (200 MHz, DMSO): δ 15.0, 45.7, 46.4, 54.4, 54.4, 118.1, 118.7, 122.4, 123.1, 123.3, 127.4, 127.8, 140.5,143.8, 153.2, 157.3. MS: *m/z* 313 (M+1); Analysis Calcd. for $C_{17}H_{20}N_4S$: C, 66.35; H, 6.45; N, 17.93. Found: C, 66.52; H, 6.49; N, 18.01.

CONCLUSIONS

In the present study we have described a practical and general method for the synthesis of 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [2,3-b][1,5]benzodiazepine, Olanzapine (1). Our synthetic pathway used for the preparation of Olanzapine involved the palladium catalyzed coupling of 2-amino-5-methylthiophene-3-carbonitrile (3) with 1-bromo-2-iodobenzene (24) in the presence of xantophos ligand and cesium carbonate as base to produce new intermediate, 2-(2-bromophenylamino)-5-methylthiophene-3-carbonitrile (23). This intermediate compound 23 was reacted with N-methyl piperazine (6) in the presence of trimethylaluminium to afford another new intermediate, imine derivative (22), which upon subsequent cyclization produced Olanzapine (1). Most of the processes mentioned in the background were protected in valid patents across the globe to have a monopoly rights by individual pharma companies. It is required to circumvent the claimed processes to market the product and particularly in less regulated countries of European region, a non-infringing process will certainly have an advantage to get an early launch opportunity. The present work is novel, involve less number of steps and does not involve any claimed steps or processes.

ACKNOWLEDGEMENT

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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TELMISARTAN AND CHLORTHALIDONE IMPURITIES IN API AND PHARMACEUTICAL FORMULATION

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ABSTRACT

A sensitive, stability-indicating gradient RP-UPLC method has been developed for the quantitative determination of impurities of Telmisartan and Chlorthalidone in API and pharmaceutical formulation. Efficient chromatographic separation was achieved on an Acquity BEH Shield-RP18, 100x 2.1mm, 1.7µm column with mobile phase containing a gradient mixture of solvents A and B. The flow rate of the mobile phase was 0.3 mL min-1 with column temperature of 25°C and detection wavelength at 235 nm. Telmisartan and Chlorthalidone formulation sample was subjected to the stress conditions of oxidative, acid, base, hydrolytic, thermal and photolytic degradation. Telmisartan and Chlorthalidone were found to degrade significantly in acid, peroxide and thermal stress conditions. The degradation products were well resolved from Telmisartan and Chlorthalidone and its impurities. The peak purity test results confirmed that the Telmisartan and Chlorthalidone peaks were homogenous and pure in all stress samples and the mass balance was found to be more than 98%.

KEYWORDS

Telmisartan, Chlorthalidone, Stability-indicating, Impurities, ICH guidelines

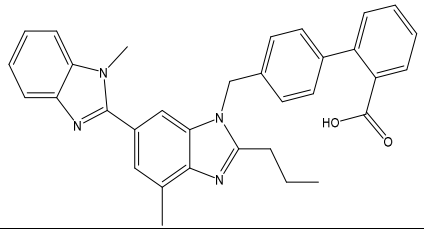
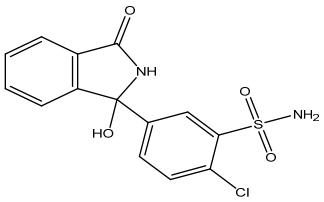
INTRODUCTION

Telmisartan (TS) is a diabetes angiotensin receptor blocker that shows high affinity for the angiotensin II type 1 (AT1) receptors, has a long duration of action and has the longest half-life of any ARB1. It is chemically named as 4'-[(1,4'-dimethyl-2'-propyl[2,6'-bi-1H-benzimidazol]-1'-yl)methyl]-[1,1'-biphenyl]-2-carboxylic acid and chemical structure is shown in Fig 1. It acts as a selective modulator of peroxisome

proliferator-activated receptor gamma (PPAR-γ), a central regulator of insulin and glucose metabolism. It is believed that TS's dual mode of action may provide protective benefits against the vascular and renal damage caused by and cardiovascular disease[1].

Chlorthalidone (CT) is thiazide-like diuretic[2], chemically known as 2-chloro-5-(1-hydroxyl-3-oxo-2,3-dihydro-1H-isoindol-1-yl) benzene-1-sulfonamide, with molecular formula $C_{14}H_{11}ClN_2O_4S$ and molecular weight 338.766 g/mol as shown in Fig 1. CT inhibits sodium ion transport across the renal tubular epithelium in the cortical diluting segment of the ascending limb of the loop of Henle. By increasing the delivery of sodium to the distal renal tubule, CT indirectly increases potassium excretion via the sodium-potassium exchange mechanism. CT is widely used in antihypertensive pharmaceutical preparations, reduces active sodium reabsorption and peripheral vascular resistance. Compared with other medications of the thiazide class, CT has the longest duration of action but a similar diuretic effect at maximal therapeutic doses. It is often used in the management of hypertension and edema.

Figure. 1. Chemical structure of Telmisartan and Chlorthalidone

Name	Chemical Name	Chemical Structure
Telmisartan	4′-[[4-Methyl-6-(1-methyl-1H-benzimidazol-2-yl)-2-propyl-1H-benzimidazol-1-yl]methyl]biphenyl-2-carboxylic acid	
Chlorthalidone	2-Chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl)benzenesulfonamide	

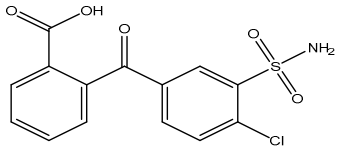
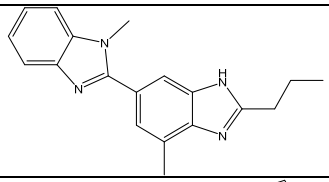
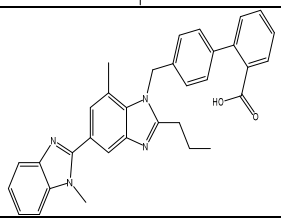
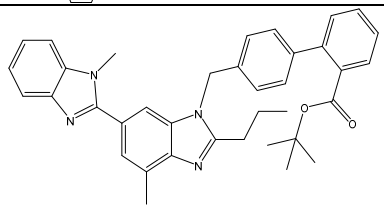
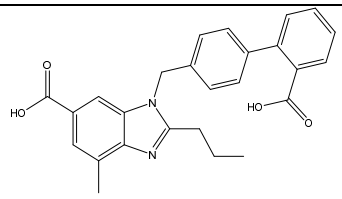
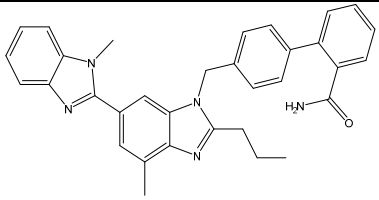
Impurity profiling of active pharmaceutical ingredients (API) in both bulk material and finalized formulations is one of the most challenging tasks of pharmaceutical analytical chemists under industrial environment[3]. The presence of unwanted or in certain cases unknown chemicals, even in small amounts, may influence not only the therapeutic efficacy but also the safety of the pharmaceutical products [4]. For these reasons, all major international pharmacopoeias have established maximum allowed limits for related compounds for both bulk and formulated APIs. As per the requirements of various regulatory authorities, the impurity profile study of drug substances and drug products has to be carried out using a suitable analytical method in the final product[5,6].

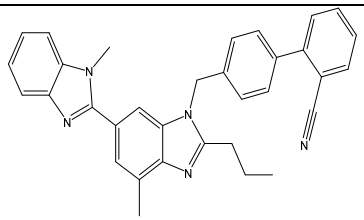
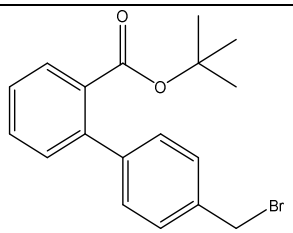
A detailed literature survey revealed that there are some analytical methods reported for estimation of TS and CT either individually like HPLC, UV spectrophotometric method, flow injection chemiluminescence analysis, or in combination with other drugs by HPLC[7-9]. Few methods are reported for estimation of Telmisartan impurities either in individual formulation or in combination with other drugs. There is not a

single method has been reported for simultaneous determination of TS and CT impurities in pharmaceuticals formulations of TS and CT. It is felt necessary to develop a stability indicating method for TS and CT related impurities combination dosage formulation.

Hence, an attempt has been made to develop an accurate, rapid, specific and reproducible method for determination of TS and CT impurities (Fig 2) in pharmaceutical dosage forms along with method validation as per ICH norms[10,11]. The stability tests were also performed on both drug substances and drug products as per ICH norms[12,13].

Figure. 2. Chemical structure of Telmisartan and Chlorthalidone impurities

	Name	Structure	IUPAC name
Chlorthalidone impurities	Impurity A		4'-Chloro-3'-sulfamoyl-2-benzophenone carboxylic acid
Telmisartan impurities	Impurity A		4-methyl-6-(1-methyl-1H-benzimidazol-2-yl)-2-propyl-1H-benzimidazole
	Impurity B		4′-[[7-methyl-5-(1-methyl-1H-benzimidazol-2-yl)-2-propyl-1H-benzimidazol-1-yl]methyl]biphenyl-2-carboxylic acid
	Impurity C		1,1-dimethylethyl 4′-[[4-methyl-6-(1-methyl-1H-benzimidazol-2-yl)-2-propyl-1H-benzimidazol-1-yl]methyl]biphenyl-2-carboxylate
	Impurity E		1-[(2′-carboxybiphenyl-4-yl)methyl]-4-methyl-2-propyl-1H-benzimidazol-6-carboxylic acid
	Impurity F		4′-[[4-methyl-6-(1-methyl-1H-benzimidazol-2-yl)-2-propyl-1H-benzimidazol-1-yl]methyl]biphenyl-2-carboxamide

Impurity G		4′-[[4-methyl-6-(1-methyl-1H-benzimidazol-2-yl)-2-propyl-1H-benzimidazol-1-yl]methyl]biphenyl-2-carbonitrile
Impurity H		1,1-dimethylethyl 4′-(bromomethyl)biphenyl-2-carboxylate

MATERIALS AND METHODS

1. Reagents and Materials

TS+CT tablets were received from formulation research and development laboratory of Dr. Reddy's Laboratories Ltd., IPDO, Hyderabad, India. TS API and its impurities were procured from Dr. Reddy's Laboratories Ltd., IPDO, Hyderabad, India. CT API and its impurities were procured from Paschim chemicals Pvt Ltd., India. Potassium dihydrogen orthophosphate and 1-hexane sulphonic acid sodium salt were purchased from Merck, Germany. HPLC grade Acetonitrile, Methanol, Triethyl amine and Ortho phosphoric acid were purchased from Merck, Germany and high pure water was prepared by using Millipore Milli Q plus purification system.

2. Instrumentation

The UPLC system used for method development and method validation was Waters with a diode array detector (Model, quaternary gradient). The output signal was monitored and processed using waters empower software. Weighing was performed with a Mettler XS 205 Dual Range (Mettler-Toledo GmbH, Greifensee, Switzerland). Photo stability studies were carried out in a photo stability chamber (SUN TEST XLS+, Atlas, USA).Thermal stability studies were performed in a dry air oven (Merck Pharmatech, Hyderabad, India).

3. Chromatographic system

UPLC measurements were carried out using a reversed phase Acquity BEH Shield-RP18, 100x 2.1mm, 1.7µm particle size column (Thermo Scientific, Runcorn, UK) operated at 25°C with gradient elution at 0.3 mL min^{-1} using a mobile phase buffer as 0.025M Potassium dihydrogen phosphate, 0.0027M 1-hexane sulphonic acid sodium salt and 1ml of triethyl amine in milli-Q water (pH 4.5 ±0.05 adjusted with diluted ortho phosphoric acid; UV absorbance at 290 nm; injection volume 3 µL. The mobile phase A consisted of pH 4.5 buffer & acetonitrile in the ratio 90:10 (v/v); mobile phase B consisted of pH 4.5 buffer & acetonitrile in the ratio 20:80 (v/v). The LC gradient program was set as: time (min)/% mobile phase B: /40, 1/95, 1.5/95, 2.5/40 and 3/40. Mixture of pH 4.5 buffer, acetonitrile and methanol in the ratio 60:20:20 (V/V/V) was used as diluent for sample preparation.

4. Preparation of standard solution and system suitability solution

A stock solution of TS (1000 µg mL^{-1}) and CT (315 µg mL^{-1}) was prepared by dissolving an appropriate amount in Methanol. Working solution was prepared from above stock solution for related substances determination (1.60 µg mL^{-1} of TS and 0.25 µg mL^{-1} of CT) in diluent. A mixture of all impurities (1.6 µg mL^{-1} of TC impurities and 0.25 µg mL^{-1} of CT impurities) along with TS and CT (1.60 µg mL^{-1} of TS and 0.25 µg mL^{-1} of CT) was prepared in diluent. Also impurity stock solutions were prepared in Methanol.

5. Preparation of Test solution

Twenty tablets (TS+CT) were weighed and average weight of tablet content was calculated. Tablet powder equivalent to 80 mg of active pharmaceutical ingredient TS (12.5 mg of active pharmaceutical ingredient CT) was transferred in to a 100 ml volumetric flask. To this added 50 ml of Methanol and sonicated for 15 minutes with intermediate shaking. The solution was then diluted to 100 ml with diluent and centrifuged at 3000 rpm for 10 min. The supernatant (800 µg mL^{-1} of TS and 125 µg mL^{-1} of CT) was collected and used as sample solution.

6. Method validation

The proposed method was validated as per ICH guidelines[11].

6a. Specificity

Stress studies were performed at an initial concentration of 800 µg mL^{-1} of TS + 125 µg mL^{-1} of CT in formulated sample to provide the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted by the stress conditions of exposed to visible light of 1.2 million lux hours, UV light of 200 watt hours m-2, heat (Exposed at 105°C for 3 h), acid (0.2N HCl for 30 min at 60°C), base (0.2 N NaOH for 30 min at 60°C), oxidation (3% peroxide for 15 min at 60ºC), water (Refluxed for 30 min at 60°C) and humidity (Exposed to 90% RH for 7 days).

6b. Precision

The precision of the determination of the impurities was checked by injecting six individual preparations of 800 µg mL^{-1} TS + 125 µg mL^{-1} CT test preparation spiked with 1.6 µg mL^{-1} of TS impurities, 0.25 µg mL^{-1} of CT impurities and calculating the % RSD of % area for each compound. The intermediate precision of the method was also evaluated using different analysts and a different instrument in the same laboratory.

6c. Accuracy

Accuracy of the method was demonstrated at five different concentration levels in triplicate. The analysis carried out by spiking all the impurities on the formulation sample at LOQ, 0.1%, 0.2%, 0.3%, 0.4% and 0.6% of the target concentration concentration (800 µg mL^{-1} TS + 125 µg mL^{-1} CT). The percentage mean recoveries at each level for all the impurities were calculated.

6d. Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ for TS, CT and its impurities were estimated at a S/N of 3:1 and 10:1 respectively, by injecting a series of dilute solutions with known concentrations. Precision study was also carried at the LOQ level by injecting six individual preparations of TS, CT, its impurities and calculated the %RSD for the areas.

6e. Linearity

Linearity of test solutions was prepared from stock solution at seven concentration levels from LOQ to 0.6% of analyte concentration. The peak area versus concentration data were subjected to least-squares linear regression analysis. The calibration curve was drawn by plotting impurities areas injections against the concentration expressed in μg mL^{-1}.

6f. Robustness

To determine the robustness of the developed method, experimental conditions were deliberately changed and the resolution between TS, CT & its impurities; tailing factor and theoretical plates of TS and CT peak were evaluated. To study the effect of the flow rate on the developed method, it was changed from 0.3 mL min^{-1} to 0.25 and 0.35 mL min^{-1}. The effect of column temperature on the developed method was studied at 20 and 30˚C (Instead of 25°C). The effect of pH was studied by varying ± 0.2 pH units (i.e. 4.3 and 4.7) and the mobile phase composition was changed ±10% from the initial composition. In all the above varied conditions, the component of the mobile phase was held constant.

6g. Stability in solution and in the mobile phase

TS+CT spiked samples (Impurities spiked at 0.2% of analyte concentration) were prepared in the diluent and leaving the test solutions at room temperature. The spiked samples were injected at 0, 24, 48 hrs time intervals. The impurity content was calculated, and the consistency in the % area of the principal peak at each interval was checked. The prepared mobile phase was kept constant during the study period. The mobile phase study was demonstrated by injecting the freshly prepared sample solution at different time intervals (0-2 days).

RESULTS AND DISCUSSION

1. Optimization of chromatographic conditions

The main criterion for developing an RP-UPLC method for the determination of impurities in TS+CT pharmaceutical dosage form in a single run, with emphasis on the method being accurate, reproducible, robust, stability-indicating, linear, free of interference from other formulation excipients and convenient enough for routine use in quality control laboratories.

A spiked solution of impurities (1.6 μg mL^{-1} for TS impurities and for 0.25 μg mL^{-1} CT impurities), TS (800 μg mL^{-1}), CT (125 μg mL^{-1}) and placebo peaks were subjected to separation by RP-UPLC. Initially, the separation of all peaks was studied using literature method conditions [7]. These conditions resulted in separation of the TS peak with the placebo peaks and impurities peaks, but CT and its impurities are merging with TS impurities. Tried with different gradient programmes by changing polarities of mobile phase to separate TS, CT and its impurities, separation was not achieved properly. Also tried with different columns like BEH phenyl, BEH Shield, BEH C8 and compared separation between the columns with BEH C18. It was observed that separation is good in BEH Shield column. Hence Acquity BEH Shield-RP18, 100x 2.1mm, 1.7μm column was selected for separation work.

Based on above experiments it was decided to change the mobile phase buffer from ammonium acetate to Potassium dihydrogen phosphate by incorporating ion pain reagent (1-hexane sulphonic acid sodium salt) for better separation. Different gradient programmes were verified at different pH with this buffer and finally the chromatographic separation is achieved with following conditions. A reversed phase Acquity BEH Shield-RP18, 100x 2.1mm, 1.7μm particle size column (Thermo Scientific, Runcorn, UK) operated at 25°C with

gradient elution at 0.3 mL min^{-1} using a mobile phase buffer as 0.025M Potassium dihydrogen phosphate, 0.0027M 1-hexane sulphonic acid sodium salt and 1ml of triethyl amine in milli-Q water (pH 4.5 ±0.05 adjusted with diluted ortho phosphoric acid; UV absorbance at 290 nm; injection volume 3 µL. The mobile phase A consisted of pH 4.5 buffer & acetonitrile in the ratio 90:10 (v/v); mobile phase B consisted of pH 4.5 buffer & acetonitrile in the ratio 20:80 (v/v). The LC gradient program was set as: time (min)/% mobile phase B: /40, 1/95, 1.5/95, 2.5/40 and 3/40. Mixture of pH 4.5 buffer, acetonitrile and methanol in the ratio 60:20:20 (V/V/V) was used as diluent for sample preparation. No chromatographic interference due to the blank (diluent) and other excipients (placebo) at the retention time of Diacerein and all impurities were observed. The typical overlay chromatogram of blank and system suitability solution, placebo and spiked test is shown in Fig 3 & 4.

2. Response factor

The measurement of response factor for each impurity determination is important when the calculations are being made on a relative percent basis. Hence authentic sample of the related substances, TS and CT were dissolved in the diluent and injected, responses were calculated.

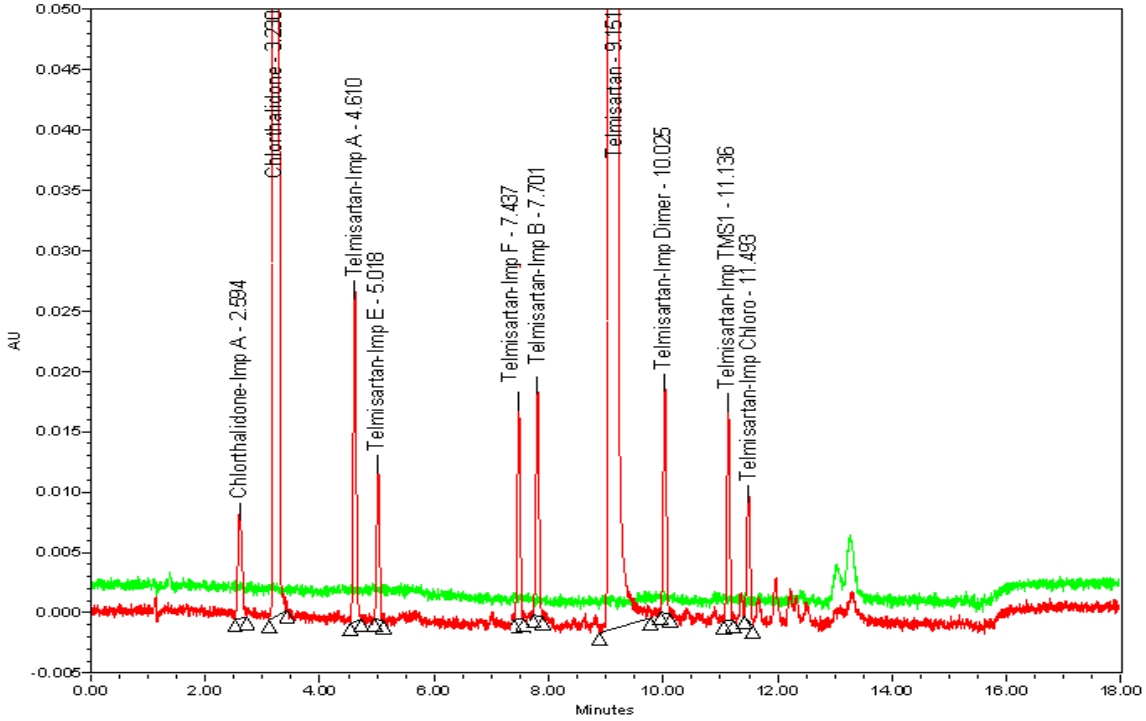


Figure 3

HPLC Overlay chromatogram of blank and system suitability solution

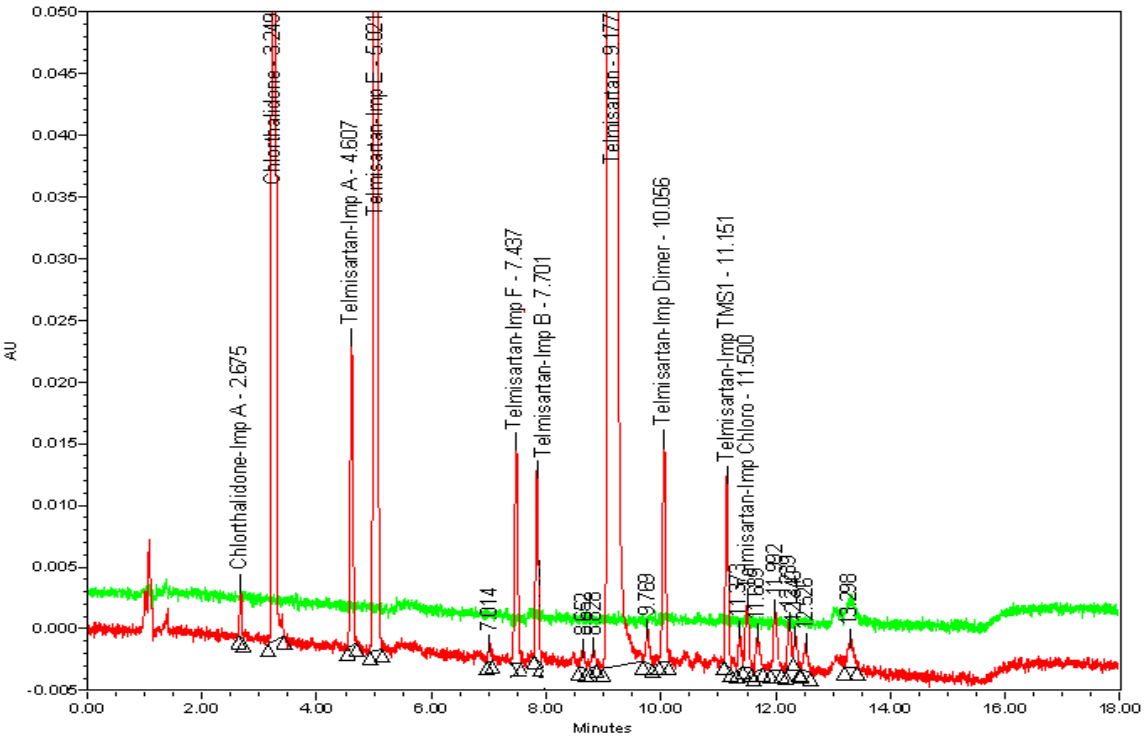


Figure 4
HPLC Overlay chromatogram of placebo and spiked sample

3. Method validation

After development of method it was subjected to method validation as per ICH guideline [11]. The method was validated to demonstrate that it is suitable for its intended purpose to evaluate adequate validation characteristics such as specificity, accuracy, precision, linearity, robustness, ruggedness, solution stability, LOD and LOQ and stability indicating capability.

a. System suitability

The percentage relative standard deviation (RSD) of area from six replicate injections was below 5.0 % (Diluted standard solution, 1.6 µg mL^{-1} of TS and 0.25 µg mL^{-1} of CT). Resolution between impurities 2.5, the tailing factor for TS and CT is not more than 1.5 and the theoretical plates are not less than 2000.

b. Specificity

All forced degradation samples were analyzed with the fore mentioned UPLC conditions using a PDA detector to monitor the homogeneity and purity of the TS, CT peaks and its related impurities. Individual impurities, placebo, TS and CT were verified and proved to be non-interfering with each other thus proving the specificity of the method.

Fig 4 shows that there is no interference at the RT (retention time) of TS, CT and all known impurities from the other excipients. Degradation was not observed in visible light, UV and humidity stress studies. Significant degradation was not shown in base hydrolysis and water hydrolysis. However, acid hydrolysis, thermal stress and oxidative conditions showed significant degradation. The specificity results are shown

Table 2a & 2b. Acid hydrolysis, thermal stress and oxidative degradation chromatograms are shown in Fig 5a, 5b & 5c. It is interesting to note that all the peaks due to degradation are well resolved from the peaks of TS, CT and its impurities. Further the peak purity of TS, CT and its impurities was found to be homogeneous based on the evaluation parameters such as purity angle and purity threshold using Waters Empower Networking Software. The verification of peak purity indicates that there is no interference from degradants, facilitating error-free quantification of TS and CT impurities. Also the mass balance of stressed samples was found to be more than 98%. Thus, the method is considered to be "Stability-indicating".

Table 1
System suitability results

Parameter	% RSD* of standard		Theoretical plates*		Tailing Factor*		Resolution 1	Resolution 2
	TS	CT	TS	CT	TS	CT		
As such method	0.3	1.0	35725	29147	1.0	1.1	2.9	1.9
At 0.25 ml/min flow rate	0.5	1.5	31256	25469	1.1	1.0	3.1	2.0
At 0.35 ml/min flow rate	1.2	0.9	32857	31256	1.0	1.2	2.8	1.9
At 25°C column temperature	0.5	2.5	25756	28565	1.0	1.2	3.2	2.1
At 35°C column temperature	0.7	3.1	28976	32756	1.1	1.3	3.3	2.3
At pH 4.8 (Buffer pH)	1.5	1.8	34156	26756	1.1	1.1	3.2	2.4
At pH 5.2 (Buffer pH)	2.1	1.7	30985	28546	1.1	1.0	2.8	2.1

* Determined on six values **Resolution 1 :** Resolution between Telmisartan Impurity B and Impurity F **Resolution 2 :** Resolution between Dimer and TMS1 impurity

Table 2a.
Forced degradation data for Telmisartan

Degradation conditions	Telmisartan			
	% degraded	Purity angle	Purity Threshold	Mass balance (%)
Refluxed with 0.2 N HCI solution for about 30 min at 60 °C	4.36	5.109	5.523	98.9
Refluxed with 0.2 N NaOH solution for about 30 min at 60 °C	0.92	5.368	6.468	99.5
Refluxed with 3% H_2O_2 solution for about 15 min at 60 °C	8.98	4.383	6.020	98.7
Refluxed with purified water for about 30 min at 60 °C	0.23	3.620	6.525	100.1
Exposed to dry heat for about 3 hours at 105 °C	6.08	3.260	7.108	99.8

Exposed to humidity at 25 °C, 90% RH for about 7 days	0.31	5.484	5.828	100.3
Exposed to Photo stress (1.2 Million lux hours followed by 200 Watt hours)	0.25	3.693	7.830	99.2

Table 2b
Forced degradation data for Chlorthalidone

Degradation conditions	Chlorthalidone			
	% degraded	Purity angle	Purity Threshold	Mass balance (%)
Refluxed with 0.2 N HCI solution for about 30 min at 60 °C	0.79	0.157	0.383	100.5
Refluxed with 0.2 N NaOH solution for about 30 min at 60 °C	0.61	0.182	27.765	99.5
Refluxed with 3% H_2O_2 solution for about 15 min at 60 °C	0.59	0.189	0.366	100.4
Refluxed with purified water for about 30 min at 60 °C	0.40	0.179	0.361	99.8
Exposed to dry heat for about 3 hours at 105 °C	1.14	0.200	0.387	99.1
Exposed to humidity at 25 °C, 90% RH for about 7 days	0.28	0.189	0.370	98.9
Exposed to Photo stress (1.2 Million lux hours followed by 200 Watt hours)	0.22	0.180	0.357	99.6

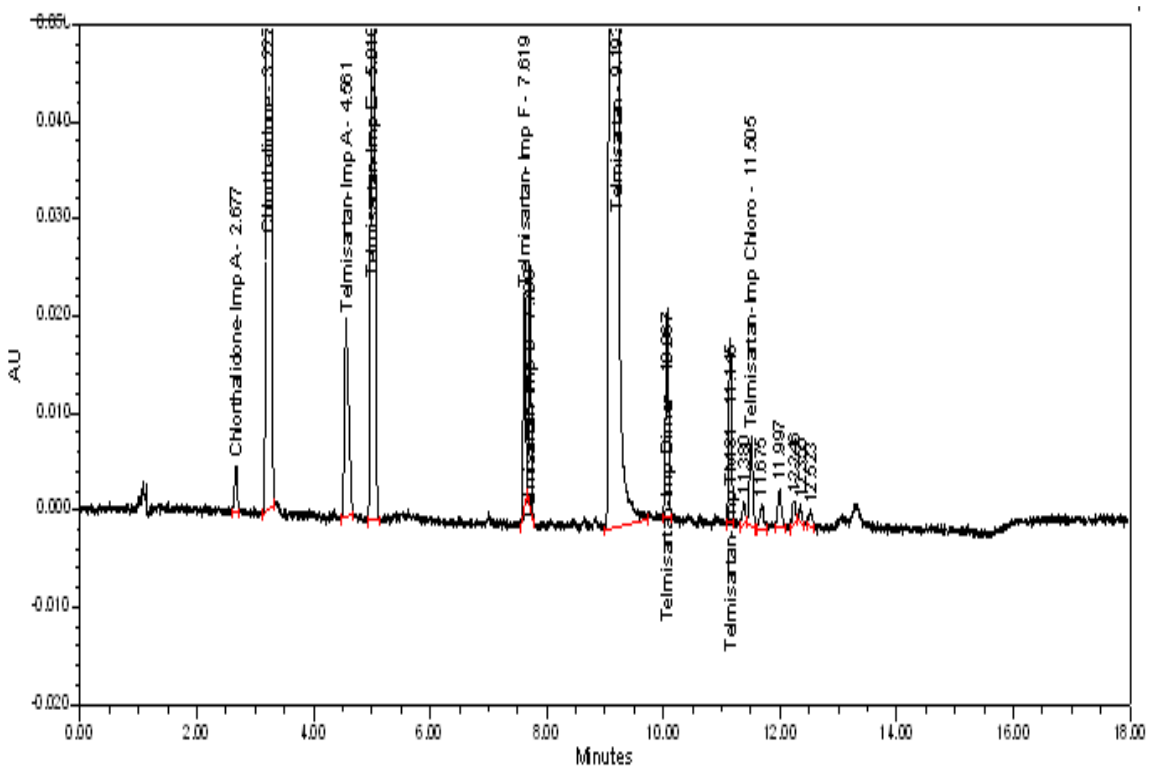


Figure 5a Typical chromatogram and purity plot of acid hydrolysis sample

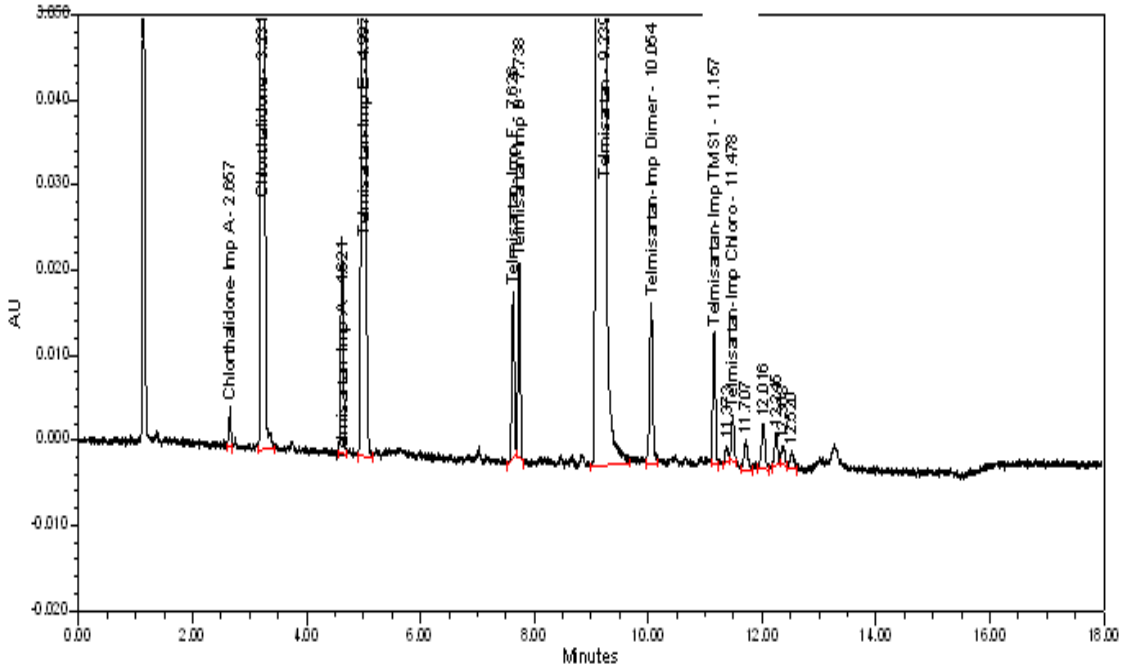


Figure 5b Typical chromatogram and purity plot of peroxide stressed sample

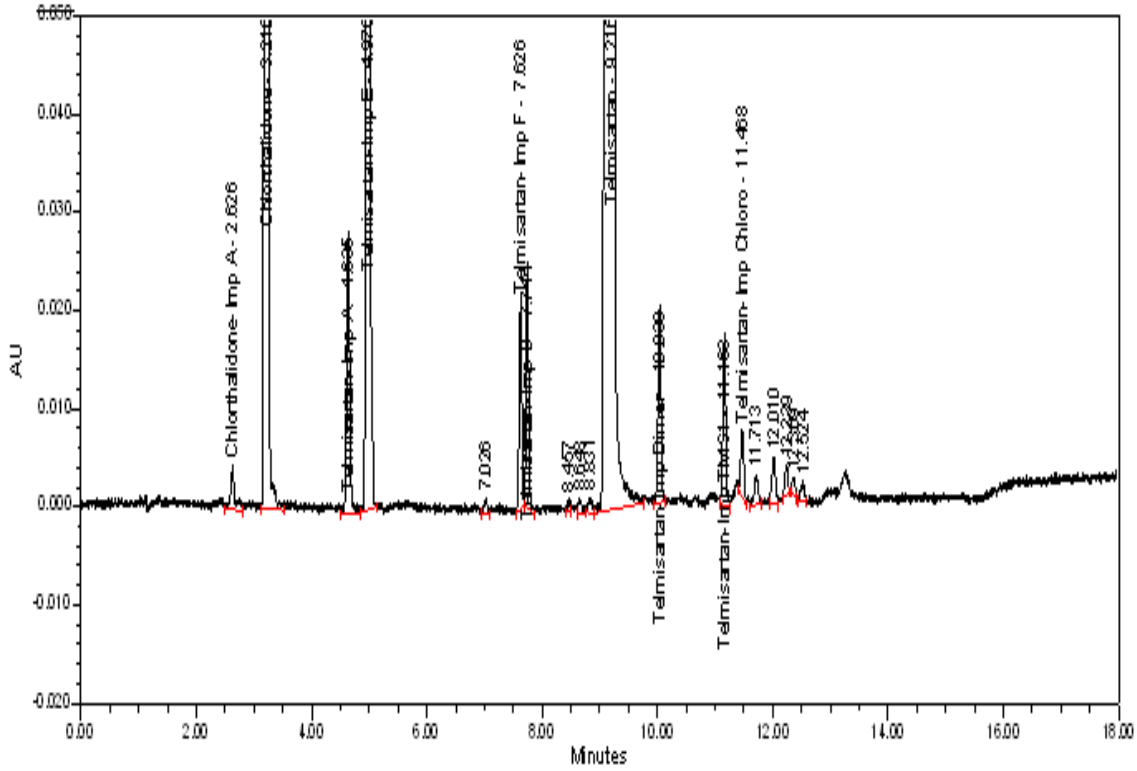


Figure 5c Typical chromatogram and purity plot of heat stressed sample

c. Precision

The % RSD for the individual % of TS, CT and its impurities in method precision study was within 1.2%. The results obtained in the intermediated precision study for the % RSD of the individual % of TS, CT and its impurities in were well within 2.7%, conforming high precision of the method. The results are shown in Table 3a & 3b.

d. Accuracy

The recovery of all the ten impurities from finished pharmaceutical dosage form ranged from 85.0 % to 115.0 %. The summary of % recovery for individual impurity was mentioned in Table 4.

Table 3a
Results of method precision

Preparation	Chlorothalidone	Telmisartan						
	Impurity A	Impurity A	Impurity B	Impurity E	Impurity F	Dimer	TMS1	Chloro analogue
Prep-1	0.196	0.196	0.202	0.194	0.200	0.205	0.197	0.201
Prep-2	0.202	0.195	0.200	0.197	0.200	0.205	0.199	0.200
Prep-3	0.200	0.198	0.200	0.194	0.200	0.206	0.202	0.212
Prep-4	0.202	0.197	0.202	0.197	0.199	0.205	0.202	0.197
Prep-5	0.202	0.197	0.202	0.192	0.202	0.208	0.2068	0.216
Prep-6	0.2022	0.196	0.202	0.196	0.201	0.204	0.204	0.197
Avg	0.201	0.197	0.201	0.195	0.200	0.206	0.202	0.204
%RSD	1.2	0.5	0.5	1.0	0.5	0.7	1.7	4.0

Table 3b.
Results of intermediate method precision

Preparation	Chlorothalidone	Telmisartan						
	Impurity A	Impurity A	Impurity B	Impurity E	Impurity F	Dimer	TMS1	Chloro analogue
Prep-1	0.199	0.201	0.194	0.208	0.198	0.202	0.212	0.197
Prep-2	0.203	0.197	0.196	0.210	0.200	0.199	0.210	0.209
Prep-3	0.195	0.201	0.200	0.199	0.203	0.202	0.215	0.205
Prep-4	0.193	0.203	0.200	0.201	0.210	0.208	0.207	0.199
Prep-5	0.202	0.201	0.203	0.199	0.198	0.210	0.213	0.201

Prep-6	0.205	0.199	0.209	0.210	0.208	0.201	0.210	0.197
Avg	0.199	0.200	0.200	0.205	0.203	0.204	0.211	0.201
%RSD	2.4	1.0	2.7	2.6	2.5	2.1	1.3	2.4

Table 4.
Accuracy of the method

Preparation	Recovery (%)[a]							
	Chlorothalidone	Telmisartan						
	Impurity A	Impurity A	Impurity B	Impurity E	Impurity F	Dimer	TMS1	Chloro analogue
LOQ level	99.8	106.5	96.5	95.4	102.5	100.1	94.5	97.8
0.1% level	102.6	104.5	101.6	99.9	98.3	102.4	96.0	98.5
0.2% level	95.4	98.5	95.5	91.9	94.8	99.5	93.5	99.6
0.3% level	103.3	95.2	96.9	94.8	99.5	98.5	93.8	100.1
0.4% level	99.0	95.5	94.4	95.4	98.8	97.9	93.8	98.5
0.6% level	94.5	95.4	93.6	93.1	100.5	95.8	94.8	99.5

[a]Mean for three determinations at each level.

e. Limit of detection (LOD) and Limit of quantification (LOQ)

LOD values were achieved at about 0.1027 µg mL^{-1} for TS, CT and its impurities. LOQ values were achieved at about 0.3112 µg mL^{-1} for TS, CT and its impurities. The % RSD of precision at LOQ concentration for TS, CT and its impurities was found to be below 1.2. The results of precision at LOQ level is shown in Table 5.

f. Linearity

Linearity regression analysis demonstrated acceptability of the method for quantitative determination range of LOQ to 0.6% of test concentration. The coefficient of correlation was found to be more than 0.999. The regression statistics are shown in Table 5, with the linearity curve for all impurities represented in Fig 6a to 6j.

Table 5. LOD, LOQ, Precision at LOQ and Regression statistics

Substance	LOQ ($\mu g\ mL^{-1}$)	LOD ($\mu g\ mL^{-1}$)	% RSD at LOQ	Linearity range ($\mu g\ mL^{-1}$)	Correlation coefficient (R^2)	Y-intercept Bias in %
CT	0.0556	0.0184	4.1	0.0556 to 0.7506	0.9998	1.9
CT impurity A	0.0532	0.0176	3.5	0.0532 to 1.1400	0.9996	1.4
TS	0.1594	0.0526	2.9	0.1594 to 4.7808	0.9994	3.1
TS impurity A	0.2383	0.0786	3.4	0.2383 to 4.2893	0.9999	2.4
TS impurity B	0.3025	0.0998	1.9	0.3025 to 6.8057	0.9999	2.0
TS impurity E	0.3066	0.1012	2.5	0.3066 to 6.4391	0.9995	1.6
TS impurity F	0.2175	0.0718	4.4	0.2175 to 4.7853	0.9999	0.6
TS Dimer impurity	0.2947	0.0973	3.7	0.2947 to 5.7470	0.9997	4.1
TS TMS1 impurity	0.3112	0.1027	2.4	0.3112 to 4.2017	0.9999	0.0
TS Chloro analogue	0.2346	0.0774	1.7	0.2346 to 4.2993	0.9996	3.5

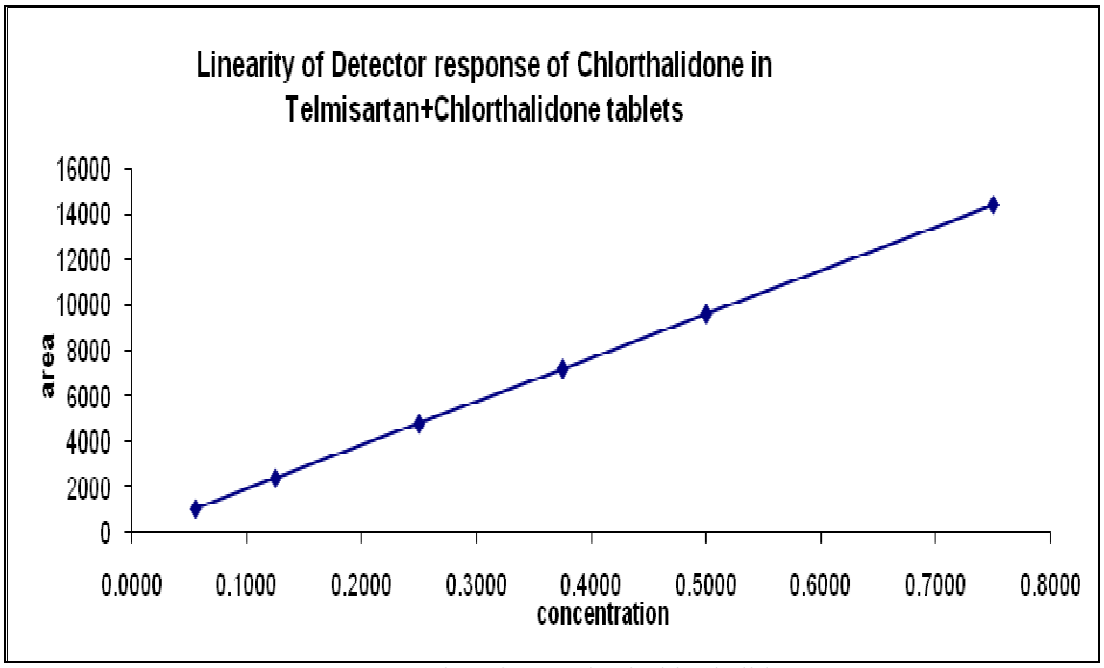


Figure 6a. Linearity graph of Chlorthalidone

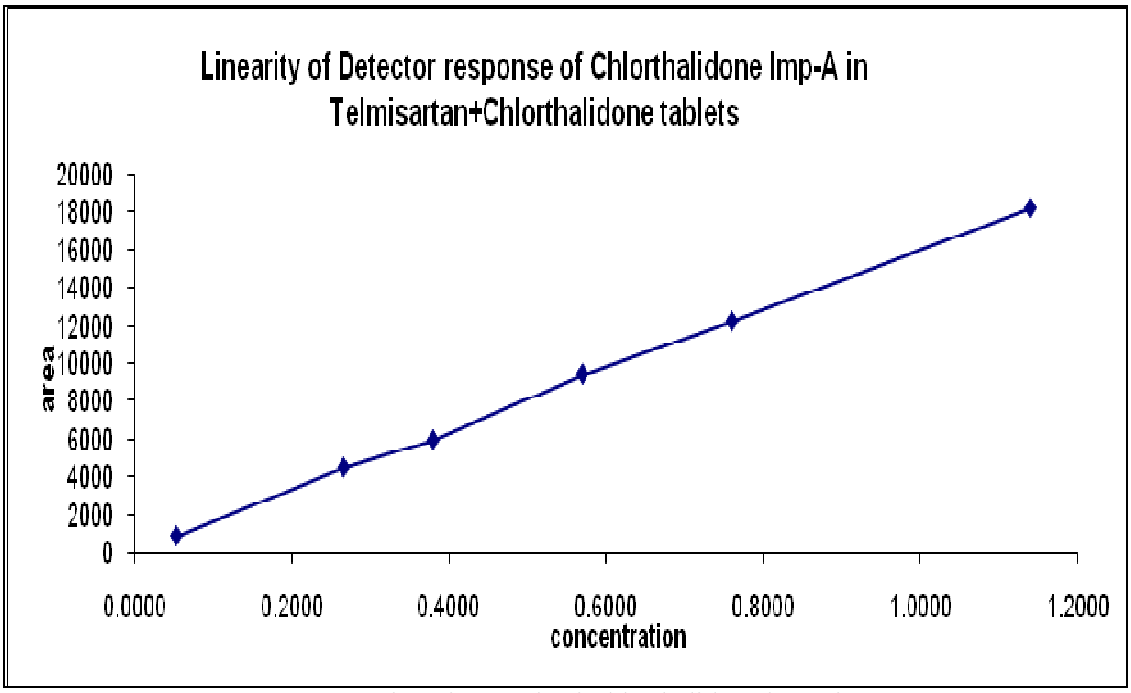


Figure 6b. Linearity graph of Chlorthalidone impurity A

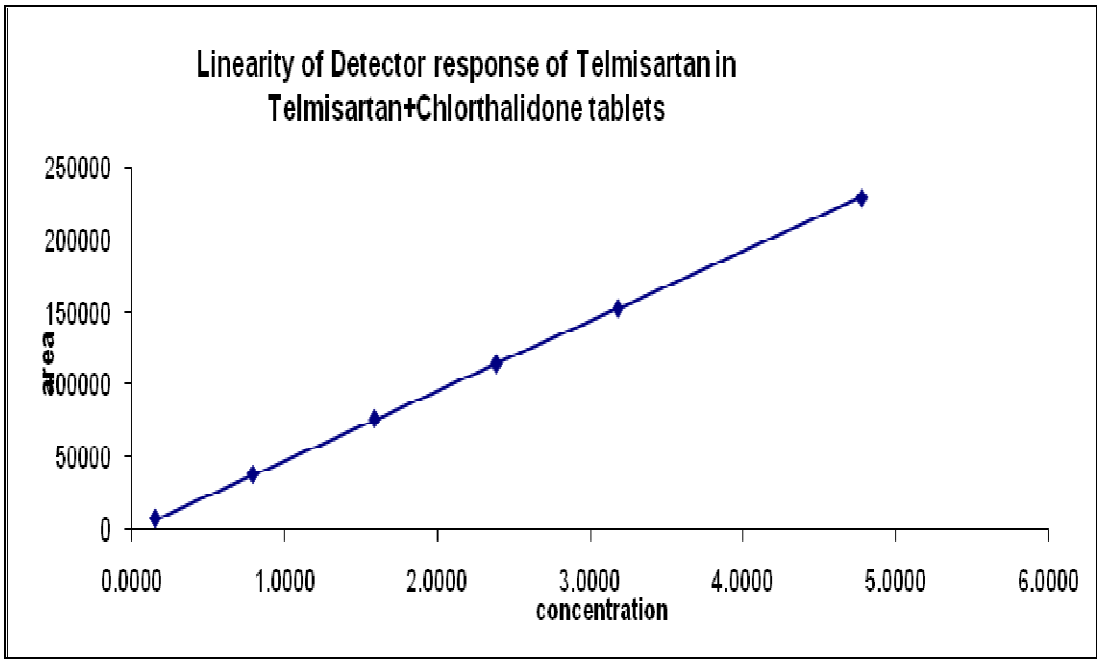


Figure 6c Linearity graph of Telmisartan

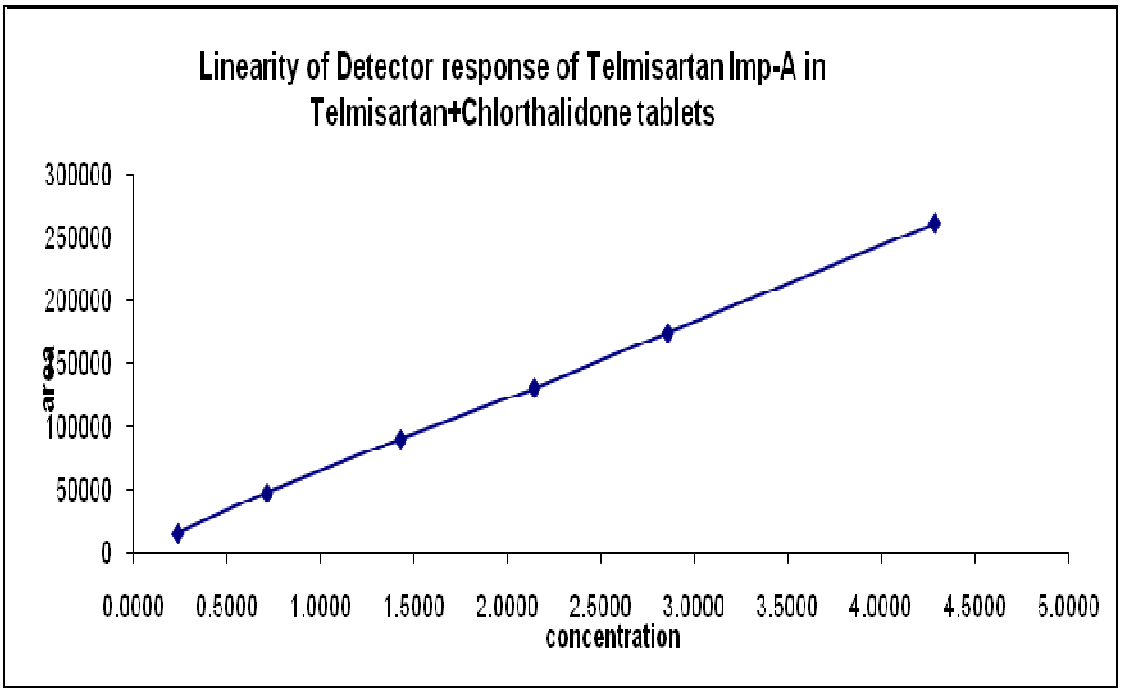


Figure 6d Linearity graph of Telmisartan impurity A

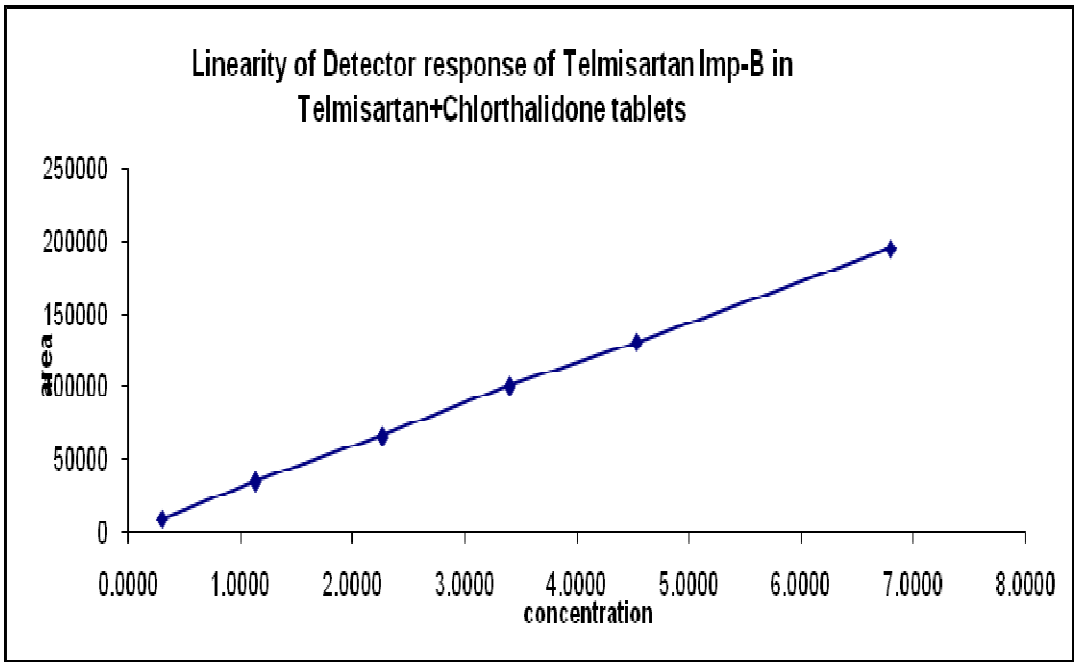


Figure 6e Linearity graph of Telmisartan impurity B

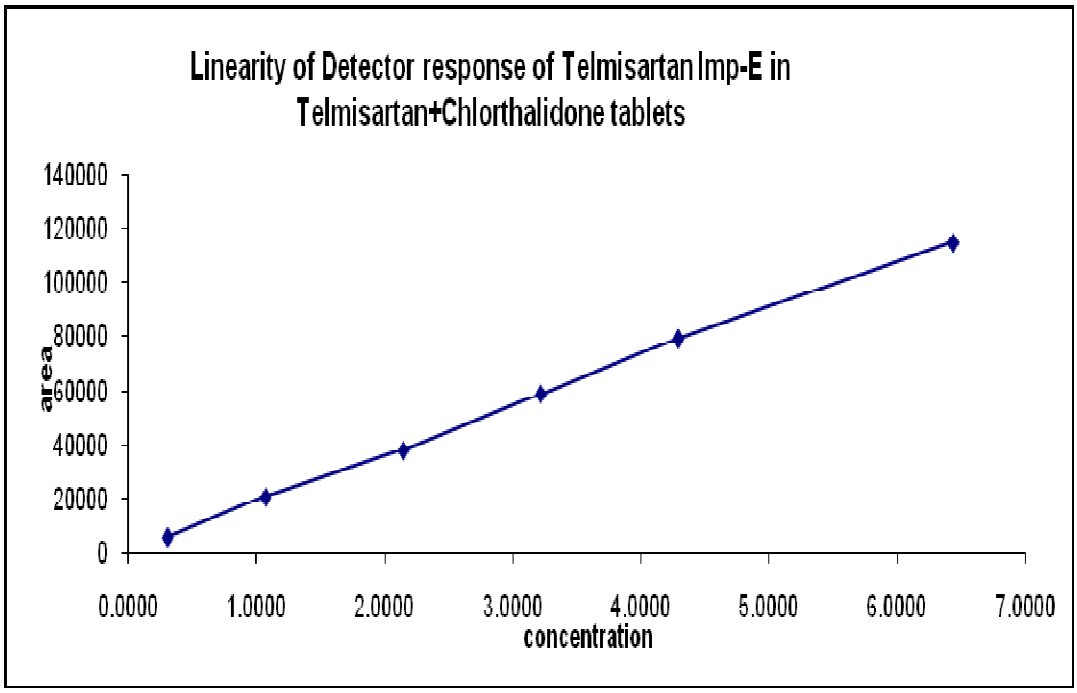


Figure 6f Linearity graph of Telmisartan impurity E

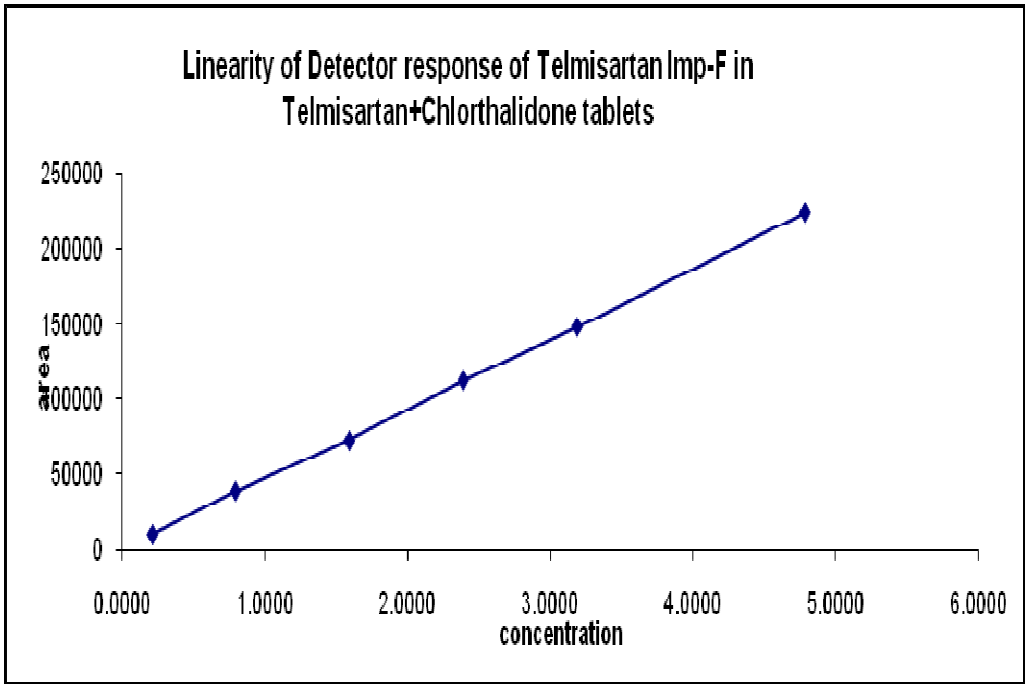


Figure 6g Linearity graph of Telmisartan impurity F

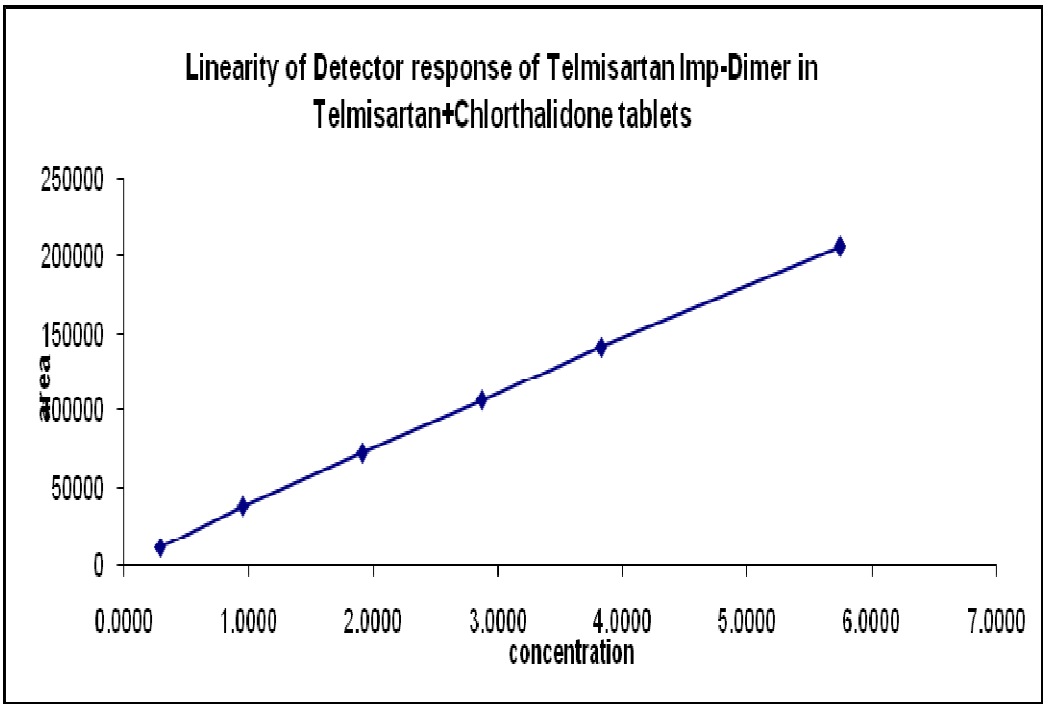


Figure 6h Linearity graph of Telmisartan Dimer impurity

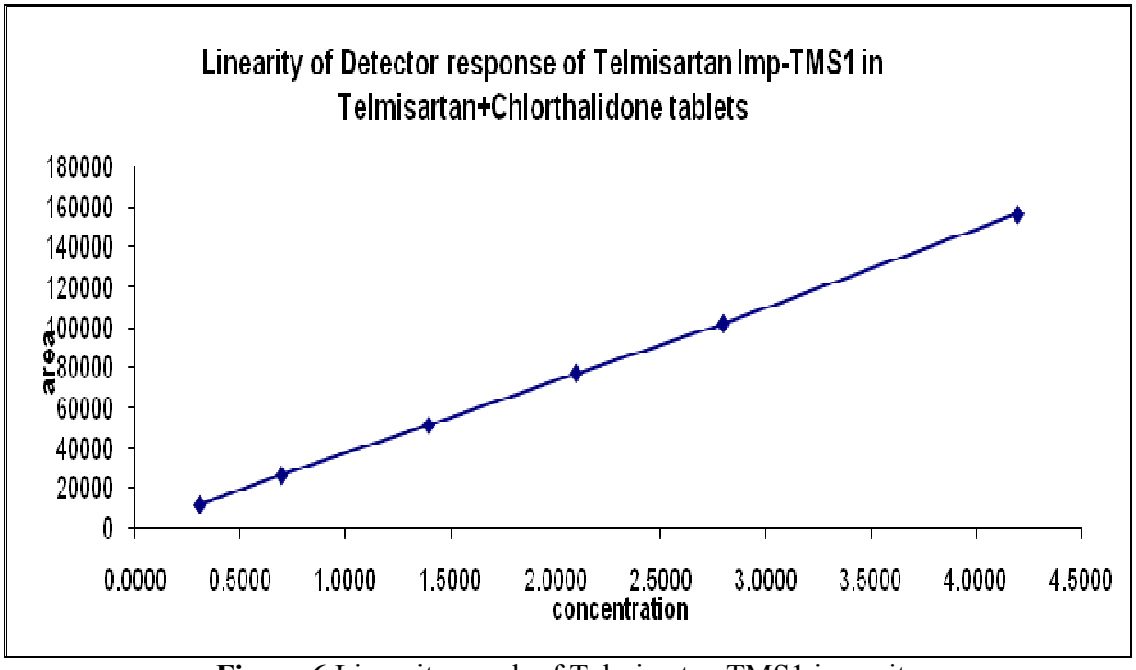


Figure 6 Linearity graph of Telmisartan TMS1 impurity

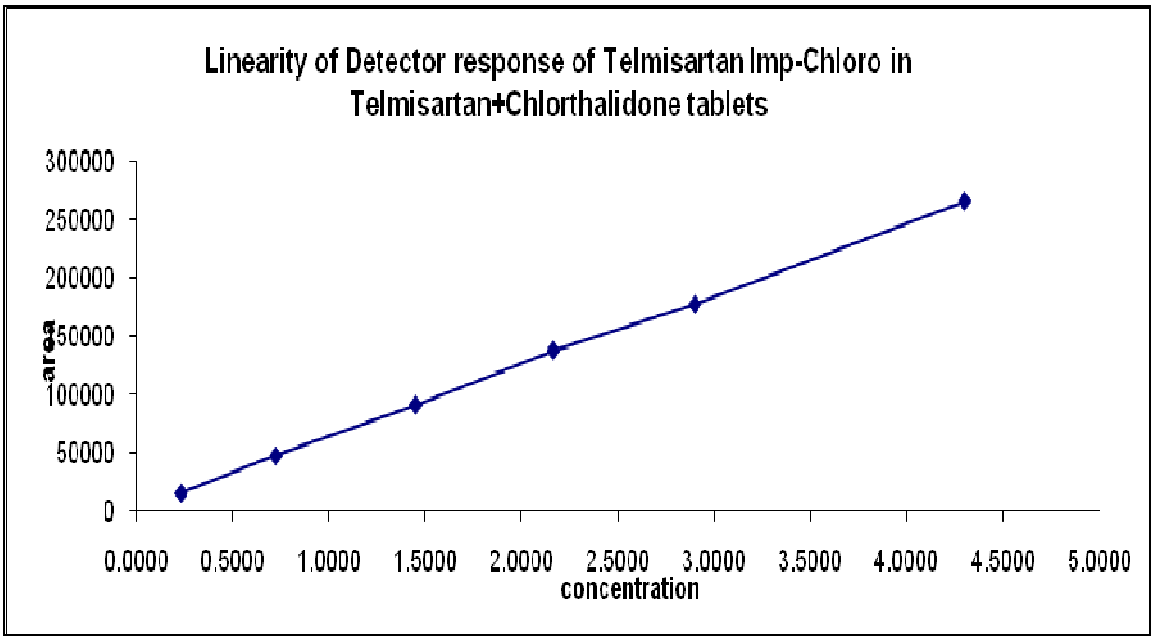


Figure 6j Linearity graph of Telmisartan Chloro analogue

g. Robustness

No significant effect was observed on system suitability parameters such as resolution, RSD, tailing factor, or the theoretical plates of TS and CT when small but deliberate changes were made to chromatographic conditions. The results are presented in Table 1, along with the system suitability parameters of normal conditions. Thus, the method was found to be robust with respect to variability in applied conditions.

h. Stability in solution and in the mobile phase

No significant changes were observed in the content of impurities of TS and CT impurities during solution stability and mobile phase stability experiments when performed using the impurities method. The solution stability and mobile phase stability experiment data confirms that the sample solution and mobile phases used during the impurity determination were stable for at least 48 h.

CONCLUSION

The gradient UPLC method developed for simultaneous determination of Telmisartan and Chlorthalidone impurities pharmaceutical dosage form was precise, accurate and specific. The method is validated as per ICH guidelines and found to be specific, precise, linear, accurate, rugged, and robust. The developed method can be used for the stability analysis of either individual or combination products of Telmisartan and Chlorthalidone formulations.

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Novel in vitro Antioxidant Bioassay, QSAR and Docking Studies of Thienopyridine Derivatives

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ABSTRACT

A novel *in vitro* antioxidant spectrophotometric method is estimated from the charge transfer complex (CTC). The method is based on the formation of a CTC between **π**-acceptor and ***n***-donor. A simple, rapid and sensitive antioxidant bioassay study of thienopyridines was monitored at 440*nm*. The source of free radicals is CTC and thienopyridine derivatives act as free radical scavengers. The IC_{50} values and antioxidant activities were predicted. The antioxidant property of thienopyridines was influenced to a great extent by the increase in the strength of electron withdrawing groups on aryl ring. semi empirical methods , AM1 and PM3 were used to estimate different physicochemical parameters. QSAR results revealed that softness and EA were responsible for high antioxidant activity. Docking studies were also performed with the active site of cyclo-oxygenase-2 to identify hydrogen bonding, hydrophobic and ionic interactions. GOLD, Auto dock and Argus lab docking results exposed the active site residues. All most all compounds showed good inhibitory activity values against cyclo-oxygenase-2 with the formation of strong hydrogen bond interactions with the residues of active site. The chemical environment may serve as a starting point for synthesis of cyclo-oxygenase-2 inhibitors with improved efficacy.

KEY WORDS

Thienopyridines, Free radical scavenging, Antioxidant, Semi empirical methods, Regression analysis, Docking studies

1. INTRODUCTION

Thienopyridine (4, 5, 6, 7-tetrahydrothieno [3, 2-c] pyridines) and their derivatives are important heterocyclic compounds (figure1) that are widely distributed in nature. Many of these compounds contain thienopyridine skeletons which were reported to have antibacterial [**1**], non-peptide GPIIb/IIIa antagonists [**2**], platelet aggregators and antithrombotic agents[**3**].The incorporation of benzylic or substituted benzylic groups on the nitrogen of the thienopyridine ring can bring an extensive modification in the biological activities of parent compound. Among the substitutions occurred at nitrogen of the thienopyridine moiety, increased the biological activity of the parent moiety, showed good antithrombotic activity in Ticlopidine and with more increased activity in Clopidogrel. Later on, the studies proved that the Prasugrel to be more efficient drug candidate than the existing Clopidogrel by making the structural modifications to the parent thienopyridine moiety **[4]**. Hence,

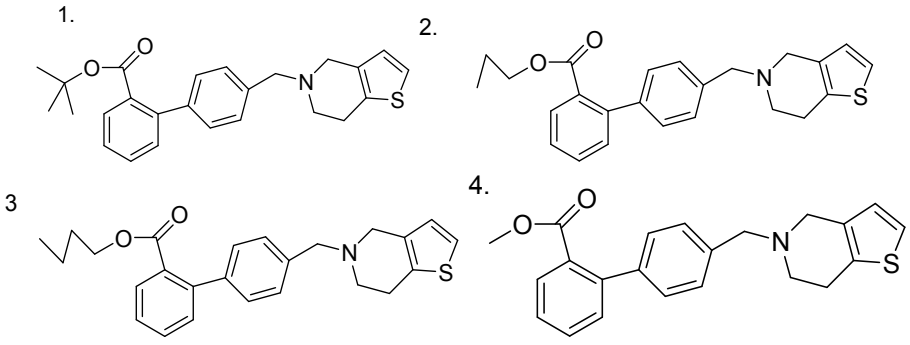
different substitutions at nitrogen of the thienopyridine moiety may bring extensive improvement in the biological activity of the new chemical entities (NCEs). Biphenyl system is the key structural unit in most of the sartans, non-peptide antagonists of angiotensin II receptors. Along with their well-known antihypertensive activity **[5, 6]**, biphenyl substituent's have also been demonstrated as stimulators of growth hormone release [**7],** metallo protease inhibitors**[8,9],**antibiotics[**10]** and chloride channel blockers [**11**]. In this context, biphenyls and its derivatives had received substantial attention as good therapeutics. Thienopyridine derivatives also provide an antioxidant protective effect that may contribute to their pharmacological activities **[12]**. The antioxidant properties of thienopyridine are known to be influenced to a greater extent by the aryl structures of the substitutions on aryl rings. Especially, the free radical donor substituents were one of the key groups to enhance greatly the antioxidant activity of thienopyridine mainly due to its easy conversion to phenoxy radicals through the hydrogen atom transfer mechanism **[13]**.

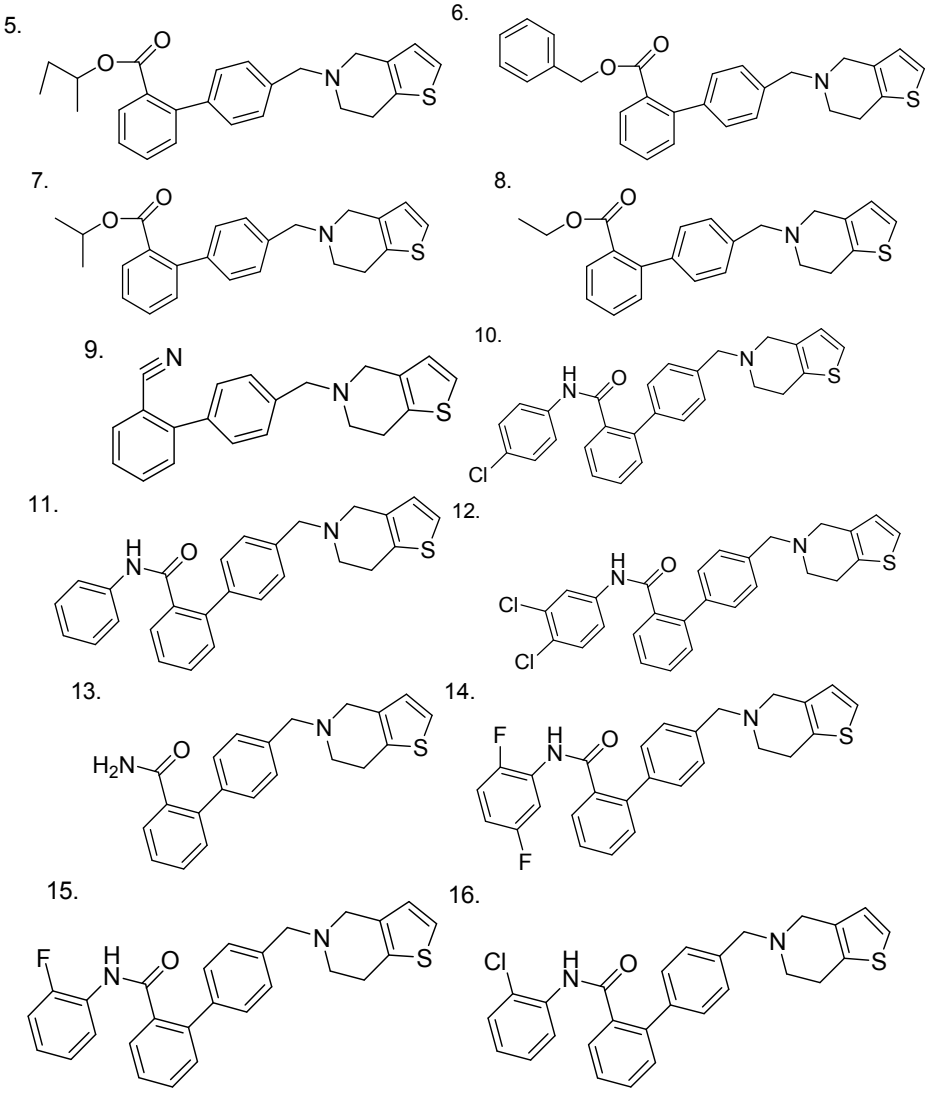
The intermolecular charge transfer complex (CTC) is formed between electron donor and electron acceptor. It is a general phenomenon in organic chemistry and Mullikan considered bond between the components of the complex being postulated to arise from the base to the empty orbital of the acceptor. The CTC have unique absorption bands in ultra violet-visible region. 2, 3-Dichloro-5, 6-dicyano 1,4-Benzoquinone(DDQ) is a ***π***-electron acceptor often it forms highly coloured electron donar-acceptor or CTC with the triethyl amine. The molecular interactions between electron donors and acceptors are generally associated with the formation of intensity coloured CTC. The photometric methods based on molecular interactions are simple and suitable since they result in the rapid formation of the complexes. Triethyl amine is a good ***n***-electron donor and forms CTC with π-acceptor, DDQ **[14,15]**. Thienopyridine derivatives may act as free radical scavengers due to their structural features.

Several methods were used for the estimation of antioxidant activity of synthetic or natural source [**16-19]**. The CTC, formed between DDQ and ***n***-donor which was used to investigate thienopyridine derivatives antioxidant activity in the present study.

The aim of the present work is to develop *in vitro* antioxidant property of thienopyridines by spectrometric method. The *in vitro* free radical scavenging activities of these derivatives may be quantitatively estimated. Molecular modeling studies further help in understanding the various interactions between the ligands and enzyme active sites.

Figure 1
Structures of thienopyridine derivatives





2. Experimental Methods

Antioxidant Bioassay

All the chemicals were used of analytical grade. A Systronics UV-Visible PC Based double beam spectrophotometer-2202 equipped with 1.0cm quartz cells with a fixed slit width (2*nm*) was used to record the absorption spectra.

Antioxidant activity of thienopyridines was measured by using spectrophotometer. To the 10mL of 3×10^{-4}M CT complex, 10mL of 10^{-4}M substituted thienopyridine was added. The mixture was allowed to stand 5 min at room temperature and then the absorbance of coloured solution was measured at 440*nm*.The capacity of free radical scavenging activity of thienopyridine was calculated using the following equation:

$$\%RSA = \frac{Ai - Af}{Ai} x100$$

RSA (radical scavenging activity) of thienopyridine, $\mathbf{A_i}$ initial absorbance of the CTC, $\mathbf{A_f}$ is the absorbance of the test/ standard compound.

The optical density was recorded as decrease in intensity of purple red colour of CTC. The antioxidant activity is expressed as IC_{50}. **(Table 1) [20]**. The antioxidant activity was compared with ascorbic acid, used as a standard.

Table 1.
Antioxidant activity of thienopyridine derivatives

c	1	2	3	4	5	6	7	8	9	1	1	1	1	1	1	1
% RSA	4	4	4	5	5	4	5	4	3	5	6	7	6	7	6	7
I	1	1	1	0	0	1	0	1	1	0	0	0	0	0	0	0
A	2	2	3	3	3	.	3	2	2	3	3	3	3	3	3	3

Comp = compound, %RSA = %Radical Scavenging Activity, Act = Activity

2.1. Computational Methodology

2.1.1. Construction of molecular structures

A series of thienopyridine compounds tested for inhibitory activity was selected for the present study and the program of window Hyperchem software Inc (http://www.warezdestiny.com/free-hyp)**[21]** was used in modeling studies. The molecules were generated and the energy was minimized using molecular modeling pro. The window version software SPSS10 (SPSS Software; Consult http://www.spss.com)**[22]** was used in the regression analysis

.

2.1.2. Calculation of quantum chemical descriptors

All of the molecular structures of the compounds were initially optimized geometrically using the semi-empirical method AM1 (Austin Model 1) and PM3 (Parameterization Model 3)[**23]**. The quantum chemical descriptors (variables)[**24-27]** obtained for model building in this work include: energy of cation (E_{cation}), energy of anion (E_{anion}), the electron affinity (EA)(calculated from $E_{neutral}$-E_{anion}), ionization potential (IP) (calculated from E_{cation}-$E_{neutral}$), electro negativity(χ), hardness(η), softness(S), electrophilic index (ω), partition coefficient (LogP), hydration energy (HE),chemical potential (μ) and polarisability (Pol)were obtained for thienopyridine derivatives.

2.2. Molecular Modeling Studies

QSAR technique was applied to the thienopyridine derivatives which were varied on aryl ring position. The appropriate descriptors or parameters for the compounds were used as independent variables for deciding in cyclo-oxygenase-2 (4COX) inhibitory activity.

Molecular docking methodologies ultimately seek to predict the best mode by which a compound fit into a binding site of a macro molecular target. This predicts the best candidate providing an insight on substitution and configuration for optimum receptor pit which leads to the development of best pharmacophore activity.

2.2.1 .GOLD2.0 Software

The GOLD2.0 (Genetic Optimization for Ligand Docking) program uses a genetic algorithm (GA) to explore the full range of ligand flexibility and the rotational flexibility of selected receptor hydrogens **[28, 29]**.The mechanism for ligand placement is based on fitting points. The program adds fitting points to hydrogen-bonding groups on the protein and ligand and maps acceptor points in the ligand, on donor points in the protein and vice versa. The docking poses are ranked based on a molecular mechanics like scoring function. There are two different built in scoring functions in the GOLD program Gold Score and Chem score. The interaction of the ligands with the receptor in the modeled complexes was investigated and observed for the fitness function ability on protein of cyclo-oxygenase-2. The 3D structure of protein cyclo-oxygenase-2 (4COX) was selected from PDB(Protein Data Bank) Bank RCSB with an X-ray resolution in the range of 2.90A^0 [**30]**. Cyclooxygenases are enzymes that take part in a complex biosynthetic cascade that results in the conversion of polyunsaturated fatty acids to prostaglandins and thromboxane(s)[31]. Their main role is to catalyze the transformation of arachidonic acid into the intermediate prostaglandin H2, which is the procursor of a variety of prostanoids with diverse and potent biological actions.COX-2 plays a major role in prostaglandin biosynthesis in inflammatory cells and in the central nervous system[32]. Prostaglandin synthesis in these sites is a key factor in the development of inflammation and hyperalgesia. COX-2 inhibitors have analgesic and anti-inflammatory activity by blocking the transformation of arachidonic acid into prostaglandin H2 selectively. The three-step mechanism explains behind the inhibitory effects of selective COX-2 inhibitors. The first step accounts for the contact of the inhibitor with the gate of the hydrophobic channel (called the lobby region)[33]. The second step could account for the movement of the inhibitor from the lobby region to the active site of the COX enzyme. The last step probably represents repositioning of the inhibitor at the active site, which leads to strong interactions of the phenylsulfonamide or phenylsulfone group of the inhibitor and the amino acids of the side pocket. It is directly inhibition to postaglanding.

The fitness function that was implemented in GOLD consisted basically of H-bonding, complexing energy, and ligand internal energy terms. The GOLD Score was calculated by defining the active site using the list of atom numbers and retaining all the other default parameters. The docking studies are frequently used to predict the binding orientations of small molecules of drug candidates to their protein targets in order to predict the affinity of the small molecules *viz;***1-16**. A population of possible docked orientations of the ligand is set up at random. Each member of the population is encoded as a chromosome, which contains information about the mapping of ligand H-bond atoms onto protein H-bond atoms, mapping of hydrophobic points, all the conformation around flexible ligand bonds and protein OH groups. All docking runs were carried out using standard default settings with a population size of 100, a selection pressure of 1.1, a maximum of 100000 operations, number of islands as 5, a niche size of 2, and a mutation and cross over rate of 95. Docking poses were obtained by applying both Chemscore and Gold score. In the present study of the GOLD Program, the performance of both Gold Score, Chemscore are found to be good. SPDBV3.7 software **[34]** was used for preparation of protein-ligand complexes by adding hydrogen atoms, removing water molecules, co-crystallization of inhibitors. Enzyme-inhibitor interactions within a radius equal to 15Å centered on reported bound inhibitors were taken into account.

2.2.2. Argus Lab

Argus Lab 4.0.1[**35]** was used for molecular modeling studies, which is very flexible and can reproduce crystallographic binding orientation. Argus lab provides a user friendly graphical interface and uses shape dock algorithm, to carry out docking studies. This helps to visualize the binding conformations of these analogues, within the active site region of cyclo-oxygenase-2 protein.

2.2.3. Auto dock

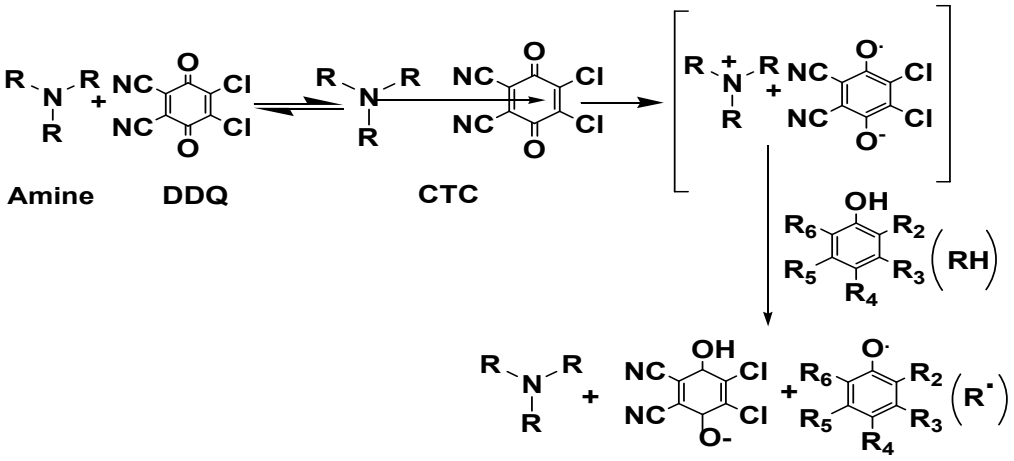
Autodock4.0 [**36]** was used to estimate binding free energy and inhibition constant (K_i).

3. Results and Discussions

3.1. Free radical scavenging activity

The photometric methods based on molecular interactions are simple and suitable since it results in the rapid formation of the complexes. The CTC is formed between triethyl amine as ***n*-**donor (D) and DDQ as π-acceptor. The Beer's law is obeyed over the concentration ranges. The described method was successfully applied to the determination of antioxidant activity. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The Lower IC_{50} value represents higher antioxidant activity. To accommodate the observed results, the following reaction mechanism is proposed in **Scheme-1.**

Scheme-1



The CTC decomposes to give DDQ free radical which in turn forms R· radical on abstraction of hydrogen from thienopyridine(RH). The R· radical then undergoes further reactions which control the overall stoichiometry *i.e*., the number of molecules DDQ reduced by RH. Mixing of DDQ solution to donor resulted in decrease in intensity of color *i.e.* shifted to shorter wave length (hypsochromic shift).

3.2. Linear regression model analysis

The biological activity data and the physicochemical properties IP, EA, ω, EN, η, *S*, LogP, HE and Pol of the thienopyridine derivatives are given in **Table 2** and **Table 3**. The data from these tables were subjected to regression analysis. The correlation matrices were generated with sixteenth ienopyridine derivatives**.** The term close to **1** indicates high co-linearity, while the value below 0.5 indicates that no co-linearity exist between more than the two parameters.

Table 2.
Antioxidant activities and molecular descriptors values of thienopyridine derivatives in AM1 method

Compd	Obs. Act.	Eq-1		Eq-2		Molecular descriptors								
		Predicted	residual	Predicted	Residual	IP (eV)	EA (eV)	EN (eV)	η (eV)	S (eV^{-1})	ω	HE (K.cal/mol)	LogP	Pol (Å^3)
1	2.93	2.96	-.03	3.08	.16	8.87	.70	4.78	4.09	.12	2.80	-5.51	-.32	53.98
2	2.93	2.90	.03	3.06	.13	8.87	.54	4.71	4.16	.12	2.66	-5.92	-.09	52.05
3	3.90	3.12	.78	3.22	-.68	8.65	.91	4.78	3.87	.13	2.96	-6.21	-.37	49.74
4	3.01	2.98	.03	3.11	.11	8.79	.68	4.74	4.05	.12	2.77	-5.64	1.28	50.22
5	3.01	3.01	.00	3.15	.15	8.66	.63	4.65	4.02	.12	2.69	-2.49	1.49	47.09
6	.86	3.03	-2.16	-	-	8.72	.73	4.72	3.99	.13	2.80	-5.66	1.51	43.42
7	3.03	3.05	-.02	3.17	.15	8.69	.76	4.72	3.96	.13	2.81	-3.29	.63	47.09
8	2.98	2.95	.03	3.07	.08	8.93	.75	4.84	4.09	.12	2.86	-6.30	.59	45.26
9	2.85	2.89	-.04	3.05	.20	8.87	.52	4.69	4.18	.12	2.64	-5.92	-.09	41.59
10	3.04	2.89	.16	3.05	.01	8.87	.51	4.69	4.18	.12	2.63	-5.92	.59	39.05
11	3	2	.	3	-	8.	.	4	4	.	2	-	-	5

1	.14	.97	16	.11	.03	79	67	.73	.06	12	.76	6.37	.09	2.05
12	3.19	2.96	.23	3.07	-.11	8.91	.74	4.83	4.09	.13	2.85	-6.30	-.61	40.47
13	3.11	2.58	.53	2.98	-.13	8.64	-.72	3.96	4.68	.11	1.67	-6.30	-.61	45.26
14	3.16	2.82	.34	2.94	-.21	9.21	.64	4.93	4.29	.12	2.83	-5.34	-1.07	47.09
15	3.13	3.46	-.33	3.60	.47	7.86	.88	4.37	3.49	.14	2.74	-6.21	-.47	50.03
16	3.18	2.69	.49	2.79	-.39	9.69	.70	5.20	4.50	.11	3.00	-6.15	-.09	49.94

Table 3
Antioxidant activities and molecular descriptors values of thienopyridine derivatives in PM3 method

		Eq-3		Eq-4		Molecular descriptors								
Compd	**Obs. Act.**	**Predicted**	**residual**	**Predicted**	**Residual**	**IP (eV)**	**EA (eV)**	**EN (eV)**	**η (eV)**	**S (eV^{-1})**	**ω**	**HE (K.cal/mol)**	**LogP**	**Pol (Aº)**
1	2.93	2.96	-.03	3.12	.20	9.09	.33	4.71	4.38	.11	2.53	-5.78	-.32	53.98
2	2.93	2.95	-.02	3.13	.20	9.08	.30	4.69	4.39	.11	2.50	-6.18	-.09	52.05
3	3.90	3.06	.84	3.14	-.76	8.98	.52	4.75	4.23	.12	2.67	-6.38	-.37	49.74
4	3.01	2.98	.02	3.04	.03	9.21	.54	4.88	4.34	.12	2.74	-5.78	1.28	50.22
5	3	3.2	-	3	.	8	.	4	4	.1	2	-	1.4	4

	.01	2	.22	.26	25	.66	63	.65	.02	2	.69	2.49	9	7.09
6	.86	2.95	-2.09	-	-	9.33	.55	4.94	4.39	.11	2.78	-5.34	1.51	43.42
7	3.03	2.30	.72	2.99	.12	10.47	-.77	4.85	5.62	.09	2.10	-2.78	.63	47.09
8	2.98	2.97	.02	3.11	.20	9.11	.38	4.75	4.37	.11	2.58	-3.57	.59	45.26
9	2.85	3.01	-.16	3.05	.07	9.18	.58	4.88	4.30	.12	2.76	-2.42	-.09	41.59
10	3.04	2.95	.09	3.11	.06	9.11	.34	4.73	4.38	.11	2.55	-2.97	.59	39.05
11	3.14	3.19	-.05	3.20	-.16	8.79	.67	4.73	4.06	.12	2.76	-6.35	-.09	52.05
12	3.19	3.00	.19	3.03	-.03	9.23	.60	4.91	4.32	.15	2.80	-6.33	-.61	40.47
13	3.11	2.93	.18	3.08	-.03	9.19	.36	4.78	4.42	.11	2.58	-5.65	-.61	45.26
14	3.16	2.91	.25	3.12	-.02	9.13	.23	4.68	4.45	.12	2.46	-7.20	-1.07	47.09
15	3.13	2.95	.17	3.10	-.09	9.12	.36	4.74	4.38	.13	2.57	-3.16	-.47	50.03
16	3.18	2.96	.22	3.09	.12	9.15	.40	4.77	4.38	.11	2.60	-2.47	-.09	49.94

The perusal of correlation matrix indicates that S and EA are the predicted parameters from AM1 method. The enter, backward, forward, removed and stepwise regression methods are used. ***S*** and **EA** were found to be explainable variable. The regression technique was applied through the origin using these explainable parameters.

Activity = 24.154 (1.307)**S* ---------- (1)
N = 16; R = 0.979; R^2 = 0.958; R^2adj=0.955; %EV = 95.80; SEE = 0.64; F= 341.566; Q =1.53;

In addition, the plot of observed activity versus predicted activity was not found to be satisfactory. Hence, the predictive ability of the model is not good. **Eq.1** shows that the values of %EV are less and to improve its value, outliers were sought and eliminated.

After the elimination of the outlier (**6**), a second model was developed. Overall, there is an increase in R (0.979-0.997) and %EV (95.80– 99.5) values, and a decrease in SEE (0.64 - 0.24).

Activity = 26.458(1.216)**S* – 0.220(0.0213)*EA---------- (2)
N =15; R= 0.996; R^2= 0.993; R^2adj =0.992; %EV = 99.6; SEE = 0.2841; F =894.019; Q =3.5058;

Eq.2 is an improved model since it explains the biological activity to the extent of (99.5%). From the correlation matrix table, it reveals ***S*** and **EA** are found to be explainable variables. A mono-parametric QSAR equation with Soft and di-parametric QSAR equation with S and **EA** were generated in PM3 method also.

Activity = 25.889(1.367)**S*---------- (3)
N =16; R = 0.980; R^2= 0.960; R^2adj =0.957; %EV = 96.0; SEE = 0.62; F = 358.871; Q = 1.56;

Eq.3 shows that the values of %EV is less and to improve its value, outliers were sought and eliminated, In addition, the plot of observed activity versus predicted activity was not found to be satisfactory. Hence, the predictive ability of the model is not good. After the elimination of the outlier (**6**), a second model was developed.

Activity = 28.910*(0.910)**S* – 0.540(0.212)*EA---------- (4)
N = 15; R = 0.997; R^2 = 0.994; R^2adj=0.994; %EV = 99.4; SEE = 0.2509; F = 1148.341; Q =3.9736;

In an attempt to investigate the predictive potential of proposed models, the cross-validation parameters ($q^2_{cv}$and PRESS) were calculated and used. The predictive power of the equations was confirmed by leave-one-out (LOO) cross-validation method (**table 2** and **table 3)**.

Eq.3 and **4** of AM1 and PM3 methods respectively give a good q^2_{cv} value, which should be always smaller than %EV. A model is considered to be significant when q^2_{cv} = (>0.82). Another cross-validation parameter, PRESS which is the sum of the squared differences between the actual and that predicted when the compound is omitted from the fitting process, also supports the predictive ability of **Eqs.2** and **4**. Its value decreases from **Eq.1** to **Eq.3.**

The quality factor Q,**[37]** is defined as the ratio of regression constants (R) to the standard error estimation (SEE), that is, Q = R/SEE. This indicates that the higher the value of R, and the lower the value of SEE, the higher is the magnitude of Q and the better will be the correlation. In present case, Q increases from 1.53 to 3.505 and 1.56 to 3.973**(Eq. 1** to **4)**.

As softness of ligand increases the activity also increases. The activity decrease with increase in electron affinity.Soft acids and bases can be explained on the HSAB principal. Softness of chemical species linked with large atomic/ionic radius, low or zero oxidation state, high polarisability, low electro negativity. Soft bases have HOMO of higher energy than hard bases, and soft acids have LUMO of lower energy than hard acids. The soft molecules are more reactive than hard molecules if electron transfer or rearrangement is necessary for the reaction. The softness is important in understanding the chemistry of large, delocalized molecules or ions **[38]**. The electron affinity is characterized by the susceptibility of the compound in relation to attacks by nucleophiles.The electron affinity of an atom or molecule is defined as the amount of energy released when an

electron is added to a neutral atom or molecule to form an egativeion. In principle, any molecule can act as an electron donor to all molecules with superior values to it. In the final AM1 and PM3 modelled **Eq-2** and **Eq-4,** contribution of the physicochemical parameters shown graphically in contribution charts (**figure 2** and **figure 3**). The correlation between actual and predicted activity for the compounds are shown in **table 2, table 3** and **figure 4-7**.

Figure 2 Plot of observed activity Vs predicted activity

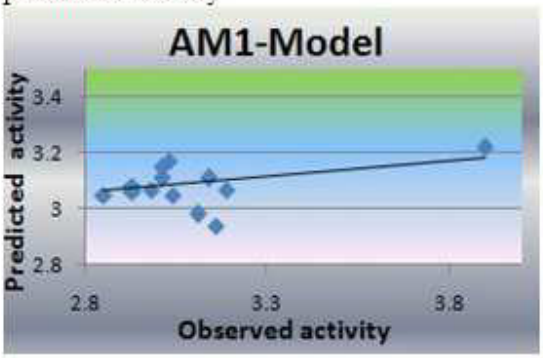


Figure 3 Plot of observed activity Vs predicted activity

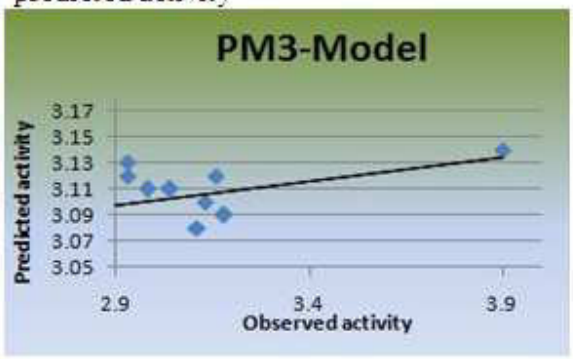


Figure 4 Plot of observed activity Vs **EA**

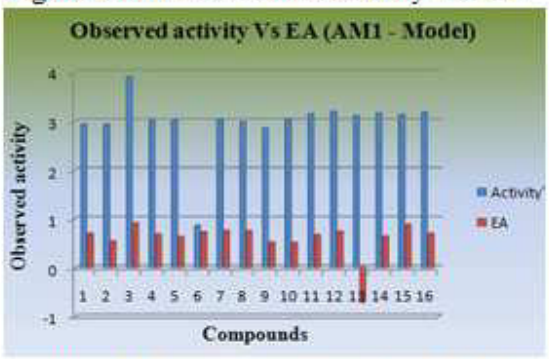


Figure 5 Plot of observed activity Vs ***S***

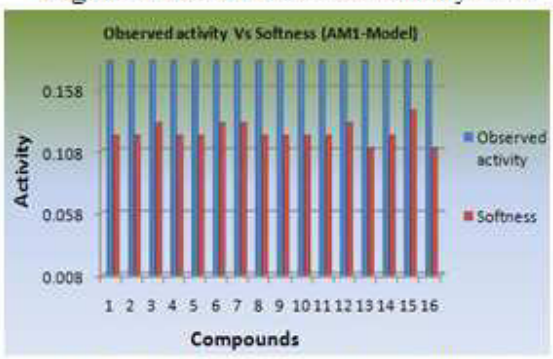


Figure 6 Plot of observed activity Vs **EA**

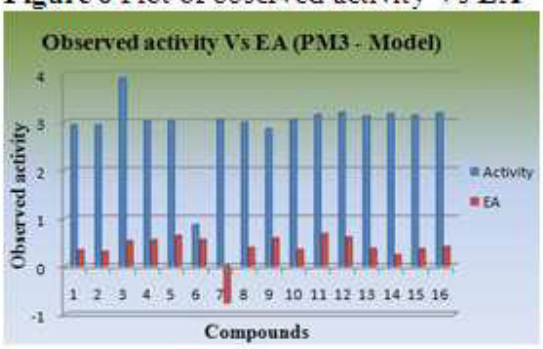
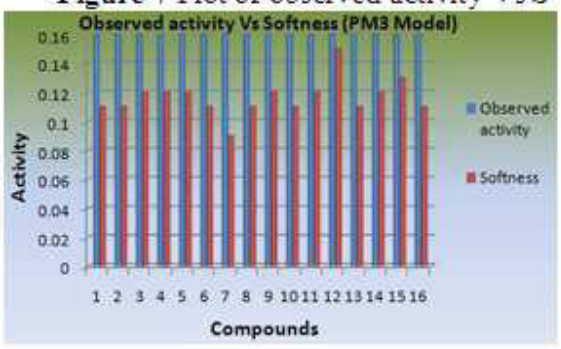


Figure 7 Plot of observed activity Vs ***S***

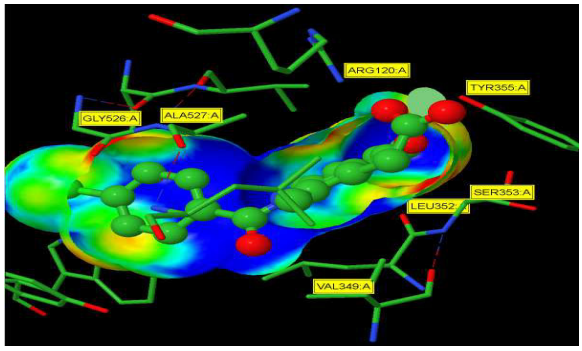


3.3. Docking Analysis

Among all the compounds tested for docking study, showed good inhibitory activity values against cyclo-oxygenase-2 (**table 4** and **table 5**).The compound **12** and **15** showed high affinities with low energy of with employed protein. It indicates the binding between 4COX and compound-**12** indicates very good

inhibition. The compounds **(1-16)** showed good inhibition with affinity ranges. In the active site of **4COX**, Thr212, Asn68, Glu67, His388, Ser 530, Tyr355, Tyr 402, Asn 382, Thr70, Glu140, Asn144 amino acids play important role and they are shown in **figure 8**.

Figure 8
Active site amino acids of crystallographic protein 4COX



The docking results from the crystal structure of cyclooxegenase-2 (4COX) in the modeling study agreed well with the observed *in vitro* data, which indicated that compound-**12 (IC_{50}=0.649)** expected to be a potent inhibitor of cyclooxegenase-2.The docked score of compound-**12** (55.19) indicates tight binding to the active site cyclooxegenase-2 and it agreed with biological activity. The high score of compound-1**2** is due to the best fitting of ligand containing electron releasing groups (-Cl) in the aromatic ring of thienopyridine derivatives with the cyclooxegenase-2 protein. The second highest score for the compound-**15** is due to electron releasing group (–F) on aromatic ring of compound-**15**.The compounds 1**4, 11,10** and **2** have next highest score due to presence of electron releasing groups on aromatic ring and highest score due to *intra* and *inter* molecular hydrogen bondings with the electron releasing groups. The remaining compounds have medium gold docking score due to presence of less capacity of electron donating groups present on the aromatic ring of thienopyridine derivatives.

Table 4
Docking Values Obtained from Gold in Fitness Score with cyclo-oxygenase-2 (PDB ID = 4COX)

Compound	Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(vdw_int)
1	46.39	0.00	37.80	0.00	-5.58
2	249.20	0.25	39.11	0.00	-4.83
3	45.71	0.16	38.43	0.00	-7.28
4	49.13	0.12	36.38	0.00	-1.01
5	545.52	0.21	36.57	0.00	-4.97
6	52.90	0.00	43.52	0.00	-6.94
7	46.76	0.18	38.31	0.00	-6.09
8	47.31	0.21	37.22	0.00	-4.08
9	44.57	0.00	33.26	0.00	-1.16
10	51.27	3.39	40.95	0.00	-8.43
11	52.00	4.48	41.78	0.00	-9.92
12	55.19	2.61	43.59	0.00	-7.35

13	46.23	0.30	37.52	0.00	-5.66
14	52.67	2.23	42.48	0.00	-7.97
15	53.73	0.78	43.71	0.00	-7.15
16	-20.33	0.24	40.03	0.00	-75.62

Table 5
Docking values obtained from GOLD in Chemscore function with cyclo-oxygenase-2 (PDB ID = 4COX)

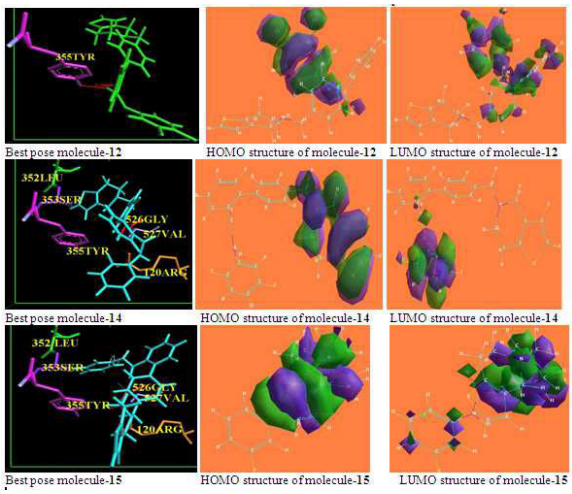
Comp	Score	DG	S(hbond)	S(metal)	S(lipo)	DE(clash)	DE(int)
1	20.17	-22.81	0.99	0.00	145.86	0.28	2.36
2	21.01	-24.32	0.99	0.00	160.01	0.20	3.10
3	21.23	-24.71	0.94	0.00	165.41	0.16	3.32
4	21.09	-23.50	0.97	0.00	152.37	0.11	2.29
5	20.51	-23.24	0.99	0.00	150.57	0.04	2.69
6	24.66	-27.85	1.00	0.00	191.04	0.71	2.48
7	20.44	-23.30	0.98	0.00	151.18	0.23	2.62
8	20.59	-24.62	0.93	0.00	163.39	1.63	2.40
9	21.34	-22.94	0.83	0.00	148.81	0.40	1.20
10	19.93	-25.06	0.92	0.00	164.72	1.72	3.41
11	21.26	-25.80	0.91	0.00	171.32	1.49	3.05
12	22.02	-29.07	1.19	0.00	191.23	2.70	4.35
13	21.06	-24.02	0.96	0.00	155.55	0.06	2.90
14	21.97	-27.92	0.96	0.00	188.76	0.30	5.65
15	21.63	-27.55	0.99	0.00	184.23	1.99	3.93
16	20.61	-24.53	0.95	0.00	159.31	0.26	3.66

Highest Occupied Molecular Orbital (HOMO) energy and Lowest Unoccupied Molecular Orbital (LUMO) energy were constructed from of HQSAR (Hologram QSAR)**[39]**.The theoretical calculations of molecular properties such as the maps of Molecular Orbitals (HOMO, LUMO), Autodock and Argus lab binding energies showed a good antioxidant activity of the title compounds (**Table 6** and **Fig.9**.). The HQSAR maps show positive (green) and negative (blue) contributions. The positive contributions of the most potent compounds-**12, 14** and **15** indicate the importance of polar contacts for biological activity. The higher energy of the HOMO and lower energy of the LUMO indicate the greater electron-donating ability and smaller resistant to accept electrons respectively. Therefore, the HOMO and LUMO energies also support the QSAR and docking results.

Table 6
HOMO, LUMO, AUTODOCK and Argus Lab energies of thienopyridine derivatives

Compound	- ε_{HOMO}	- ε_{LUMO}	Auto dock K_i in uM	Auto dock B.E in K cal/mol	Argus B.E in K cal/mol(elapsed time in seconds)
1	-9.03	-0.45	68.56	-5.68	-15.99(7)
2	-9.02	-0.44	-	+12.77	-14.19(8)
3	-9.03	-0.45	+40.59	-	-15.91(6)
4	-9.03	-0.35	50.40	-5.86	-13.47(6)
5	-9.03	-0.41	+41.19	-	-15.66(8)
6	-3.59	-0.22	+62.01	-	-16.69(8)
7	-9.02	-0.41	65.61	-5.71	-14.35(7)
8	-9.03	-0.42	99.25	-5.46	-14.34(6)
9	-9.26	-0.65	20.51	-6.40	-15.10(8)
10	-8.98	-0.40	124.59	-5.33	-16.09(8)
11	-9.14	-0.35	21.35	-6.37	-15.11(7)
12	-9.03	-0.43	152.95	-5.21	-14.82(6)
13	-9.12	-0.38	34.13	-6.09	-13.88(5)
14	-9.14	-0.63	82.14	-5.57	-14.46(8)
15	-9.01	-0.49	8.33	-6.93	-16.77(7)
16	-8.79	-0.61	-	+227.44	-12.99(7)

Figure 9
Best docking poses of molecule 12,14 and 15.HOMO,LUMO energy maps of molecule (12,14 and 15) and green colour indicate favorable regions, while blue colour indicate unfavorable region for the activity.



4.CONCLUSIONS

The antioxidant activity of thienopyridine derivatives was determined using CTC of DDQ. In our present study, it was established the predictive QSAR models that are quite reliable to the experimental antioxidant activity of thienopyridines. The main contribution of the high score compounds to the cyclo-oxygenase-2 enzyme is due to hydrophobic interactions. These findings demonstrated that these compounds could be developed into novel antioxidant pharmacophore. QSAR shows good predictive performance and has ability to provide some insight into the relative importance of the individual compounds involved in determining the biologic activity. Based on the activity data, from the series **12, 15, 14, 11, 10** and **2** serve as an important pharmacophore for the design and development of new lead as antioxidant agent. Therefore, it gives insight into the pharmacophore and residues of cyclo-oxygenase-2active site.The docking studies helped in understanding the various interactions between the ligands and enzyme active sites. The QSAR studies revealed the indicative physicochemical parameters and type of substituents are responsible for high antioxidant efficacy of thienopyridine derivatives.

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A LINEAR QSAR AND DOCKING APPROACH TO MODEL INHIBITORY ACTIVITY OF CAFFEIC ACID AMIDES AS LIPOXYGENASE PROTEIN INHIBITORS

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ABSTRACT

Molecular modeling studies of twenty seven caffeicacid derivatives are carried out to optimize their inhibitory activity against lipoxygenase. The biological activities of these analogs are correlated to different molecular properties. The AM1and PM3 semi empirical methods are used to estimate vertical ionization potentials (IPv's), electron affinity (EA) ,electro negativity (χ), hardness (η), softness (S), electrophilic index (ω), partition coefficient (LogP),hydration energy(HE), ionization potential(IP) and charges. The different modeled equations are proposed by regression analysis and validated. The biological activity depends on mainly η, S and ω. Docking studies of Caffeic acid with lipoxygenase are also made to support the finding of QSAR studies. Analysis of results of both QSAR and docking studies suggested that remarkable inhibitory activity is exhibited by molecule **10.**Thehydrogen bond interactions along with hydrophobic and electrostatic interactions are mapped to confirm their potencies.

KEYWORDS

Semi empirical Methods, Caffeic Acid Amides, Inhibitor, QSAR, Regression analysis, Docking.

1. INTRODUCTION

Caffeic acid amides show antibacterial, antiviral, vasoactive, antiatherogenic, antiproliferative, anticancer, anticardiovascular and anti-inflammatory properties[1]. These activities are at least partially related to their antioxidant properties.

The caffeicacid derived compounds occur in various beverages and foodstuffs. The atherosclerosis preventive activity is attributed due to the presence of these compounds and analogous polyphenols.[2]Lipid peroxidation is indeed an initial step in the atherosclerosis pathology. Hence, these compounds are used aspreventive cardiovascular drugs. Their use is however, compromised by the low metabolic stability of esters. Although literature data on caffeic esters are scarce and mainly concern the metabolism of the phenolic groups. It is clear that the ester group is metabolically very labile.[3-8]

The antioxidant activity was determined by measuring the inhibition of the microtonal lipid peroxidation being the most biomimetic antioxidant test[9-12]Caffeic acid amides **1-27** (**table 1 and**

figure1) were synthesized recently by varying different hydroxyl and amine groups and their bioassay was carried against Lipid peroxidation. The study helps in discovering and filtering effective compounds as Lipid peroxidation inhibitors.[13-14]

Figure 1
Derivatives of amides of Caffeic acid(R=OH) and p-coumaric acid(R=H).

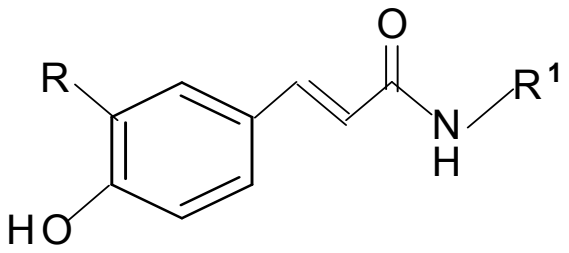


Table1
Structural Skeleton and Inhibition Effect of Caffeic Acid Amides Lipid Peroxindation Activity (figure 1).

Comp	Acid	Amine (R^1)	IC_{50}	Activity
1	Caffeic	3-Methylbut-2-enyl amine	3.4	3.4685
2	Caffeic	Ammonia	2.2	3.6576
3	Caffeic	Hydroxylamine	2.1	3.6778
4	Caffeic	Methylamine	6.0	3.2220
5	Caffeic	Ethylamine	2.7	3.5687
6	Caffeic	Isopropylamine	3.9	3.4090
7	Caffeic	Isobutylamine	2.2	3.6576
8	Caffeic	Isopentylamine	1.4	3.8539
9	Caffeic	Allylamine	2.2	3.6576
10	Caffeic	Aniline	0.38	4.4202
11	Caffeic	2-Aminophenol	0.29	4.5376
12	Caffeic	3-Aminophenol	0.37	4.4317
13	Caffeic	4-Aminophenol	0.63	4.2006
14	Caffeic	Benzylamine	1.02	3.9915
15	Caffeic	Phenethylamine	0.85	4.0705
16	Caffeic	Dopamine	0.59	4.2291
17	Caffeic	Tyrosine-OCH_3	3.2	3.4949
18	Caffeic	Diethylamine	4.1	3.3873
19	Caffeic	Pyrrolidine	2.4	3.6199
20	Caffeic	PIperdine	3.6	3.4438
21	Caffeic	Morphine	6.1	3.2146
22	p-CoumaricCaffeic	3-Methyl-2-butenyl amine	29.1	2.5366
23	Caffeic	3-Methyl-2-butenol	3.5	3.4560
24	Caffeic Acid	-	3.3	3.4815
25	p-CoumaricCaffeic		100	1.0000

26	Trolox	-	2.8	3.5528
27	Quercetin	-	0.95	4.0222

The presentQSAR studyof Caffeic acid amide analogs is to elucidate lipoxygenase inhibitors using physicochemical parameters like vertical ionization potentials (IPv's), electron affinity (EA) , electro negativity (χ), hardness (η), softness (S), electrophilic index (ω), partition coefficient (LogP),hydration energy(HE),and ionization potential(IP).Recently, it is reported on QSAR study of phenols with antioxidant activity by employing descriptors calculated by semi empirical methods AM1 and PM3 (**table 2, 3**). This study was also made on quantitative basis in which four computational methods viz. density functional (DF), HF (Hartree-Fock) and AM1 and PM3 were employed to explore and determine various electronic descriptors with better accuracy to make the necessary improvement in the QSAR models.

Table 2
Values obtained fromthe AM1 computational method.

Comp	IPv = - $\varepsilon_{HOMO(AM1)}$	IP	EA	EN	η	S	ω	LogP	HE	Pol(A^{o3})
1	-8.8046	-1.6562	-9.6275	-5.6419	3.9857	.1255	3.9931	-.0300	-14.5800	27.4400
2	-8.8033	-1.7707	-9.8173	-5.7940	4.0233	.1243	4.1720	-1.5200	-19.1900	18.4600
3	-8.0607	-1.6649	-8.4263	-5.0456	3.3807	.1479	3.7652	-1.0400	-19.0400	19.1000
4	-8.7836	-1.7270	-9.7587	-5.7429	4.0158	.1245	4.1063	-1.2800	-15.8500	20.2900
5	-8.9440	-1.5542	-9.8944	-5.7243	4.1701	.1199	3.9289	-.9400	-15.2200	22.1300
6	-8.8380	-1.5873	-9.5145	-5.5509	3.9636	.1261	3.8869	-.5200	-14.7900	23.9600
7	-8.6690	-1.5364	-9.3336	-5.4350	3.8986	.1283	3.7884	-.5200	-14.3000	23.9600
8	-8.7010	-1.5215	-9.3510	-5.4363	3.9148	.1277	3.7745	.3400	-13.8900	27.6300
9	-9.0359	-1.4142	-9.3254	-5.3698	3.9556	.1264	3.6448	-.8000	-17.6800	23.7700
10	-8.7120	-1.4682	-9.2856	-5.3769	3.9087	.1279	3.6983	-.7900	-18.7000	28.1200
11	-8.8676	-1.1761	-8.4997	-4.8379	3.6618	.1365	3.1959	-1.8100	-24.1400	28.7500
12	-7.9885	-1.1325	-9.2301	-5.1813	4.0488	.1235	3.3153	-1.8100	24.5200	28.7500
13	-7.9379	-1.0763	-9.1988	-5.1376	4.0612	.1231	3.2495	-1.8100	24.8100	28.7500
14	-9.0197	-1.4299	-9.8192	-5.6246	4.1947	.1192	3.7709	-.2700	-21.6700	29.9500
15	-8.5601	.5098	-6.7487	-3.1195	3.6293	.1378	1.3406	-.0100	-19.2000	31.7900
16	-8.8686	-1.2464	-9.6849	-5.4657	4.2193	.1185	3.5401	-2.0600	-30.2700	33.0600
17	-8.9205	-1.3839	-9.5666	-5.4753	4.0914	.1222	3.6636	-1.6800	-11.9900	36.1800
18	-8.1429	-1.1746	-8.2680	-4.7213	3.5467	.1410	3.1425	.4100	-12.3700	27.6300
19	-8.7213	-1.7243	-9.5802	-5.6523	3.9279	.1273	4.0667	-.7100	-12.0700	25.0200
20	-8.7862	-1.7592	-9.6242	-5.6917	3.9325	.1271	4.1189	-.3100	-13.8600	26.8600
21	-8.8260	-1.7324	-9.5387	-5.6356	3.9032	.1281	4.0684	-1.3700	-16.0500	25.6600
22	-8.8991	-1.5417	-9.7667	-5.6542	4.1125	.1216	3.8869	1.3700	-8.9400	26.4800
23	-8.4034	-.6622	-8.7027	-4.6825	4.0203	.1244	2.7269	.4000	-15.9000	29.2800
24	-9.0005	-1.7963	-9.7382	-5.7673	3.9710	.1259	4.1881	-.6600	-20.9800	17.7400
25	-9.1119	-1.7843	-9.9855	-5.8849	4.1006	.1219	4.2228	.3600	-15.3200	17.1100
26	-8.5949	-1.4656	-8.3103	-4.8880	3.4224	.1461	3.4906	.0400	6.3800	26.3400
27	-8.7294	-1.3289	-9.0388	-5.1838	3.8550	.1297	3.4854	-4.0100	-32.4600	28.5400

Table 3. Values obtained from the **PM3** computational method.

Comp	IPv = $-\varepsilon_{HOMO(PM3)}$	IP	EA	EN	η	S	ω	Log P	HE	Pol(A^{o3})
1	-8.8864	-1.6476	-9.6486	-5.6481	4.0005	.1250	3.9871	-.0300	-14.7200	27.4400
2	-8.9233	-1.8106	-9.7426	-5.7766	3.9660	.1261	4.2069	-1.5200	-19.2300	18.4600
3	-8.5141	-.9783	-8.9270	-4.9527	3.9743	.1258	3.0859	-1.0400	-20.2000	19.1000
4	-8.8434	-1.7871	-9.6855	-5.7363	3.9492	.1266	4.1661	-1.2800	-15.9400	20.2900
5	-8.7986	-1.7181	-9.6468	-5.6825	3.9644	.1261	4.0726	-.9400	-15.3100	22.1300
6	-8.9324	-1.5430	-9.5534	-5.5482	4.0052	.1248	3.8428	.5200	-14.8800	23.9600
7	-8.8857	-1.3935	-9.5176	-5.4556	4.0621	.1231	3.6635	.0500	-14.3900	25.8000
8	-8.8903	-1.4061	-9.5053	-5.4557	4.0496	.1235	3.6750	.3400	-13.9000	27.6300
9	-8.3547	-1.8451	-8.3650	-5.1051	3.2600	.1534	3.9972	-.8000	-17.9700	23.7700
10	-8.7368	-1.4656	-9.2272	-5.3464	**3.8808**	**.1288**	**3.6827**	-.7900	-18.7100	28.1200
11	-8.7505	-1.6061	-8.1354	-4.8708	3.2647	.1532	3.6335	-1.8100	-24.0900	28.7500
12	-8.1160	-.8301	-9.2935	-5.0618	4.2317	.1182	3.0274	-1.8100	-24.5900	28.7500
13	-7.9874	-1.0170	-9.2473	-5.1321	4.1152	.1215	3.2002	-1.8100	-24.9000	28.7500
14	-8.8425	-1.7495	-9.5867	-5.6681	3.9186	.1276	4.0993	-.2700	-21.8000	29.9500
15	-6.6951	-1.3668	-5.4774	-3.4221	2.0553	.2433	2.8489	.0100	-20.3800	31.7900
16	-9.0096	-1.4689	-9.7348	-5.6019	4.1330	.1210	3.7964	-2.0600	-36.4700	33.0600
17	-8.9721	-1.4978	-9.4888	-5.4933	3.9955	.1251	3.7763	-1.6800	-11.5800	36.1800
18	-8.4324	-1.1194	-8.4927	-4.8061	3.6866	.1356	3.1327	.4100	-12.0100	27.6300
19	-8.7073	-1.7342	-9.5412	-5.6377	3.9035	.1281	4.0712	-.7100	-12.0500	25.0200
20	-8.7356	-1.7067	-10.345	-6.0263	4.3196	.1158	4.2036	-.3100	-13.5500	26.8600
21	-8.8129	-1.7580	-9.4911	-5.6246	3.8666	.1293	4.0909	-1.3700	-16.0500	25.6600
22	-8.9857	-1.5268	-9.8704	-5.6986	4.1718	.1199	3.8921	1.3700	-8.9100	26.4800
23	-8.1702	-.7761	-8.4980	-4.6371	3.8609	.1295	2.7846	.4000	-15.8400	29.2800
24	-8.9967	-1.8541	-9.7481	-5.8011	3.9470	.1267	4.2631	-.6600	-20.0900	17.7400
25	-9.1352	-1.8429	-9.9944	-5.9186	4.0757	.1227	4.2974	.3600	-15.1800	17.1100
26	-8.5005	-1.4971	-8.0431	-4.7701	3.2730	.1528	3.4760	.0400	6.9000	26.3400
27	-8.7137	-1.3950	-8.9980	-5.1965	3.8015	.1315	3.5517	-4.0100	-32.2200	28.5400

This prompted us to correlate the biological activity of Caffeic Acid Amide analogs with ionization potentials, electron affinity ,electronegativity, hardness(η), partition coefficient (LogP), softness(S), hydration energy(HE) and Polarisability(Pol) from computational methods AM1 and PM3 **(table 4,5)**. GOLD and Argus lab 4.0.1 is Molecular modeling and Drug Docking softwares are used. This helps in computational virtual screening to find the lead compounds. Molecular docking started with Fischer's lock and key theory, where, every receptor has its unique ligand to catalyze the reaction.

Table 4. Observed activity and predicted activity values (**AM1** method)**.**

Compound	Observed	Eq. (1)		Eq. (2)	
		Predicted	Residual	Predicted	Residual
1	3.4685	3.4333	.0352	3.6474	-.1789
2	3.6576	3.3546	.3030	3.6185	.0391
3	3.6778	3.6082	.0696	3.4295	.2483
4	3.2220	3.3835	-.1615	3.6320	-.4100
5	3.5687	3.4658	.1029	3.7629	-.1942
6	3.4090	3.4803	-.0713	3.6636	-.2546
7	3.6576	3.5252	.1324	3.6580	-.0004
8	3.8539	3.5308	.3231	3.6692	.1847
9	3.6576	3.5869	.0707	3.7253	-.0677
10	4.4202	**3.5645**	.8557	3.6867	.7335
11	4.5376	3.8049	.7327	-	-

12	4.4317	3.7318	.6999	3.8631	.5686
13	4.2006	3.7610	.4396	3.8873	.3133
14	3.9915	3.5366	.4549	3.8192	.1723
15	4.0705	4.6256	-.5551	4.1896	-.1191
16	4.2291	3.6397	.5894	3.8951	.3340
17	3.4949	3.5794	-.0845	3.7917	-.2968
18	3.3873	3.8459	-.4586	3.6664	-.2791
19	3.6199	3.4019	.2180	3.5973	.0226
20	3.4438	3.3788	.0650	3.5850	-.1412
21	3.2146	3.4019	-.1873	3.5842	-.3696
22	2.5366	3.4818	-.9452	-	-
23	3.4560	3.9905	-.5345	4.0072	-.5512
24	3.4815	3.3477	.1338	3.5865	-.1050
25	1.0000	3.3337	-2.3337	-	-
26	3.5528	3.7186	-.1658	3.5202	.0326
27	4.0222	3.6604	.3618	3.7174	.3048

Table 5. Observed activity and predicted activity values (**PM3** method).

Comp	Observed	**Eq. (3)**		**Eq. (4)**	
		Predicted	Residual	Predicted	Residual
1	3.4685	3.4647	.0038	3.6511	-.1826
2	3.6576	3.3456	.3120	3.5766	.0810
3	3.6778	3.9072	-.2294	3.8974	-.2196
4	3.2220	3.3615	-.1395	3.5818	-.3598
5	3.5687	3.4122	.1565	3.6135	-.0448
6	3.4090	3.5380	-.1290	3.6930	-.2840
7	3.6576	3.6438	.0138	3.7654	-.1078
8	3.8539	3.6344	.2195	3.7577	.0962
9	3.6576	3.3446	.3130	3.4534	.2042
10	**4.4202**	**3.5852**	.8350	**3.6954**	.7248
11	4.5376	3.5261	1.0115	-	-
12	4.4317	4.0149	.4168	4.0121	.4196
13	4.2006	3.8910	.3096	3.9168	.2838
14	3.9915	3.3868	.6047	3.5904	.4011
15	4.0705	4.4893	-.4188	4.0481	.0224
16	4.2291	3.5991	.6300	3.7546	.4745
17	3.4949	3.5685	-.0736	3.7084	-.2135
18	3.3873	3.8181	-.4308	3.7918	-.4045
19	3.6199	3.3970	.2229	3.5933	.0266
20	3.4438	3.4583	-.0145	3.7142	-.2704
21	3.2146	3.3781	-.1635	3.5752	-.3606
22	2.5366	3.5637	-1.0271	-	-
23	3.4560	4.0286	-.5726	3.9444	-.4884
24	3.4815	3.3125	.1690	3.5539	-.0724
25	1.0000	3.3316	-2.3316	-	-
26	3.5528	3.6048	-.0520	3.6035	-.0507
27	4.0222	3.6322	.3900	3.7077	.3145

2. METHODOLOGY

2.1 Calculated Properties

Quantum chemical calculations at the DFT/RB3LYP/631G* (restricted B3LYP), RHF/6-31G* (restricted Hartree-Fock)[15], AM1[16] and PM3[17] semi empirical theory levels, are employed for full optimization of the selected neutral compounds. The geometrical structures of the radicals studied are optimized independently from the neutral molecules prior to the calculation of energies, treated as open shell systems. All calculations are performed by using the program of window hyper chem. software Inc.

The calculated vertical ionization potential (IPv's) and electron affinity (EA) is corrected for zero-point energy, assuming a negligible error and thus saving computer-time.TheIPv are calculated as the energy differences between a radical cation and the respective neutral molecule; IPv $(E_{cation} - E_{neutral})_{DFT}$ and Koopmans's theorem $(IPv = -\varepsilon_{HOMO})$. The EA are computed as the energy differences between a neutral form and the anion molecule; $EA=(E_{neutral} - E_{anion})$. The AM1 and PM3-based reactivity descriptors are used.[18-21]

2.2 Correlation Analysis

A relation between biological activity, expressed as $Log(1/IC_{50})$, and the physicochemical parameters and QSAR is analyzed statistically by fitting the data to correlation equations consisting of various combinations of these parameters. The statistical optimization was used to propose the best correlation model.

The matrix correlation uses the Pearson product moment correlation to measure the degree of linear relationship between two variables. The coefficient assumes a value between -1 and +1 .If one variable tends to increase the other decreases, the correlation coefficient is negative. Conversely, if the two variables tend to increase together the correlation coefficient is positive. We obtained the correlation matrix between inhibitory activity and respective calculated properties for **27**caffeic acid amides. The more relevant regression models were selected from the following criteria; correlation coefficient (R), Fisher ratio values (F) and the standard deviations(s),standard error estimate (SEE), percentage of effective variable(%EV) and R^2adjusted(R^2_{adj}).

Finally the performance of the model (its predictive ability) is given by PRESS (Predictive Sum of Squares) and S_{PRESS} (Standard deviation of cross validation)[22]. The predictive ability of the model is also quantified in terms of the Q^2. [23]

3.3 Docking Studies and Validation

The GOLD Score is calculated by defining the site using the list of atom numbers and retaining all the other default parameters. Now a day's docking is frequently to predict the binding orientations of small molecules of drug candidates to their protein targets in order to predict the affinity of the small molecules.[24]The 3D structure of lipoxygenase (LOX-3) was retrieved from Protein Data Bank (PDB ID 1IK3) with an X-ray resolution of $2A^O$(**http://www.rcsb.org/pdb**). Docking poses are obtained by applying both Chemscore and Goldscore, fitness functions available for scoring. As easily interpretable results were obtained based on a recently published work,[25] all the results reported in the present paper are referred to the Chemscore fitness functions.

These complexes are prepared for docking studies by adding hydrogen atoms, removing water molecules and co-crystallized inhibitors and refined by using the DeepView/SwissPdbViewer3.7 (SP5).[26] Enzyme-inhibitor interactions within a radius equal to 15 Å centered on reported bound inhibitors are taken into account. As a conclusive part of docking we expect ,generated results should yield RMSD values below 1.5 Å. Successful docking has been performed for the selected set of **27**caffeic acid amide inhibitors and their corresponding Chemscore with their respective RMSD have been produced in the **table 6**.

Table6. Energy and Chemscore values of the docked ligands.

Comp	GOLD Data (Chemscore)	ArgusLab (Energy value)
1	23.59	0
2	16.18	-8.86
3	16.77	0
4	18.55	0
5	16.79	-9.63
6	18.92	0
7	20.42	-10.65
8	20.32	0
9	20.46	-9.83
10	**21.59**	**-12.04**
11	21.03	-11.48
12	20.01	-11.33
13	18.92	-10.58
14	20.50	-12.13
15	15.66	-12.91
16	9.67	-11.42
17	-27.58	0
18	-14.51	0
19	-3.12	0
20	21.95	-10.24
21	19.88	-8.73
22	21.48	-11.62
23	-10.07	-10.78
24	9.78	-8.99
25	12.27	-9.21
26	10.87	0
27	-25.80	0

Argus Lab 4.0.1 is Molecular modeling and Drug Docking software. It is very flexible and can reproduce crystallographic binding orientations. Argus lab, which provides a user-friendly graphical interface and uses Shape Dock algorithm, is used to carry out docking studies of the lipoxygenase.

4. RESULTS AND DISCUSSIONS

4.1. Simple linear regression model

The biological activity data and the physicochemical properties IPv, IP, EA, EI, EN, Hard, Soft, LogP, HE and Pol of the caffeic acid amides are given in **tables 1-3.** The data from these tables are subjected to regression analysis. The correlation matrices were generated with **27** analogs. The term close to 1 indicates high co-linearity, while the value below 0.5 indicates that no co-linearity exist between more than the two parameters.

The perusal of correlation matrix indicates that Hard, Soft and EI are the predicted parameters from AM1 method. From regression methods backward, forward, removed and stepwise. Hard, Soft and EI are found to be explainable variable. The regression technique was applied through the origin using these explainable parameters.

Activity = 0.648 x Hard(0.358) +20.786 x Soft(8.692) – 0.440 x EI(0.232) ------(1)

N = 27; R = 0.986; R^2 = 0.972; R^2adj=0.968 ; %EV = 97.20; SEE = 0.6514 ; F= 276.00; Q =1.5136;

In addition, the plot of observed activity versus predicted activity is not found to be satisfactory. Hence, the predictive ability of the model is not good. **Eq.1**show that the values of %EV are less and to improve its value, outliers are sought and eliminated.

After the elimination of the outlier (**11,22**and **25**), a second model was developed. Overall, there is an increase in R and %EV (97.2 – 99.3) values, and a decrease in SEE (0.6514 - 0.3273).

Activity = 0.853 x Hard(0.183) +10.564 x Soft(4.614) – 0.270 x EI(0.118) ----- (2)

N = 24; R= 0.997; R^2= 0.993; R^2adj =0.992; %EV = 99.3; SEE = 0.3273;

F = 1032.895; Q =3.0461;

Eq.2 is an improved model since it explains the biological activity to the extent of (99.3%). In this way, the predictive molecular descriptors Hard, Soft and EI are considered as variables. From the correlation matrix table, it reveals Hard, Soft and EI are found to be explainable variables. A tri parametric QSAR equation with Hard, Soft and EI was generated in PM3 method also.

Activity = 0.821 x Hard (0.256) +17.361 x Soft (3.870) – 0.499 x EI (0.306) ----- (3)

N = 27; R = 0.985; R^2= 0.971; R^2adj =0.967; %EV = 97.10; SEE = 0.6631;

F = 266.657; Q = 1.4854;

Eq.3 shows that the values of %EV is less and to improve its value, outliers are sought and eliminated.In addition, the plot of observed activity versus predicted activity is not found to be satisfactory. Hence, the predictive ability of the model is not good. After the elimination of the outlier (**11,22 and 25**), a second model is developed.

Activity = 0.777 x Hard (0.131) +13.400 x Soft (1.984) – 0.284 x EI (0.157) ----- (4)

N = 24; R = 0.997; R^2 = 0.993; R^2adj=0.992; %EV = 99.7; SEE = 0.3302;

F = 1014.698; Q =3.0193;

In an attempt to investigate the predictive potential of proposed models, the cross-validation parameters ($q^2_{cv}$and PRESS) are calculated and used. The predictive power of the equations is confirmed by leave-one-out (LOO) cross-validation method. The cross-validation evaluates the validity of a model by how well it predicts the data rather than how well it fits the data. The cross-validation parameter**,** q^2_{cv}, is mentioned in the respective equations **(table 4 and 5).**

Eq.**3** and 4 of AM1 and PM3 methods respectively give goodq^2_{cv} values, which should be always smaller than %EV. A model is considered to be significant when q^2_{cv}>0.7.

Another cross-validation parameter, PRESS which is the sum of the squared differences between the actual and that predicted when the compound is omitted from the fitting process, also supports the predictive ability of **Eqs.2 and 4**. Its value decreases from **Eq.1 to Eq.3.**

4.2. DOCKING ANALYSIS

The compounds are then docked using each of the three docking softwares. The Chemscore from GOLD and the energy values from the two docking softwares are indicated in **table 6**. The binding energies obtained in

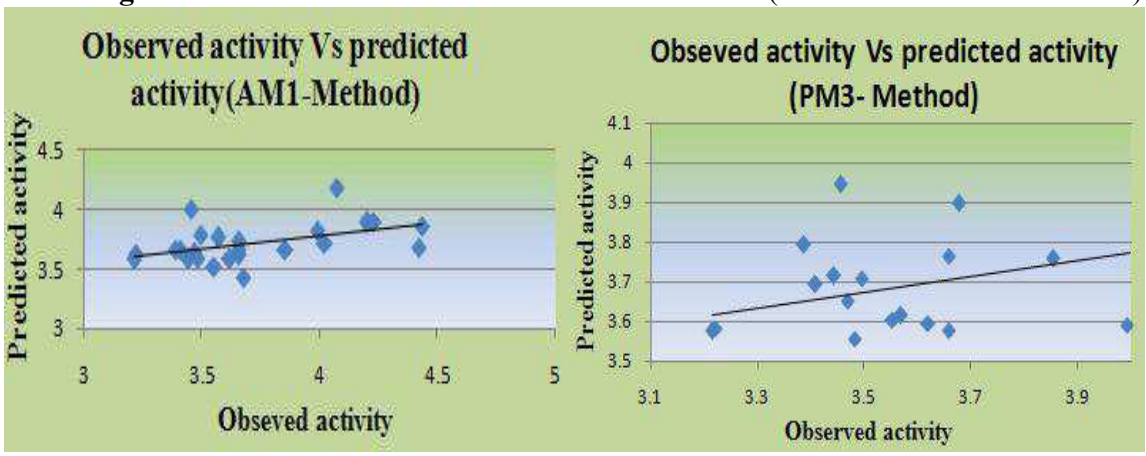
Argus Lab ranged from -8.86 to -12.91 kJ/mol. The results of GOLD can be analyzed in terms of energy values ranging from -27.58 to 23.59.

The docking simulation of the most active N-phenyl compound **10** toward LOX (PDB ID **1IK3**) showed that the most enzyme–inhibitor complex is stabilized by hydrophobic interactions occurring between the aromatic moieties of the ligand and lipophilic residues of the binding site. In particular the N-phenyl compound **10** group is oriented towards the hydrophobic region lined by Ile857,Arg726,His523,His518,His513 and Ser510.In addition, the carboxylic functional group is supposed to coordinate the iron(Fe(II)) ion as it showed a similar structural arrangement compared to the peroxide group of the co-crystallized Ligand. Result of docking studies has proved that the molecule numbered **10** shows Chemscore and RMSD values as 21.59 and 1.5 Å respectively**(table 6).**The molecule **10**has been reported with appreciable IC_{50} values of 0.38µM. All the poses of themolecule (chosen as best in docking studies) and its interactions in the active pocket of LOX have been illustrated in **figure3.**

5. CONCLUSION

The presentQSAR studies of Caffeic acid amides as LOX inhibitors are successfully modeled using tri parametric equations. The **Eq.2** and **Eq.4** from AM1 and PM3 calculations reveal Hard, Soft and EI cause the inhibitory activity. Higher values of Hard, Soft and EI are responsible for higher inhibitory activitynature for LOX enzyme. The linear dependence of inhibitory nature on Hard, Soft and EI are evident from **figure 2.**

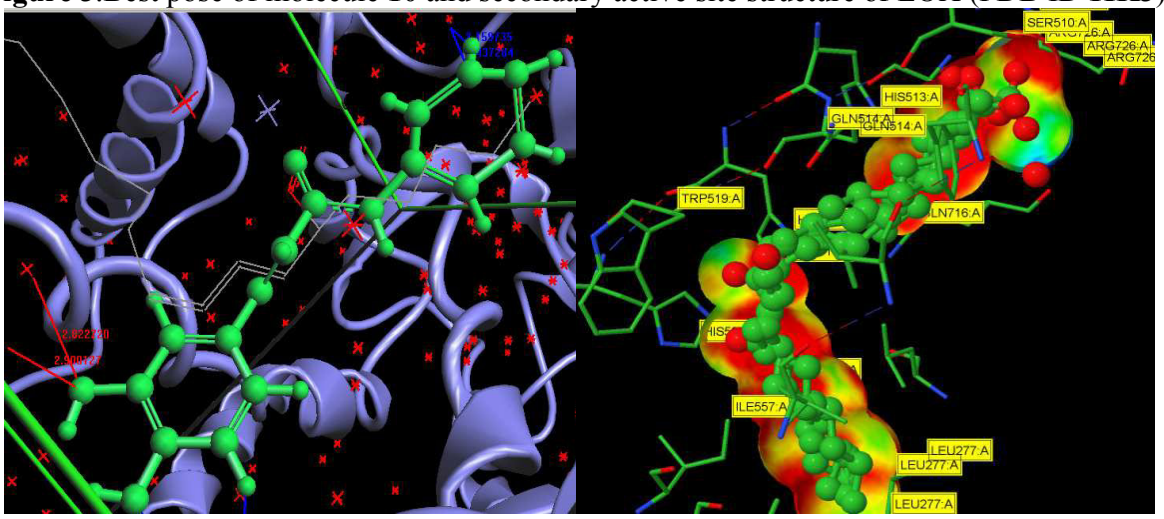
Figure 2. Plot of Observed Verses Predicted activities (**AM**-Method&**PM3**-Method).



The most active compounds docked successfully into the active site of the inhibited enzyme. Inhibitory activity of the most potent compounds was explained mostly by hydrophobic interactions. The compound **10** was found to present a promising with a high antioxidant activiy, siginificant inhibitory activity on LOX and good hydroxyl (˙OH) radical scavenging activity.

The spin population defined as **NS(A)=NA↑-- NA↓**illustrates the effect of the N-substituent on the caffeoyl group on the unpaired spin density distribution showing an additional mesomeric effect with the second aromatic ring offering a positive spin density to theC4'-nucleus and responsible for the more effective spin delocalization and the higher activity of the N-phenyl compound **10 (figure 3)**.

Figure 3.Best pose of molecule **10** and secondary active site structure of LOX (PDB ID **1IK3**).



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MICELLE FORMING SURFACTANTS AS EFFICIENT CATALYSTS FOR THE OXIDATION OF XANTHINE ALKALOIDS BY CERIC AMMONIUM NITRATE IN ACETONITRILE – A KINETIC AND MECHANISTIC STUDY

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ABSTRACT

Oxidation of Xanthine alkaloids such as Xanthine (**XAN),** hypoxanthine (**HXAN),** caffeine (**CAF),** theophylline (TPL), theobromine (TBR) with ceric ammonium nitrate **(CAN)** is too sluggish in acetonitrile media even at elevated temperatures, but significantly accelerated in presence of micelle forming surfactants such as Tx-100, SDS, and CTAB. Reaction followed first order kinetics in both [CAN] and [Xanthine alkaloid]. Rate of oxidation is accelerated with an increase in [Surfactant] linearly. Mechanism of oxidation in Tx-100 micellar media has been explained by Menger- Portnoy enzymatic model with the oxidation of micelle-bound substrate (MS) by Ceric nitrate (CAN) appropriately. However, in SDS and CTAB mediated reactions micelle bound-CAN is formulated as oxidizing species.

INTRODUCTION

During the past several decades single electron transfer (SET) oxidizing agents have received quite some attention as a means to promote bond-forming reactions in organic synthesis[1]. The Ce (IV) reagents in general and ceric ammonium nitrate (CAN) in particular have emerged as valuable SET reagents[2] due to their relative abundance, ease of preparation, low cost, and low toxicity. A recent review reported by Jih and King[3] substantiated the role, efficiency, and advantages of ceric ammonium nitrate in different chemical transformations. It also provided excellent bibliography on a variety of CAN reactions such as carbon–carbon bond formation, oxidative carbon– carbon bond cleavage, nitration, and removal of protecting groups. CAN oxidation of primary alcohols to aldehydes, secondary alcohols to ketones and benzylic alcohols to benzaldehyde has also been discussed.

Xanthine (3,7-dihydro-1H-purine-2,6-dione) belongs to the purine group, and possesses a critical biological importance, because it plays a prominent role as an intermediate in adenine and guanine degradation to uric acid[4,5]. The N-methyl derivatives of xanthine, including theophylline (3,7-dihydro-1,3-dimethyl-1H-purine- 2,6-dione), theobromine (3,7-dihydro-3,7-dimethyl- 1H-purine-2,6-dione), and caffeine (3,7-dihydro-1,3,7-

trimethyl-1H-purine-2,6-dione), are alkaloids that are widely distributed in plant products and beverages and are known to have many physiological effects, such as gastric acid secretion, dieresis, and stimulation of the central nervous system[6,7].

Surfactants are amphiphilic molecules that contain both hydrophilic (water loving) and hydrophobic (water hating) characteristics, which can be thus used as catalysts in aqueous and non aqueous reaction media. Depending on the nature of reaction medium, surfactant molecules aggregate to form micelles (in water) or inverted micelles (in nonaqueous solvents). In view of these reasons micellar solutions can provide cleaner reaction media to carrying out organic reactions[8-10]. With the growing need for sustainability, continuous interest has developed for the application of surfactant-based reaction media during the last few decades. A number of noble-metal catalyzed homogeneous reactions such as hydrogenation, hydroformylation, oxidation and coupling reactions have been conducted in micellar media. Since oxidation of Xanthine alkaloids by CAN are too sluggish to monitor the progress of reaction, surfactants are being used to enhance the reaction rate. Thus, kinetic studies of CAN oxidation of Xanthine alkaloids, which are taken up in this study may throw light the mechanisms and probably serve as bio oxidation model mechanisms.

EXPERIMENTAL DETAILS

All chemicals used were of analytical grade. Doubly distilled water (distilled over alkaline $KMnO_4$ and acid dichromate in an all glass apparatus) was used whenever required. Acetonitrile and other solvents were HPLC grade and used as such throughout the work. Xanthine (**XAN),** hypoxanthine(**HXAN) ,**caffeine (**CAF),** theophylline (TPL), theobromine (TBR), ceric ammonium nitrate (CAN), sodium dodecyl sulfate (SDS), cetyl trimethyl ammonium bromide (CTAB) and triton-X 100 (Tx) were procured from Aldrich, E-merck or SD fine chemicals.

1. Kinetic method of following the reaction:

One flask containing known amount of ceric ammonium nitrate (CAN) in acetonitrile solvent and another flask containing the substrate (Xanthine Derivative) and suitable amount of surfactant (Micelle) solutions were clamped in a thermostatic bath, which maintained desired temperature with an accuracy of ± 0.1^{o}C. Reaction was initiated by mixing requisite amount of CAN to the other contents of the reaction vessel. Immediately after thorough mixing of the reactants, aliquots of the reaction mixture were withdrawn into a cuvette and placed in the cell compartment of the Elico made visible spectrophotometer. Cell compartment was provided with an inlet and outlet for circulation of thermostatic liquid at a desired temperature. Absorbance of unreacted [CAN] was recorded at 400nm. The CAN content could be estimated from the previously constructed calibration curve showing Absorbance vs [CAN]. Absorbance values were in agreement to each other with an accuracy of ±3% error.

RESULTS & DISCUSSION

1. Stoichiometry of the reaction:

The Stoichiometry of the CAN- Xanthine alkaloid reactions, one mole of substrate (S) consumed two moles of CAN and exhibited 1[S]: 2[Ce (IV)] in all the micellar media.

2. Order of the Reaction:

(i) When [S] = [CAN], the plots of $1/(A_t)$ or [1/(a-x)] vs time were straight lines with positive slopes and definite intercepts on ordinate indicating over all second order kinetics (figures 1and 2). (ii) Under the conditions [S] >> [CAN], the plots of ln (A_0/A_t) i.e. ln[a/(a-x)] vs time were straight lines with positive slopes passing through the origin indicating first order (x) with respect to [CAN] as could be seen from figures 3and 4. The first order rate constant (k') were obtained from the slopes of these linear plots. (ii) Since the reaction followed second order kinetics (figures 1 and 2) and first order with respect to [CAN] is one (figures 3 and 4), it is clear that order with respect to [Substrate] is also one in all the Ce (IV)-Xanthine alkaloid reactions (iv) Rate accelerations are observed with an increase in the [surfactant] when CAN oxidation of Xanthine alkaloids were studied in SDS indicated as shown in the tables 1 to 5. (v) Addition of olefin monomer (acryl amide and acrylonitrile) to the reaction mixture decreased the reaction rate. When heated, the contents of the reaction mixture turned viscous and indicated dense polymer formation. This observation can be explained due to the induced vinyl polymerization of added monomer, showing the presence of free radicals in the system. Thus, the rate law for oxidation of Xanthine Alkaloid (S) reaction by ceric ammonium nitrate (CAN) could be represented as,

$$CAN + S \longrightarrow \text{Products} \qquad (1)$$

Rate of the reaction (V) could be given as, $V = k_1[CAN]^x [S]^y$

The entire CAN - Xanthine alkaloid reactions were found to enhance with an increase in temperature. The kinetic data have been collected at three different temperatures within the range of 300 to 320°K. Eyring's equation is used to evaluate activation parameters such as $\Delta H^{\#}$ and $\Delta S^{\#}$, which can be rewritten as:

$$k = \left(\frac{k_B T}{h}\right) \exp\left(\frac{\Delta S^{\ddagger}}{R}\right) \exp\left(-\frac{\Delta H^{\ddagger}}{RT}\right) \qquad (2)$$

To find the linear form of the Eyring-Polanyi equation:

$$\ln\frac{k}{T} = \frac{-\Delta H^{\ddagger}}{R} \cdot \frac{1}{T} + \ln\frac{k_B}{h} + \frac{\Delta S^{\ddagger}}{R} \qquad (3)$$

Where: k'' = reaction rate constant, T = absolute temperature, **$(\Delta H^{\#})$** = enthalpy of activation, R= gas constant, k_B= Boltzmann constant, h = Planck's constant and **$(\Delta S^{\#})$** = entropy of activation. The plot of ln(k''/T) versus (1/T) afforded a straight line with negative slope (from which the enthalpy of activation $((\Delta H^{\#})$ is derived, while the intercept being equal to entropy of activation $(\Delta S^{\#})$ (Figures 5 and 6). Free energy of activation $(\Delta G^{\#})$ is obtained from Gibbs – Helmholtz equation

$$\Delta G^{\#} = \Delta H^{\#} - T\,\Delta S^{\#}. \qquad (4)$$

3. Mechanism of Oxidation of xanthine derivatives by Ce (IV) Nitrate (CAN) in MeCN Medium:

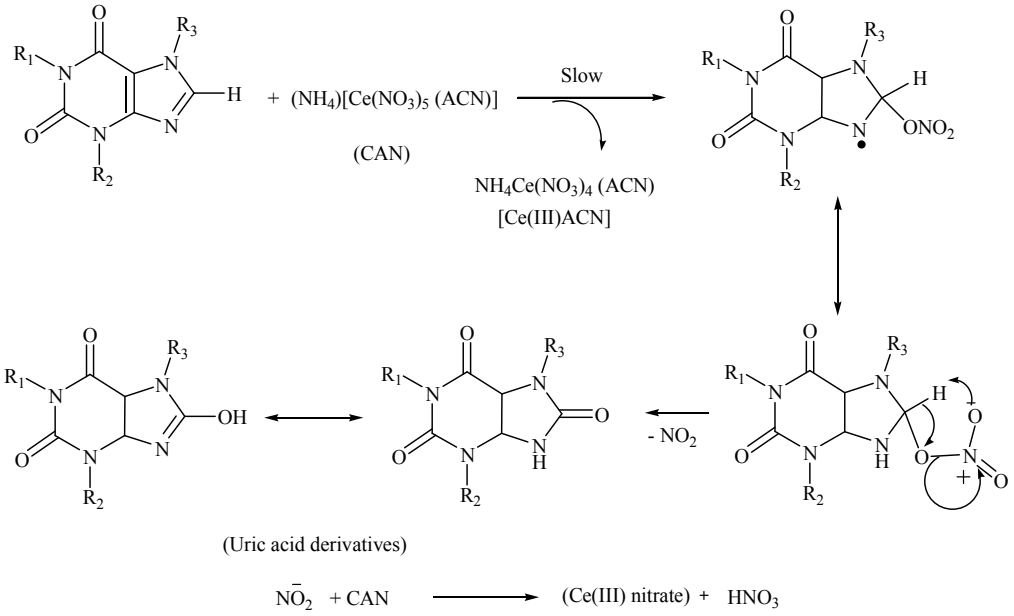
In order to gain an insight into the mechanistic aspects of CAN-Substrate reaction in MeCN medium, the knowledge of distribution of CAN species in HNO_3 medium could be useful. In aqueous HNO_3 medium Ce(IV) mainly exists as $Ce(NO_3)_6^{2-}$, $Ce(NO_3)_5^{-}$,$Ce(OH)(NO_3)_4^{-}$, $Ce(NO_3)_4$, which could arise from the following equilibria as cited by earlier workers and also from our laboratory[11-16]. But the CAN species in the present study could be entirely different because the reactions are conducted in large excess of MeCN

medium. Since MeCN is large excess over [CAN], it may penetrate into the coordination spheres of Ce (IV) and form *solvated CAN species* according to the following equilibrium,

$$(NH_4)_2Ce(NO_3)_4 + CH_3CN \rightleftharpoons NH_4[Ce(NO_3)_4(CH_3CN)] + NH_4NO_3 \quad (5)$$

(Solvated CAN)

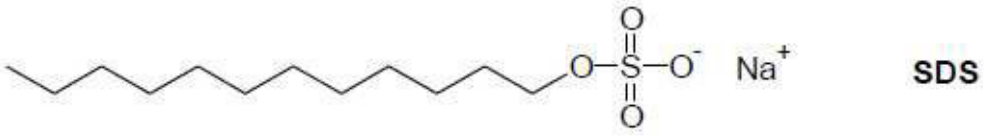
Solvated CAN thus formed oxides Xanthine alkaloid (S) uric acid derivative as shown in the following sequence of steps:



4. Mechanism of oxidation in Micellar media:

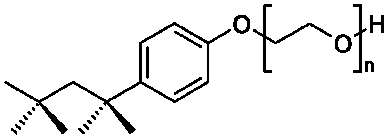
Highly sluggish reactions are enhanced to fairly good extent in the presence of sodium dodecylsulfate (SDS), cetyl trimethyl ammonium bromide (CTAB) and Triton- X-100 (Tx-100), a set of anionic, cationic and non-ionic surfactants respectively. Reaction time reduced from several hours (> 24 hrs) to only few hours. The catalytic activity was found to be in the increasing order: CTAB< SDS < TX- 100. In order to understand the role of micelles in chemical reactions the properties of surfactants are specifically important. Surfactants exhibit a wide variety of applications in chemical, biological, materials and engineering sciences [8-10]. These molecules are said to be amphipathic; that is, they have distinct hydrophilic (polar) and hydrophobic (nonpolar) regions. The polar region called the head group, may be neutral, cationic, anionic or zwitterionic. The hydrophobic tail has one or more chains of varying length, composed usually of a hydrocarbon. Common examples are:

Cetyl trimethylammonium bromide, $CH_3(CH_2)_{15}(CH_3)_3N^+Br^-$ ($C_{16}TAB$, cationic)



Sodium dodecyl sulfate, $CH_3(CH_2)_{11}OSO_3^-Na^+$ (SDS, anionic)

Triton X-100 ($C_{14}H_{22}O(C_2H_4O)_n$) is a nonionic surfactant which has a hydrophilic polyethylene oxide group (on average it has 9.5 ethylene oxide units) and a hydrocarbon lipophilic or hydrophobic group. The hydrocarbon group is a 4-(1,1,3,3-tetramethylbutyl)-phenyl group. It is related to the Pluronic range of detergents (triblock copolymers of ethylene oxide and propylene oxide. The part formed from ethylene oxide is more hydrophilic than the part from propylene oxide.

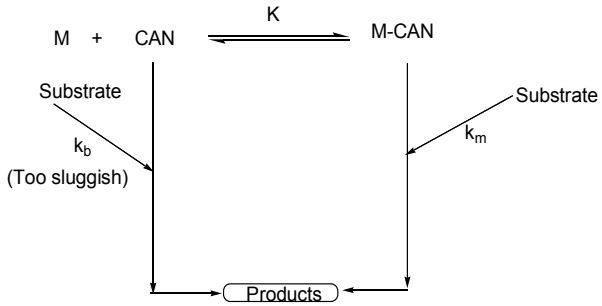


(Triton X-100)

Ionic surfactants such as, SDS and CTAB generally dissolve completely in water at very low concentrations, but above certain level, the critical Micelle concentration (CMC), the molecules form globular aggregates, called Micelles. The hydrophobic tails group together to create a non-polar interior with the head groups located at the surface of the glob in contact with the aqueous environment. Micelles vary in size and shape, but are commonly rough-surfaced spheres with aggregation numbers on the order of 50-100. The acceleration of a chemical reaction in solution by the addition of a surfactant at a concentration higher than its critical micelle concentration (CMC) so that the reaction can proceed in the environment of surfactant aggregates (Micelles). Rate enhancements could be either due to the higher concentration of the reactants in that environment, more favorable orientation and solvation of the species, or enhanced rate constants in the micellar pseudo phase of the surfactant aggregate. Micelle formation can also lead to a decreased reaction rate depending on the nature of reactive species. In non-aqueous the structure of Micelle is inverted / reversed and therefore, called as inverted/reverse Micelles and still affect the reaction rates. Further, it is also interesting note that the absorbance of solvated Ce(IV) species is increased in presence of TX and CTAB, when surfactant is added to [CAN] solution. This observation may indicate that solvated [CAN] species could bind with Tx moiety to form Tx supported CAN species according to the following equilibrium,

$$\underset{\text{Tx-100}}{H\text{-}(\text{-}OCH_2\text{-}CH_2)_n\text{-}O\text{-}Ar} + \underset{\text{CAN}}{(NH_4)[Ce(NO_3)_5(ACN)]} \overset{K}{\rightleftharpoons} \underset{\text{[Tx-CAN]}}{[Ar\text{-}O\text{-}(OCH_2\text{-}CH_2\text{-}O\text{-})_n\text{-}(NH_4)[Ce(NO_3)_5(ACN)]}$$

In the presence of Tx-100, micellar-substrate interaction is more likely because both of them contain phenyl groups, which favor hydrophobic interactions. The plots of k" (rate constant of Micelle mediated reaction) Vs $C_{Micelle}$ (concentration of Micelle) indicated almost linear rate enhancements with an increase in the concentrations of Tx-100. A perusal of literature shows that the mechanisms of micelle mediated reactions could be explained in the lines of enzyme catalysis because Micelle resembles the structure of enzyme. The first and widely applied model is that of Menger and Portnoy[8-10] which closely resembles to that of an enzymatic Catalysis. Efforts have been made to interpret micellar effects on the mechanism of Tx-100 mediated CAN-Substrate reaction in the same lines cited in Menger – Portnoy model. According to this model, formation of Micelle bound substrate (Micelle- S) could occur in the pre-equilibrium step due to the interaction of substrate (xanthine alkaloid) with micelle. The complex thus formed may possess higher reactivity to give products. A general mechanism is proposed by considering the bulk phase and micellar phase reactions as shown in scheme-2.



Considering the total concentration of (C_S) as the algebraic sum of free species and Micelle bound substrate complex (MS) species,

$C_S = [S] + [MS]$ (6)

From Micelle-substrate binding equilibrium,

$K = [MS]/[M][S]$ or $[S] = [MS]/K[M]$

Substitution of [S] in eq. (6) gives

$$C_S = \frac{[MS]}{K[M]} + [MS] = \frac{[MS] + K[M][MS]}{K[M]}$$

$$\text{or} \quad [MS] = \frac{K[M]C_S}{1 + K[M]}$$

Similarly free substrate [S] is written as, $[S] = C_S - [MS]$

$$[S] = C_S - \frac{K[M]C_S}{1 + K[M]}$$

After simplification, above equation reduces to,

$$[S] = \frac{C_S}{1 + K[M]}$$

Substitution of [MS] and [S] in eq. gives,

$$k' = \frac{k_0 C_S}{1 + K[M]} + \frac{k_m K[M]C_S}{1 + K[M]} \qquad (7)$$

$$\text{or} \quad k_\varphi = \frac{(k_0 + k_m K[M])}{1 + K[M]} \qquad (8)$$

Where $k_\varphi = (k'/ C_S)$, the second order rate constant in micellar media. Subtracting k_0 from both the sides of equation and rearranging

$$(k_m - k_w)K[M]$$

$$k_\varphi - k_0 = \frac{}{1 + K[M]} \quad (9)$$

But, since the reactions are too sluggish in the absence of [Micelle], the rate constant (k_0) would be much smaller than (k_m K[M]) i.e., ($k_0 <<< k_m$ K[M]). Therefore the (k_0) term could be neglected in the above equation. The rate law (9) could be then considered as,

$$k_\varphi = \frac{k_m K[M]}{1 + K[M]} \quad (10)$$

This rate –law resembles Michaelis –Menten type rate law that is used for enzyme kinetics. Interestingly the plots of rate constant (k_φ) i.e second order rate constant of Micelle mediated reaction vs [Micelle] indicated continuous increase in the k" values with an increase in [Micelle] passing through a maximum point in the profile. In view of this reaction kinetics are studied in detailed at various Micellar concentrations and at various temperatures in order to have an insight into the variation in the enthalpies and entropies of activation with [Micelle]. Reciprocals of the above plot take the form,

$$\frac{1}{k} = \frac{1}{k_m K[M]} + \frac{1}{k_m} \quad (11)$$

Reciprocal plot of (1/k") versus (1/[M]) afforded a straight line with positive gradient and intercept on ordinate indicating the consistency of the proposed mechanism in the present study. From the slope and intercepts of reciprocal plots, binding constant (K) and rate constants (k_m) are presented in tables 6, 7 and 8 respectively for Tx-100, SDS and CTAB mediated reactions.

Efforts have also been made to interpret SDS and CTAB micellar effects on the mechanism of CAN-Substrate reaction by Menger – Portnoy model. In this case, formation of Micelle bound reagent (Micelle-CAN)) could occur in the pre-equilibrium step due to the interaction of CAN with micelle. As a typical example complex between (SDS) anion **$[CH_3(CH_2)_{11}OSO_3^-]$** and CAN is shown in the following sequence of steps given in Scheme3.

Scheme-3:

$$\underset{\text{SDS}}{[CH_3(CH_2)_{11}OSO_3Na]} + \underset{\text{CAN}}{NH_4[Ce(NO_3)_5(CH_3CN)]} \underset{-\,NaNO_3}{\overset{K}{\rightleftharpoons}} \underset{[\text{SDS-CAN}]}{NH_4[Ce(NO_3)_4(CH_3(CH_2)_{11}OSO_3)(CH_3CN)]}$$

$$[\text{SDS-CAN}] + \text{Substrate} \xrightarrow{k_m\ (\text{slow})} \text{Products}$$

The complex thus formed could possess higher reactivity than CAN to give products with enhanced reaction rates. Rate law is the same as rate equation (10); where K represents [Micelle- CAN] binding constant and k_m represents rate constants for micelle phase reaction. Since the rate of oxidation in bulk phase reaction is too sluggish, rate law for the above mechanism, is more likely. Binding constant and rate constants are presented in tables 7 and 8 respectively for SDS and CTAB mediated reactions. When CAN has been used to oxidize the compounds in presence of CTAB in acetonitrile medium the rate enhancement occurred with increase [CTAB] that can be attributed to the formation of Cetyl trimethylammonium ceric nitrate (CTACN).

In the case of CTAB reactions pre-equilibrium step of Scheme could be written as,

$$[C_{16}H_{33}(CH_3)_3NBr] + NH_4[Ce(NO_3)_4(CH_3CN)] \underset{-\ NH_4Br}{\overset{K}{\rightleftharpoons}} [C_{16}H_{33}(CH_3)_3N\text{-}\ Ce(NO_3)_4\ (CH_3CN)]$$

CTAB CAN CTA-CAN

The ((K/k_m) values presented in tables 6 to 8 are fairly high in the case of caffeine according to the following trend: Theobromine >= Theophylline > Xanthine > Caffeine > Hypoxanthine. This trend probably indicates that the ease of binding and decomposition are effective in the case of theobromine and theophylline over other xanthine alkaloids used in the present study. These observations coupled with positive entropies of activation indicate reorganization of species in the transition state leading to the formation of products.

Effect of structure on enthalpy & entropy changes:

The enthalpy of activation ($\Delta H^{\#}$) and entropy of activation ($\Delta S^{\#}$) are the two parameters typically obtained from the temperature dependence of a reaction rate, when the rate constant data are analyzed using the Eyring's equation. The entropy of activation ($\Delta S^{\#}$) provides clues about the molecularity of the rate determining step in a reaction, i.e. whether the reactants are bonded to each other, or not. Positive values of $\Delta S^{\#}$ suggest that entropy increases upon achieving the transition state, which often indicates a dissociative mechanism. Negative values for $\Delta S^{\#}$ indicate that entropy decreases due to the formation of more ordered transition state, which often indicates an associative mechanism. Values near zero are difficult to interpret[12,13]. Almost similar magnitude of $\Delta G^{\#}$ in a series of closely related reactions generally indicates a similar type of mechanism for the reactions under study. Free energy of overall reaction (ΔG) may be considered to be the driving force of a chemical reaction. When $\Delta G < 0$ the reaction is spontaneous; $\Delta G = 0$ the system is at equilibrium and no net change occurs; $\Delta G > 0$ the reaction is not spontaneous.

Data compiled in tables 6 to 8 of the present study show that enthalpy of activation values are in the range of moderately fast reactions, while entropy of activation values are highly negative indicating a more ordered transition state, which are by and large in accordance with the above contention. Further, the entropies of activation could also probably support the reorganization of the transition state species due to solvation prior to decomposition. Almost similar magnitudes of activation free energy values indicate a similar type of mechanism of oxidation of the xanthine derivatives used in this study. Changes in $\Delta H^{\#}$ are paralleled by changes in $\Delta S^{\#}$ in such a direction that the resulting effect on reactivity is less than it would be if controlled by either $\Delta H^{\#}$ or $\Delta S^{\#}$ alone (compensation effect). Its cause is seen in steric or solvent parameters affecting simultaneously the geometry of the transition state and the force constants. On the basis of foregoing discussions the reactions of the present investigation appeared to be controlled by $\Delta H^{\#}$ as well as $\Delta S^{\#}$ factors[13].

Figure-1: Second Order Kinetic Plots of Caffeine with MeCN at 310K; [CAF] = 0.002 mol dm^{-3}; [CAN] = 0.002 mol dm^{-3}; [TX-100] = 0.0375 mol dm^{-3}

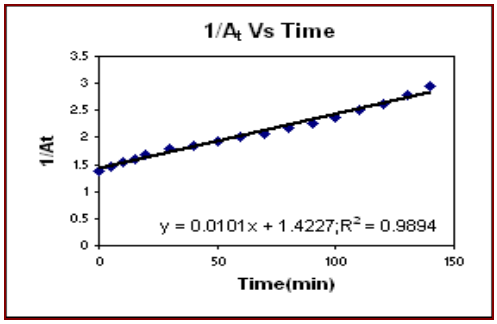


Figure-2: Second Order Kinetic Plots of Xanthine with MeCN at 300K; [XAN] = 0.002 mol dm^{-3}; [CAN] = 0.002 mol dm^{-3} ; [TX-100] = 0.0375 mol dm^{-3}

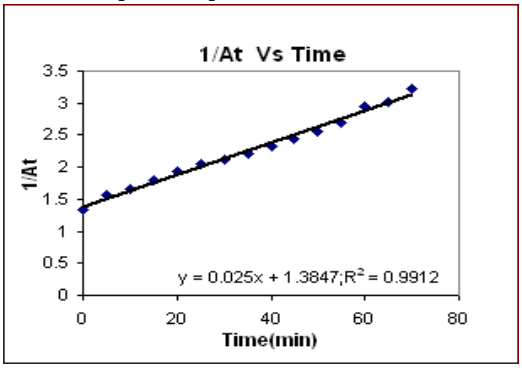


Figure-3: Pseudo First Order Kinetic Plots of Caffeine with MeCN at 310K; [CAF] = 0.016 mol dm^{-3} ; [CAN] = 0.0041 mol dm^{-3} ; [TX-100] = 0.0125 mol dm^{-3}

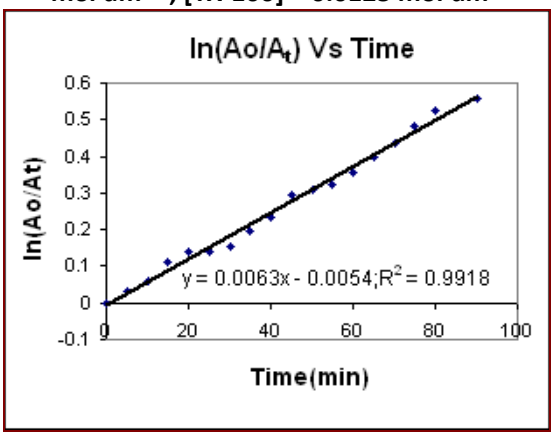


Figure-4: Pseudo First Order Kinetic Plots of Theophylline with MeCN at 310K; [TPL] = 0.016 mol dm^{-3}; [CAN] = 0.0041 mol dm^{-3}; [TX-100] = 0.0375 mol dm^{-3}

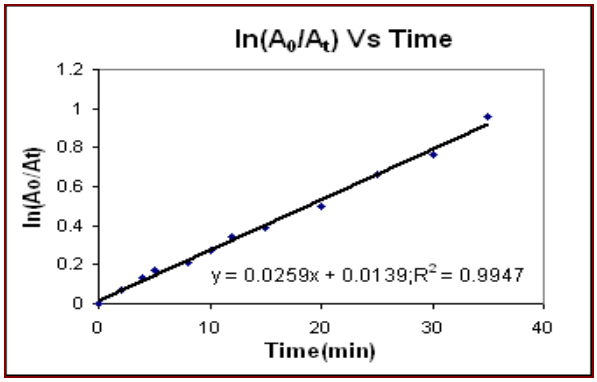


Figure-5: Eyring's Plot: Tx-100 catalysed oxidation of Caffeine by CAN

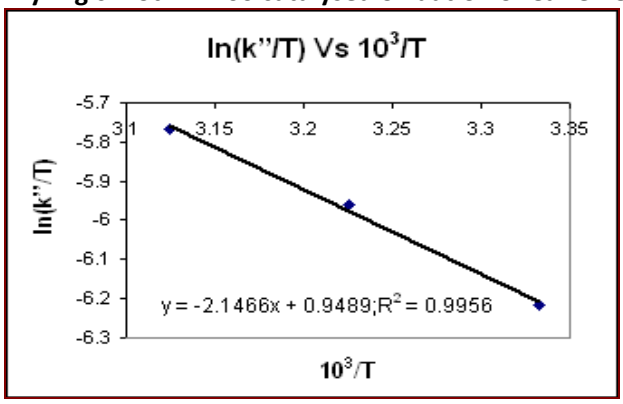


Figure-6: Eyring's Plot : TX-100 catalysed oxidation of Xanthine by CAN

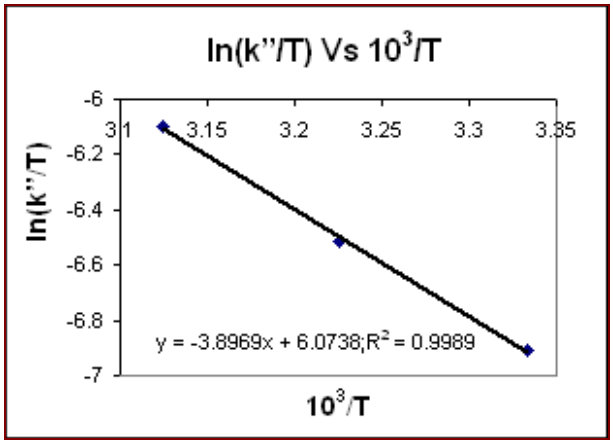


Table-1
Activation Parameters of Caffeine in different micellar media

Type of micelle	Micelle %(V/V)	k'' at 300 K	Equation	R^2	$\Delta H^{\#}$ kJ /mol	$\Delta G^{\#}$	$-\Delta S^{\#}$ J/ Kmol
TX-100	0.5	0.2	y = -3.028x + 2.797	0.992	25.1	77.3	174
	1.0	0.4	y = -4.104x + 7.088	0.985	34.1	75.5	138
	2.0	0.5	y = -1.308x - 2.031	0.998	10.8	75.0	214
	3.0	0.8	y = -2.382x + 2.025	0.995	19.8	73.8	180
	4.0	0.7	y = -0.899x - 3.060	0.999	7.47	74.3	223
	5.0	1.2	y = -0.432x - 4.078	0.999	3.59	72.8	231
SDS	0.5	0.3	y = -2.146x + 0.255	0.995	17.8	76.3	195
	1.0	0.6	y = -2.146x + 0.948	0.995	17.9	74.6	189
	2.0	0.4	y = -1.640x - 1.146	0.997	13.6	75.7	207
	3.0	0.7	y = -1.862x + 0.153	0.996	15.4	74.2	196
	4.0	0.7	y = -1.399x - 1.412	0.963	11.6	74.3	209
	5.0	1.0	y = -1.308x - 1.338	0.998	10.8	73.2	208
CTAB	0.5	0.1	y = -4.956x + 6.155	0.969	41.2	85.0	146
	1.0	0.2	y = -3.750x + 5.546	0.954	31.1	76.4	151
	2.0	0.2	y = -3.028x + 3.490	0.992	25.1	75.5	168
	3.0	0.3	y = -1.634x - 0.784	0.967	13.5	70.8	191
	4.0	0.4	y = -1.399x - 1.412	0.963	11.6	74.3	209
	5.0	0.5	y = -0.654x - 3.627	0.999	5.42	73.5	227

Table-2
Activation Parameters of Xanthine in different micellar media

Type of Micelle	[Micelle] %(V/V)	k'' at 300 K	Equation	R^2	$\Delta H^{\#}$ kJ /mol	$\Delta G^{\#}$	$-\Delta S^{\#}$ J/ Kmol
TX-100	0.5	0.30	y = -3.8969x + 6.073	0.998	32.3	76.4	147
	1.0	0.42	y = -2.7669x + 2.627	0.997	20.5	73.0	175
	2.0	0.56	y = -1.6408x - 0.809	0.997	13.6	74.8	204
	3.0	0.58	y = -1.5868x - 0.941	0.964	13.1	74.6	205
	4.0	0.67	y = -1.1642x - 2.221	0.998	9.66	74.4	216
	5.0	0.75	y = -0.8738x - 3.082	0.993	7.24	74.1	223
SDS	0.5	0.25	y = -1.8334x - 0.973	0.997	15.2	76.1	205
	1.0	0.30	y = -2.0558x - 0.027	0.952	17.0	76.1	197
	2.0	0.7	y = -1.4213x - 1.949	0.983	11.7	75.6	213
	3.0	0.51	y = -1.8018x - 0.585	0.984	15.5	76.1	202
	4.0	0.60	y = -0.5062x - 4.443	0.974	4.19	74.3	234
	5.0	0.65	y = -0.5062x - 4.442	0.973	4.17	74.3	235

Table-3
Activation Parameters of Theophylline in different Micellar media

Type of Micelle	Micelle %(V/V)	k'' at 300 K	Equation	R^2	$\Delta H^{\#}$	$\Delta G^{\#}$	$-\Delta S^{\#}$ J/ Kmol
					kJ /mol		
TX-100	0.5	0.23	y = -1.705x - 1.496	0.996	14.1	77.1	210
	1.0	0.37	y = -1.046x - 3.202	0.976	8.67	75.8	224
	2.0	0.35	y = -1.6842x - 1.134	0.997	13.9	76.0	207
	3.0	0.63	y = -0.5228x - 4.426	0.980	4.33	74.5	234
	4.0	0.51	y = -0.8562x - 3.521	0.999	7.10	74.9	226
	5.0	0.72	y = -0.5434x - 4.220	0.999	4.50	74.1	232
SDS	0.5	0.37	y = -1.046x - 3.202	0.976	8.67	75.8	224
	1.0	0.58	y = -0.2364x - 5.457	0.959	1.96	74.5	242
	2.0	0.49	y = -0.4993x - 4.755	0.993	4.14	75.2	237
	3.0	0.63	y = -0.9565x - 2.977	0.999	7.93	74.5	222
	4.0	0.65	y = -0.6252x - 4.049	0.999	5.18	74.4	231
	5.0	0.89	y = -0.1544x - 5.303	0.962	1.28	73.5	241
CTAB	0.5	0.21	y = -1.7125x - 1.569	0.982	14.2	77.2	210
	1.0	0.37	y = -0.5231x - 4.957	0.988	4.33	75.7	238
	2.0	0.32	y = -0.6431x - 4.703	0.989	5.33	76.1	236
	3.0	0.46	y = -0.6331x - 4.375	0.978	5.24	75.1	233
	4.0	0.35	y = -0.5686x - 4.853	0.981	4.71	75.8	237
	5.0	0.46	y = -0.8015x - 3.813	0.989	6.65	75.3	229

Table-4
Activation Parameters of Hypoxanthine in different Micellar media

Type of Micelle	Micelle %(V/V)	k'' at 300 K	Equation	R^2	$\Delta H^{\#}$	$\Delta G^{\#}$	$-\Delta S^{\#}$ J/ Kmol
					kJ /mol		
TX-100	0.5	0.35	y = -4.74x + 9.061	0.996	39.3	75.9	122
	1.0	0.65	y = -2.5954x + 2.514	0.999	22.8	75.6	176
	2.0	0.51	y = -6.8274x + 16.38	1.0	56.6	74.9	61.3
	3.0	0.42	y = -8.4606x + 21.52	0.957	70.2	75.7	18.6
	4.0	0.49	y = -10.671x + 29.00	0.951	88.5	100	39.6
	5.0	1.12	y = -9.44x + 25.96	0.972	78.3	85.2	23.3
SDS	0.5	0.21	y = -4.5787x + 8.019	0.992	38.0	77.0	130
	1.0	0.23	y = -4.1347x + 6.608	1.00	34.3	76.9	142
	2.0	0.30	y = -7.3229x + 17.48	0.998	60.7	76.3	52.2
	3.0	0.46	y = -3.4108x + 4.844	0.955	28.2	75.3	157
	4.0	0.56	y = -5.263x + 11.23	0.994	43.6	74.8	104
	5.0	0.65	y = -5.6499x + 12.66	0.991	46.8	74.4	92.3

Table-5
Activation Parameters of Theo bromine in different Micellar media

Type of micelle	Micelle %(V/V)	k'' at 300 K	Equation	R^2	$\Delta H^{\#}$ kJ /mol	$\Delta G^{\#}$ kJ /mol	$-\Delta S^{\#}$ J/ Kmol
TX-100	0.5	0.25	y = -1.3086x - 2.725	0.998	10.8	76.8	220
	1.0	0.37	y = -1.0072x - 3.324	0.981	8.35	75.8	225
	2.0	0.37	y = -1.046x - 3.202	0.976	8.67	75.8	224
	3.0	0.58	y = -0.1635x - 5.702	0.999	1.35	74.5	244
	4.0	0.51	y = -0.1386x - 5.917	0.986	1.14	74.9	246
	5.0	0.60	y = -0.566x - 4.331	0.982	4.69	74.5	233
SDS	0.5	0.39	y = -0.9833x - 3.359	0.977	8.16	75.6	225
	1.0	0.58	y = -0.5949x - 4.269	0.983	4.93	74.8	233
	2.0	0.35	y = -1.6882x - 1.1067	0.963	14.0	75.8	206
	3.0	0.58	y = -0.731x - 3.810	0.999	6.06	74.7	229
	4.0	0.42	y = -0.809x - 3.869	0.990	6.71	75.4	229
	5.0	0.60	y = -0.7592x - 3.684	0.999	6.30	74.7	228

Table-6
Temperature dependent Rate constant (k) and Binding constant (K) data of Xanthine derivatives in TX-100 media

Xanthine Derivative	Temp (K)	Equation: y= mx +c	Regress Coeff (R^2)	Rate constant (k_m)	Binding constant (K)	(K/k_m) ratio
Caffeine	300	y = 0.0304x + 0.6454	0.978	1.54	21.2	13.7
	310	y = 0.0186x + 0.6267	0.962	1.59	33.6	21.1
	320	y = 0.0131x + 0.5668	0.956	1.76	43.2	24.5
Xanthine	300	y = 0.015x + 1.256	0.984	0.798	83.7	105
	310	y = 0.0075x + 1.1428	0.988	0.875	152	174
	320	y = 0.0025x + 1.0773	0.952	0.928	430	464
Hypoxanthine	300	y = 0.0156x + 0.5894	0.982	1.69	37.7	22.2
	310	y = 0.0066x + 0.1655	0.861	6.04	25.0	4.15
	320	y = 0.0066x + 0.1655	0.861	6.04	25.0	4.15
Theophylline	300	y = 0.0233x + 1.0622	1.00	0.941	45.5	48.4
	310	y = 0.018x + 1.0326	0.999	0.968	57.3	59.2
	320	y = 0.0125x + 1.1512	0.929	0.868	92.0	106
Theobromine	300	y = 0.0188x + 1.3605	0.998	0.735	72.3	98.4
	310	y = 0.0142x + 1.3183	0.998	0.758	92.8	122
	320	y = 0.0113x + 1.2637	0.997	0.791	111	141

Table-7
Temperature dependent Rate constant (k) and Binding constant (K) data of Xanthine derivatives in SDS media

Xanthine Derivative	Temp (K)	Equation: y= mx +c	Regress Coeff (R^2)	Rate constant (k_m)	Binding constant (K)	(K/k_m) ratio
Caffeine	300	y = 0.001x + 0.9123	0.921	1.09	912	832
	310	y = 0.0007x + 0.6591	0.930	1.51	941	620
	320	y = 0.0006x + 0.4637	0.940	2.15	772	358
Xanthine	300	y = 0.0008x + 2.2077	0.959	0.452	275	609
	310	y = 0.0007x + 1.5084	0.977	0.662	215	325
	320	y = 0.0005x + 1.3758	0.915	0.726	275	378
Hypoxanthine	300	y = 0.0029x + 1.0317	0.929	0.969	355	367
	310	y = 0.0018x + 0.6104	0.916	1.63	339	206
	320	y = 0.0013x + 0.1063	0.944	9.40	81.7	8.69
Theophylline	300	y = 0.0007x + 0.91	0.994	1.09	130	118
	310	y = 0.0006x + 0.934	0.999	1.10	155	145
	320	y = 0.0005x + 0.9314	0.994	1.11	186	173
Theobromine	300	y = 0.0004x + 1.562	0.999	0.640	390	609
	310	y = 0.0003x + 1.4143	1.00	0.707	471	666
	320	y = 0.0003x + 1.2686	0.999	0.788	422	536

Table-8
Temperature dependent Rate constant (k) and Binding constant(K) data of Xanthine derivatives in CTAB media

Xanthine Derivative	Temp (K)	Equation: y= mx +c	RegressCoeff (R^2)	Rate constant (k_m)	Binding constant (K)	(K/k_m) ratio
Caffeine	300	y = 0.0017x + 1.0266	0.962	0.974	912	936
	310	y = 0.0011x + 0.9693	0.960	1.03	941	912
	320	y = 0.0003x + 0.9049	0.962	1.10	772	699
Theophylline	300	y = 0.0012x + 1.6687	0.943	0.599	275	460
	310	y = 0.001x + 1.6063	0.967	0.622	215	346
	320	y = 0.0006x + 1.6221	0.974	0.616	275	446

CONCLUSIONS

We have studied oxidation of Xanthine alkaloids such as Xanthine (**XAN),** hypoxanthine (**HXAN),** caffeine (**CAF),** theophylline (TPL), theobromine (TBR), by a versatile chemical reagent CAN in catalytic amounts. Oxidation of xanthine derivatives afforded uric acid derivatives. Even though the reaction is too sluggish in acetonitrile media even at elevated temperatures, it underwent smoothly in presence of micelle forming

surfactants such as Tx-100, SDS, and CTAB. Reaction followed first order kinetics in both [CAN] and [Xanthine alkaloid]. Rate of oxidation is accelerated with an increase in [Surfactant] linearly. Mechanism 0f oxidation in Tx-100 micellar media has been explained by Menger- Portnoy enzymatic model with the oxidation of micelle-bound substrate (MS) by Ceric nitrate (CAN) appropriately. However, in SDS and CTAB mediated reactions micelle bound-CAN is formulated as oxidizing species.

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MICROWAVE ASSISTED HIGH-SPEED PARALLEL SYNTHESIS OF (N-PHENYL MALONIC) ACID ESTERS UNDER SOLVENT FREE CONDITION

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ABSTRACT

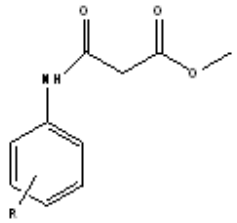
A new synthetic approach to make malonamic esters using micro wave conditions without any solvent. These building blocks are usefull to have new chemical entities to serve medicinal chemistry

KEY WORDS

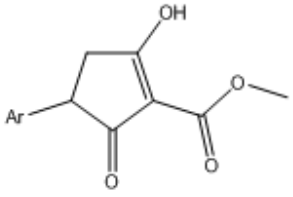
Malonamic acid esters, quinolinone heterocycles, microwave- assisted synthesis.

INTRODUCTION

N-Phenyl malonamic acid esters (Fig – 1 **I**) are useful intermediates in the synthesis of pharmacologically active compounds which are used as potent inhibitors of plasminogen activator inhibitor-1 (PAI-1, Fig. 2, **II**),[1a] cytostatic agent (breast cancer BT-549 cell line, colon cancer HCT-15 cell line, non-small cell lung NCI-H23 cell line, Fig. 2, **III**),[1b] glycine NMDA receptor antagonists (Fig. 2, **IV**),[1c] antibacterial agents, β-lactam class of compounds,[1d] and serotonin ($5HT_3$) receptor antagonists.[1e] In addition, *N*-phenyl malonamic acid esters have been foundto serve as key intermediates in the synthesis of novel heterocycles.[2]



I Figure 1



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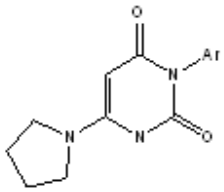
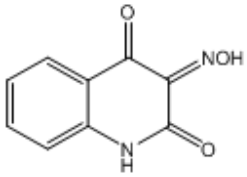


III

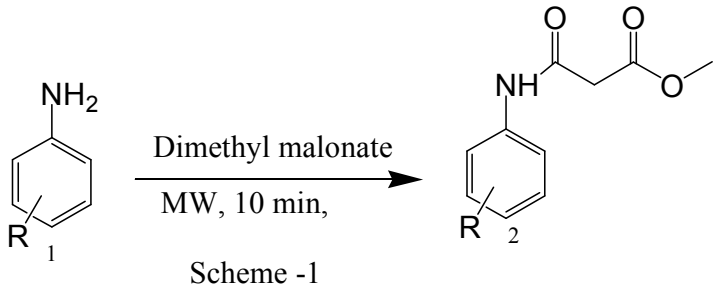


IV

Figure 2

PRESENT WORK

Due to their enormous importance in chemical as well as pharmaceutical research, a number of methods are reported in literature for the synthesis of (**I**). The existing methods consist of the use of anilines and dimethylmalonate in benzene,[3] addition of chlorocarbonyl acetic acid ethyl ester to anilines in presence of base,[4] malonic acid mono methyl ester and anilines in presence of DCC/EDC.[5] Apart from these, they are also obtained as side product from different reactions.[6] However, all these methods require carcinogenic organic solvents such as benzene, heating for a long time, and utilize expensive reagents such as EDC, CDI etc. Therefore, there is a need for an improved and alternative procedure for synthesis of (**I**). The solvent free reactions are useful in organic synthesis and in particular, use of microwave irradiation has been well documented in the literature.[7-9] In continuation of our interest in microwave assisted reactions, we applied this technique for the synthesis of *N*-phenylmalonamic acid methyl esters. Herein, we report for the first time, microwave enhanced formation of *N*-phenyl malonamic acid esters from substituted anilines and malonic ester using solvent free conditions and without any supported reagents. The present work was an observation during the preparation of tricyclic oxazolo compounds.[10] Optimization of reaction conditions were performed using 4-fluoroaniline and dimethyl malonate (entry 7 in Table 1). Optimal conditions for this synthesis were found to be 10 min reaction time using 40% Microwave oven level (1000 W), from the house hold Microwave oven. These optimal conditions were then applied for the synthesis of a range of 2,3 and 4 substituted *N*-phenyl malonamic acid esters, as depicted in Table -1. It is interesting to note that the electron withdrawing containing anilines also worked well and afforded good yields. Our synthetic procedure involves irradiation of mixture of aniline compound (substituted anilines) and dimethyl malonate in house hold Microwave oven. The reactions were carried out in open vessel so as to enable escape of methanol formed during the reaction.

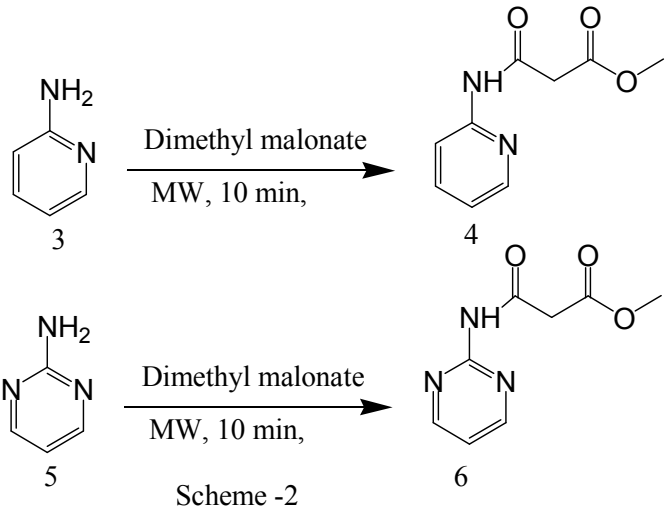


Scheme -1

Table 1
Reaction products and the spectral data obtained from substituted anilines and dimethyl malonate.

Entry	Product	R	Yield (%)
1.	**2a**	H	92
2.	**2b**	3,4-Dimethoxy	92
3.	**2c**	2,4-Dimethoxy	96
4.	**2d**	2-Methoxy	90
5.	**2e**	4-Methoxy	95
6.	**2f**	3-Fluoro	93
7.	**2g**	4-Fluoro	96
8.	**2h**	4-Acetyl	89
9.	**2i**	2-Methyl	85
10.	**2j**	4-Carboxy	91

We extended this work to prepare 2-amino pyridine and 2-amino pyrimidine derivatives (Scheme -2), out of which 2-amino pyrimidine derivative (6) was reported as an anti inflammatory (carrageenin-induced Paw edema in Rats) molecule.[10]



Scheme -2

EXPERIMENAL PREOCEDURES

Typical procedure for the synthesis of *N*-phenyl malonamic acid methyl esters

*Preparation of (**I**):* A solution of 3,4-dimethoxy aniline (3.0 g, 19.6 mmol) and dimethyl malonate (6.75 mL, 58.82 mmol) in 100 ml flat bottomed round flask, was irradiated in the house hold Microwave oven for 10 min at 40 % power level . Cooled the reaction mixture and stirred with *n*-hexane at 0 ^{0}C for 10 min. The light brown solid product was filtered, washed with hexane and dried to furnish 2a – 2j.

Parallel Synthesis of malonamic esters

The appropriate anilines (1.0 g, Table 1) and dimethyl malonate (3.0 eq) were placed in six individual 100 ml flat bottomed round flasks. These all reaction flasks were irradiated in the house hold Microwave oven for 10 min at the 40 % power level. A similar work-up as described above, provided excellent yields of the desired products (Table 1).

All the products were characterized from their spectral (IR, ^{1}H NMR and MS) data. The spectral data of the all compounds are given below.

1. N-Phenylmalonamic acid methyl ester (2a, Entry 1)

   Solid; mp 49-51 °C. IR (KBr) 3271, 1867, 1741, 1601, cm^{-11}H NMR ($CDCl_3$, 200 MHz) δ 9.11 (bs, 1H, D_2O exchangeable), 7.53 (s, 2H), 7.32 (m, 2H), 7.12 (s, 1H), 3.8 (s, 3H), 3.48 (s, 2H). MS (CI Method): *m/z* = 194 [100%, $(M+1)^+$].

2. N-(3,4-Dimethoxyphenyl)-malonamic acid methyl ester (2b, Entry 2):

   Solid; mp: 103 – 105 °C., IR (KBr): 3268, 2951, 1752, 1649 cm^{-1}. ^{1}H NMR: δ 9.16 (bs, 1H, D_2O exchangeable), 8.20 (d, *J* = 9.0 Hz, 1H), 6.48 – 6.43 (m, 2H), 3.88 (s, 3H), 3.79 (s, 6H), 3.48 (s, 2H).MS (CI Method): *m/z* = 254 (100) [M^+1] 253 (30) [M^+].

3. N-(2,4-Dimethoxyphenyl)-malonamic acid methyl ester (2c, Entry 3)

   Solid; mp: 112 – 114 °C. IR (KBr): 3270, 2954, 1748, 1647 cm^{-1}. ^{1}H NMR: δ 9.16 (bs, 1H, D_2O exchangeable), 8.20 (d, *J* = 9.0 Hz, 1H), 6.48 – 6.43 (m, 2H), 3.88 (s, 3H), 3.79 (s, 6H), 3.48 (s, 2H). MS (CI Method): *m/z* = 254 (100) [M^++1], 253 (30 %) [M^+].

4. N-(2-methoxyphenyl) malonamic acid methyl ester (2d, Entry 4):

   Solid; mp: 162 – 164 °C.IR (KBr): 3342, 2955, 1740, 1688 cm^{-1}.^{1}H NMR: 9.36 (bs, 1H, D_2O exchangeable), 8.33 (d, *J* = 7.3 Hz, 1H), 7.08 – 6.86 (m, 3H), 3.89 (s, 3H), 3.78 (s, 3H), 3.73 (s, 2H).MS (CI Method): *m/z* = 209 (100), 163, 121.

5. N-(4-methoxyphenyl) malonamic acid methyl ester (2e, Entry 5):

   Solid; mp: 190 – 192 °C. IR(KBr): 3274, 2925, 1744, 1645 cm^{-1}.^{1}H NMR: δ 9.00 (bs, 1H, D_2O exchangeable), 7.45 (d, J = 8.66 Hz, 2H), 6.87 (d, *J* = 8.60 Hz, 2H), 3.79 (s, 6H), 3.47 (s, 2H).MS (CI Method): *m/z* = 224 [M^++1].

6. N-(3-fluorophenyl) malonamic acid methyl ester (2f, Entry 6):

   Solid; mp: 152 – 154 °C.IR(KBr): 3317, 3088, 2957, 1744, 1676 cm^{-1}.^{1}H NMR: δ 9.30 (bs, 1H D_2O Exchangeable), 7.53 (d, *J* = 11.2 Hz, 1H), 7.28 – 7.16 (m, 2H), 6.82 (t, *J* = 7.8 Hz, 1H), 3.8 (s, 3H), 3.49 (s, 2H). MS (CI Method): *m/z* = 212 (100) [M^++1].

7. N-(4-fluorophenyl) malonamic acid methyl ester (2g, Entry 7):

   Solid; mp: 124 – 126 °C. IR (KBr): 3301, 3102, 2924, 1731, 1659 cm^{-1}.^{1}H NMR: δ 9.18 (bs, 1H D_2O Exchangeable), 7.55 – 7.48 (m, 2H), 7.02 (d, *J* = 8.3 Hz, 2H), 3.81 (s, 3H), 3.48 (s, 2H). MS (CI Method): *m/z* = 212 (M^++1, 100%).

8. N-(4-acetylphenyl) malonamic acid methyl ester (2h, Entry 8):

Solid; mp: 220 – 222 °C.IR (KBr): 3300, 3195, 2954, 1738, 1672 cm^{-1}.^{1}H NMR: δ 9.49 (bs, 1H D_2O Exchangeable), 7.95 (d, *J* = 8.3 Hz, 2H), 7.66 (d, *J* = 8.8 Hz, 2H), 3.82 (s, 3H), 3.51 (s, 2H), 2.36 (s, 3H).MS (CI Method): *m/z* = 236 (100) [M^++1].

9. N-(2-methylphenyl) malonamic acid methyl ester (2i, Entry 9):

Solid; mp: 132 – 134 °C.IR (KBr): 3266, 3037, 2954, 1737 cm^{-1}.^{1}H NMR: δ 9.21 (bs, 1H D_2O Exchangeable), 7.95 (d, *J* = 8.8 Hz, 1H), 7.17 (m, 3H), 3.81 (s, 3H), 3.52 (s, 2H), 2.32 (s, 3H).MS (CI Method): *m/z* = 208 (100) [M^++1].

10. N-(4-carboxyhenyl) malonamic acid methyl ester (2j, Entry 10):

Solid; mp: 249 – 251 °C.IR (KBr): 3271, 1867, 1741 cm^{-1}.^{1}H NMR: δ 9.11 (bs, 1H D_2O Exchangeable), 7.53 (s, 2H), 7.32 (m, 2H), 7.12 (s, 1H), 3.8 (s, 3H), 3.48 (s, 2H). MS (CI Method): *m/z* = 194 (100) [M^++1].

11. Methyl 2-(2-pyridylcarbamoyl)acetate (4):

Solid; mp: 185 – 187 °C, IR (KBr): 3310, 2955, 1744, 1696 cm^{-1}.^{1}HNMR(CDCl3, 200 MHz): δ 9.53 (bs, 1H D_2O exchangeable), 8.29 (d, *J* = 4.0 Hz, 1H), 8.16 (d, *J* = 8.3 Hz, 1H), 7.69 (t, *J* = 7.0 Hz, 1H), 7.05 (t, *J* = 5.0 Hz, 1H), 3.78 (s, 3H), 3.5 (s, 2H).MS (CI Method): *m/z* = 195 (100) [M^++1], 163, 121, 94. Yield: 43 %.

12. Methyl 2-(2-pyrimidinylcarbamoyl) acetate (6):

Solid; mp: 216 – 218 °C. IR (KBr): 3143, 3073, 2953, 2921, 1731, 1681 cm^{-1}. ^{1}HNMR(CDCl3, 200 MHz): 10.81 (s, 1H, D_2O exchangeable), 8.64 (d, *J* = 4.88 Hz, 2H), 7.18 (t, *J* = 4.88 Hz, 1H), 3.71 (s, 2H), 3.63 (s, 3H). MS (CI Method): *m/z* =196 (100) [M^++1), 164 (75).

CONCLUSIONS

In conclusion, we described here a reagent free rapid and practical procedure for the synthesis of *N*-phenyl malonamic acid esters from substituted anilines and malonic acid methyl ester under microwave condition.

ACKNOWLEDGEMENT

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ONE POT SYNTHESIS OF SUBSTITUTED 3,4-DIHYDRO-2H-BENZO[B][1,4]THIAZINE-3-ONES AND 1,2,3,4-TETRAHYDRO-2-QUINOXALINONE

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ABSTRACT

3,4-dihydro-2*H*-benzo[*b*][1,4]thiazine-3-ones &1,2,3,4-tetrahydro-2-quinoxalinones are important scaffolds in pharmaceutical industry. We report here new methods to make new heterocycles benzothiazines & quinoxalines conventional, parallel and microwave conditions to have scaffolds to make NCEs with good purity & more yields

.

KEY WORDS

One pot synthesis, microwave assisted protocol.

INTRODUCTION

The wide occurrence of benzo-fused [1,4]thiazines and 2-quinoxalinones in bioactive pharmaceuticals and their interesting chemical applications has made them important synthetic targets. These are an integral part of a range of pharmacologically relevant compounds such as antidiabetic[1], antimicrobial[2], aldose reductase inhibitors[3], muscle relaxants[4], potassium channel openers[5], high-affinity ligands for GABA[6],anxiolytic antagonist[7] etc., and are also found to be useful intermediates in the synthesis of variety of heterocycles[8]. In view of their enormous importance in chemical and pharmaceutical research, a variety of synthetic methods have been reported in the literature for the synthesis of benzo[b] thiazine[9] and 2-quinoxalinones[10].

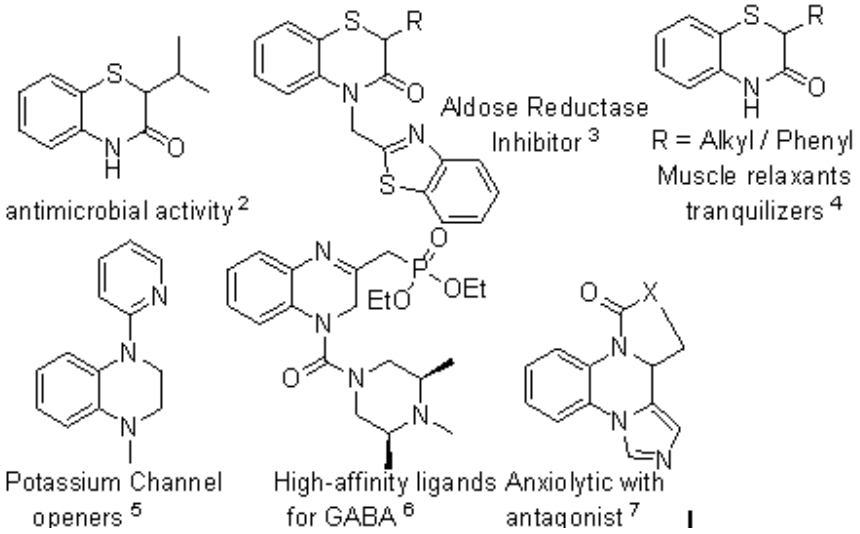


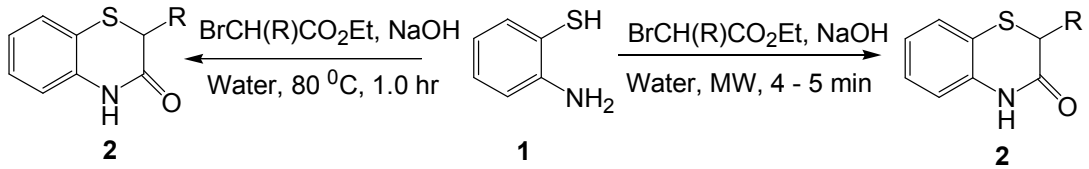
Figure 1

Selected known bioactive compounds prepared from benzothiazine-3-ones and 2-quinoxalinones

PRESENT WORK

As part of our continuing interest in the development of economically viable synthesis of heterocyclic systems, we investigated the utility of the microwave irradiation in the synthesis of both these classes of compounds. Typically, benzo[1,4]thiazines and 1,2,3,4-tetrahydro-2-quinoxalinones are made by reaction of 2-haloacetates with 2-aminothiophenol or 1,2-diaminobenzene in the presence of different bases and in a variety of solvents. All the reported methods involve more than one step and requires fairly long reaction times. Therefore, there is a need for the development of an improved procedure for synthesis of substituted benzothiazines and 2-quinoxalinones.

Here we report the synthesis of 2-alkyl/aryl Benzo[1,4]thiazine-3-ones, from 2-aminothiophenol and 2-bromo-2-alkyl/aryl acetic acid esters in presence of aqueous sodium hydroxide solution. The reactions were performed in three different methods: (A) conventional, (B) parallel synthesis and (C) microwave irradiation methods. In method A, 2-amino thiophenol was treated with 2-bromo-2-alkyl acetates in the presence of sodium hydroxide in water at 100 °C for 1.0 hr. In method C, a similar mixture was irradiated with microwave using house hold microwave oven for 5.0 min. In method B, 2-aminothiophenol was treated with a variety of 2-bromo-2-alkyl acetates in the presence of sodium hydroxide in water and was stirred using parallel synthesizer at 100^{o}C. In this method, eight 2-substituted benzothiazines were synthesized in one lot. The results are shown in the Table 1.



Scheme 1

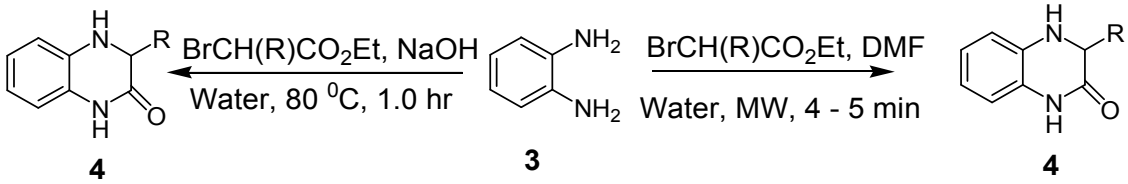
Table 1

Synthesis of benzothiazine-3-ones (2a-h). And ^{1}H NMR spectroscopic data & Mass spectrometric Data of substituted malonamic acid esters.

Entry	Product	R	Yield (%)		
			Method A	Method B	Method C
1.	**2a**	H	94	96	96
2.	**2b**	Methyl	60	65	77
3.	**2c**	Dimethyl	62	65	71
4.	**2d**	Ethyl	68	67	73
5.	**2e**	Propyl	53	59	61
6.	**2f**	Isopropyl	51	62	65
7.	**2g**	Hexyl	39	42	57
8.	**2h**	Phenyl	63	69	74

[a] A: Conventional, B: Using Parallel synthesizer, C: Microwave irradiation.

For the preparation of 1,2,3,4-tetrahydro-2-quinoxalinones, 1,2-diaminobenzene **(3)** was reacted with ethyl 2-bromo-2-alkyl/arylacetate in the presence of sodium hydroxide in water and the reaction mixture was refluxed for one hour to give 3-substituted quinoxaline-2-ones. The other protocol used was irradiation with microwaves using microwave oven for 5.0 min in *N,N*-dimethylformamide instead of refluxing in water to yield **4a-f**. **(Scheme - 2)** Preparation of **4a-f** was also performed in the presence of diisopropylethylamine as base in DMF and also neat by microwave irradiation. However, better yields were achieved in the presence of 10 % *N,N*-dimethylformamide in water as solvent.



Scheme 2

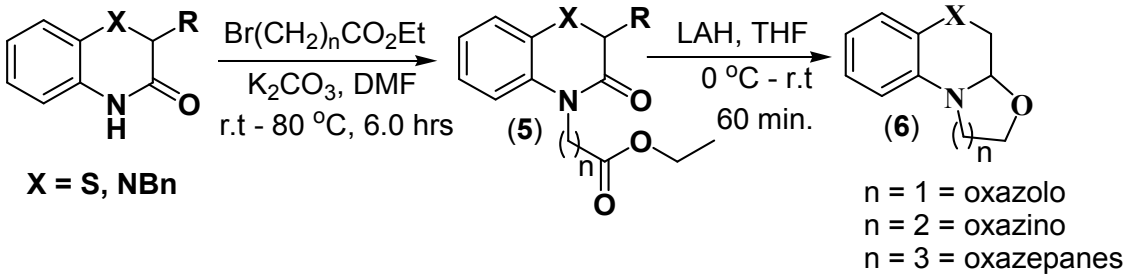
Table2
Synthesis of 2-quinoxalinones (4a-h)

Entry	Product	R	Yield (%)	
			Method A	Method B
9.	**4a**	H	74	79
10.	**4b**	Methyl	69	75
11.	**4c**	Dimethyl	49	73
12.	**4d**	Ethyl	65	73
13.	**4e**	Propyl	68	76
14.	**4f**	Isopropyl	59	68
15.	**4g**	Phenyl	63	75

[a] A: Using Parallel synthesizer, B: Microwave irradiation

APPLICATION OF THE METHODOLOGY

The developed methodology was successfully utilized for the synthesis of a number of novel compounds having interesting biological properties. Thus, our protocol was used as one of the crucial steps during the synthesis of novel tricyclic oxazolo/oxazine compound [11]**6**, an interesting and novel heterocycle in organic chemistry, which can serve as new scaffold for developing different bioactive compounds. (Scheme - 3)



Scheme – 3

EXPERIMENTAL PROCEDURE

Preparation of 2a -h

Method A:

To a solution of 2-aminothiophenol (5.0 g, 40.0 mmol) in water was added powdered sodium hydroxide (1.5 eq) followed by ethyl 2-bromo-2-alkyl acetate at room temperature. The reaction mixture was stirred for 1.0 hour at 80 ^{o}C and then poured into dil. HCl (100 % v/v). The precipitated solid was filtered, washed with water and dried to obtain **2a-h** (Table-1).

Method B: To a solution of 2-aminothiophenol (0.250 g, 2.0 mmol) in water was added sodium hydroxide (0.12 g, 3.0 mmol) followed by ethyl 2-bromo-2-alkyl acetate (1.2 eq) at room temperature. The mixture was irradiated with microwave using household microwave oven for 5.0 min. The mixture was cooled and stirred with colddil. HCl (100 mL, 50 % v/v). The separated solid was filtered, washed with water and dried to obtain **2a-h** (Table-1).

Method C:

Similar to method A, but all the substituted 2-bromoethyl acetates were separately reacted with 2-aminothiophenol in different vessels at a time using parallel synthesizer.

Preparation of 4a-g:

Method A:

To a solution of *o*-phenylenediamine (**3**, 5.0 gms, 46.0 mmol) in water was added powdered sodium hydroxide followed by addition of ethyl 2-bromo-2-alkyl acetate at room temperature. The reaction mixture was stirred for one hour at 80 $^{\circ}$C, cooled to room temperature and neutralized with aq. HCl (50 %, v/v). The separated solid was filtered, washed with water and dried to obtain **4a-g** (Table-2)**.**

Method B:

To a solution of *o*-phenylenediamine (3, 0.5 gms, 4.6 mmol) in DMF was added powdered sodium hydroxide (1.5 eq) followed by ethyl 2-bromo-2-alkyl acetate (1.2 eq) at room temperature. The mixture was irradiated with microwaves using household microwave oven for 5.0 min. Then, the mixture was cooled, and neutralized with aq. HCl (50 %, v/v). The separated solid was filtered, washed with water and dried to obtain 4a-f (Table-2). All the products were characterized from their spectral (IR, ^{1}H NMR and MS) data. The spectral data of

the all compounds are given below.

1.4H-Benzo[1,4]thiazin-3-one (2a, Entry 1):

Solid; mp: 162 – 164 °C.IR(KBr):3058, 2928, 2864, 1588 cm^{-1}.^{1}H NMR:(DMSO d_6): δ 10.56 (bs, 1H, D_2O exchangeable), 7.27 (t, *J* = 7.52 Hz, 1H), 7.15 (t, *J* = 7.52 Hz, 1H), 6.99 (m, 2H), 3.44 (s, 2H).MS (CI Method): *m/z* =166 (100) [M^++1], 165 (30) [M^+].

2.2-Methyl-4H-benzo[1,4]thiazin-3-one (2b, Entry 2):

Solid; mp: 216 – 218 °C.IR(KBr):3196, 3055, 2977, 2925, 1665 cm^{-1}. ^{1}H NMR: ($CDCl_3$) δ 9.25 (bs, 1H, D_2O Exchangeable), 7.29 (d, *J* = 7.52 Hz, 1H), 7.15 (d, *J* = 7.52 Hz, 1H), 7.03 (t, *J* = 7.52 Hz, 1H), 6.93 (t, *J* = 7.52 Hz, 1H), 3.60 – 3.50 (q, *J* = 6.98 Hz, 1H), 1.49 (d, *J* = 6.99 Hz, 3H).MS (CI Method): *m/z* =180 (100) [M^++1].

3. 2-Dimethyl-4H-benzo[1,4]thiazin-3-one (2c, Entry 3):

Solid; mp: 220 – 222 °C. IR(KBr): 3422, 3199, 2973, 2922, 1667, 1584 cm^{-1}. ^{1}H NMR: ($CDCl_3$) δ 10.57 (bs, 1H, D_2O Exchangeable), 7.29 (d, *J* = 7.33 Hz, 1H), 7.18 (d, *J* = 7.81 Hz, 1H), 7.00 (d, *J* = 7.82 Hz, 2H), 1.34 (s, 6H). MS (CI Method): *m/z* =194 (100) [M^+ +1].

4. 2-Ethyl-4H-benzo[1,4]thiazin-3-one (2d, Entry 4):

Solid; mp: 178 – 180 °C. IR(KBr): 3195, 3054, 2963, 1662, 1582 cm^{-1}. ^{1}H NMR: ($CDCl_3$) δ 10.56 (bs, 1H, D_2O Exchangeable), 7.31 (d, *J* = 7.81 Hz, 1H), 7.16 (d, *J* = 7.81 Hz, 1H), 7.00 – 6.94 (m, 2H), 3.45 – 3.38 (dd, *J* = 5.86 & 8.31 Hz, 1H), 1.81 – 1.70 (m, 1H), 1.54 – 1.39 (m, 1H), 0.96 (t, *J* = 7.33 Hz, 3H). MS (CI Method): *m/z* = 194 (100) [M^++1];

5. 2-Propyl-4H-benzo[1,4]thiazin-3-one (2e, Entry 5):

Solid; mp: 188 – 190 °C. IR(KBr): 3050, 2960, 2924, 1670, 1583 cm^{-1}. ^{1}H NMR: ($CDCl_3$) δ 10.56 (bs, 1H, D_2O Exchangeable), 7.31 (d, *J* = 7.81 Hz, 1H), 7.16 (m, 1H), 7.00 – 6.94 (m, 2H), 3.52 – 3.45 (d, *J* = 7.81 Hz, 1H), 1.77 – 1.67 (m, 2H), 1.52 – 1.33 (m, 2H), 0.89 – 0.83 (t, *J* = 7.33 Hz, 3H).MS (CI Method): *m/z* = 208 (100) [M^++1]

.

6. 2-Isopropyl-4H-benzo[1,4]thiazin-3-one (2f, Entry 6):

Solid; mp: 152 – 154 °C. IR(KBr): 3412, 2962, 2923, 1685, 1581 cm^{-1}; ^{1}H NMR: δ 9.17 (bs, 1H, D_2O Exchangeable), 7.30 (d, *J* = 7.82 Hz, 1H), 7.16 (t, *J* = 7.81 Hz, 1H), 6.99 (t, *J* = 7.33 Hz, 1H), 6.88 (d, *J* = 7.81 Hz, 1H), 3.11 (d, *J* = 8.79 Hz, 1H), 1.97 – 1.90 (m, 1H), 1.05 (d, *J* = 6.83 Hz, 6H). MS (CI Method): *m/z* = 208 (100) [M^++1].

7. 2-Hexyl-4H-benzo[1,4]thiazin-3-one (2g, Entry 7):

Solid; mp: 88 - 90 °C. IR(KBr): 2960, 2853, 1663, 1583 cm^{-1}.^{1}H NMR: ($CDCl_3$) δ 9.10 (bs, 1H, D_2O Exchangeable), 7.30 (d, *J* = 7.33 Hz, 1H), 7.17 (t, *J* = 7.33 Hz, 1H), 7.00 (t, *J* = 7.81 Hz, 1H), 6.89 (d, *J* = 7.81 Hz, 1H), 3.39 (t, *J* = 7.08 Hz, 1H), 1.93 – 1.84 (m, 1H), 1.66 – 1.50 (m, 1H), 1.25 (m, 8H), 0.86 – 0.82 (m, 3H).MS (CI Method): *m/z* =250 (100) [M^+ +1].

8. 2-Phenyl-4H-benzo[1,4]thiazin-3-one (2h, Entry 8):

Solid; mp: 204 - 206 °C. IR(KBr): 3430, 3190, 351, 2973, 1677 cm^{-1}. ^{1}H NMR (DMSOd_6, 200 MHz): δ 10.43 (bs, 1H, D_2O Exchangeable), 7.33 – 7.26 (m, 6H), 7.17 – 7.09 (m, 1H), 7.02 – 6.91 (m, 2H), 4.62 (s, 1H). MS (CI Method): *m/z* = 242 (100) [M^+ +1]; 241 (20) [M^+].

9. 3,4-Dihydro-1H-quinoxalin-2-one (4a, Entry 9):

Solid; mp: 152 - 154 °C. IR(KBr): 3367, 2924, 1681 cm^{-1}; ^1H NMR (In DMSO d_6): δ 10.21 (bs, 1H, D_2O Exchangeable), 6.78 – 6.54 (m, 4H), 5.93 (bs, 1H, D_2O Exchangeable), 3.38 (s, 2H). MS (CI Method): *m/z* = 149 (100) [M^+ +1].

10. 3-Methyl-3,4-dihydro-1H-quinoxalin-2-one (4b, Entry 10):

Solid; mp: 112 - 114 °C IR(KBr): 3448, 2921, 2850, 1688 cm^{-1}; ^1H NMR (DMSO d_6): δ 12.17 (bs, 1H, D_2O Exchangeable), 7.72 (d, *J* = 7.81 Hz, 1H), 7.5), 7.70 – 7.21 (m, 3H), 2.95 (m, 1H), 2.55 (s, 3H). MS (CI Method): *m/z* =162 (10) [M^+], 161 [100];.

11. 3,3'-Dimethyl-3,4-dihydro-1H-quinoxalin-2-one (4c, Entry 11):

Solid; mp: 170 - 172 °C. IR(KBr): 3440, 2924, 2853, 1659 cm^{-1}; ^1H NMR (CDCl3): 10.57 (bs, 1H, D_2O Exchangeable), 7.29 (d, *J* = 7.33 Hz, 1H), 7.18 (d, J = 7.79 Hz, 1H), 7.00 (d, *J* = 7.79 Hz, 2H), 1.34 (s, 6H). MS (CI Method): *m/z* = m/z 194 (20) [M^+ +1].

12. 3-Ethyl-3,4-dihydro-1H-quinoxalin-2-one (4d, Entry 12):

Solid; mp: 186 - 188 °C. IR(KBr): 3440, 2924, 2853, 1659 cm^{-1}; ^1H NMR ($CDCl_3$): δ 11.99 (bs, 1H, D_2O Exchangeable), 8.10 (d, *J* = 7.81 Hz, 1H), 7.78 – 7.71 (m, 1H), 7.59 – 7.52 (m, 2H), 3.32 – 2.21 (m, 1H), 1.72 – 1.56 (m, 2H), 1.44 – 1.36 (t, *J* = 7.33 Hz, 3H). Mass: 177 (10) [M^+ +1], 175 (100) [M^+].

13. 3-Propyl-3,4-dihydro-1H-quinoxalin-2-one (4e, Entry 13):

Solid; mp: 158 - 160 °C. IR(KBr): 3316, 2959, 1659 cm^{-1}; ^1H NMR (DMSO d_6): δ 12.16 (bs, 1H, D_2O Exchangeable), 7.70 (d, *J* = 7.82 Hz, 1H), 7.45(t, *J* = 7.81 Hz, 1H), 7.29 – 7.22 (m, 2H), 2.75 (t, *J* = 7.33 Hz, 1H), 2.53 (t, *J* = 5.36 Hz, 2H), 1.73 (t, *J* = 7.32 Hz, 2H), 0.95 (t, *J* = 7.32 Hz $3H_3$). MS (CI Method): *m/z* = 191 (50) [M^++1], 189 (100) [M^+].

14. 3-Isopropyl-3,4-dihydro-1H-quinoxalin-2-one (4f, Entry 14):

Solid; mp: 218 - 220 °C. IR (KBr): 3433, 2964, 2853, 1686 cm^{-1}. ^1H NMR (DMSO d_6): δ 11.92 (bs, 1H, D_2O Exchangeable), 7.71 (d, J = 7.82 Hz, 1H), 7.45(d, J = 7.33 Hz, 1H), 7.29 – 7.22 (m, 2H), 3.49 – 3.42 (m, 1H), 1.23 (s, 3H), 1.19 (s, 3H). MS (CI Method): *m/z* = 191 (10) [M^++1], 189 (100) [M^+], 188 (40).

15. 3-Phenyl-3,4-dihydro-1H-quinoxalin-2-one (4g, Entry 15):

Solid; mp: 184 - 186 °C,IR(KBr): 3437, 2925, 1684, 1446 cm^{-1}.^1H NMR (DMSO d_6): δ 12.57 (bs, 1H, D_2O Exchangeable), 8.32 – 8.27 (m, 2H), 8.29 – 8.27 (d, J = 7.33 Hz, 1H), 7.59 –7.49 (m, 4H), 7.74 (d, J = 7.81 Hz, 1H), 7.51 (s, 3H).MS (CI Method): *m/z* =224 (10) [M^+ +1], 223 (100) [M^+], 194 (10);

CONCLUSIONS:

In the present study we have described a practical and general method for the synthesis of 3,4-dihydro-2*H*-benzo[*b*][1,4]thiazine-3-ones and 1,2,3,4-tetrahydro-2-quinoxalinones. These compounds could be utilized for the synthesis of bioactive molecules and also synthetically important heterocycles. The advantages are: (i) Use of aqueous medium is a safe alternative to organic solvents under microwave exposure. (ii) Mild reaction conditions (iii) excellent yields and reproducibility. (iv) Simple operational procedure.

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PHYTOCHEMICAL EXPLORATION AND THEIR BIOLOGICAL EVALUATION FROM THE STEM BARK OF ALANGIUM SALVIFOLIUM

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ABSTRACT

Extraction, isolation and chemical characterization of bioactive molecules from the bark of *Alangium salviifolium* lead to the separation of four phytochemicals Stigmasterol **(1),** Stigmasterol-3-O-β-D-glucoside **(2),** Rutin **(3)** and α-D-glucopyranoside-β-fructofuranosyl **(4)** respectively. This is the first report of compounds **2-4** from the plant. Total phenolic and flavonoid assays revealed the methanol extract (88.47 mg GAE and 395.8 mg QE) as rich in these contents. The ethylacetate extract (IC_{50} 65.7 µg/ml) showed significant anti-oxidant activity using the DPPH method. Hexane extract showed better anti-bacterial activity against *Streptococcus aureus* while methanol extract was better against *Chromobacterium violaceum.*

KEYWORDS

Alangium salviifolium; Total phenolic assay; Total flavonoid assay; Anti-oxidant activity; Anti-bacterial activity.

INTRODUCTION

Alangium salviifolium (L.f.) Wang (family Alangiaceae) is a tree which grows to a height of 3-10 meters. The plant has been used as a folk medicine for the treatment of diarrhea, asthma and coughs, hemorrhoids and for the expulsion of intestinal worms traditionally. The phytochemical screening of *A. Salviifolium leaves* reported the presence of flavonoids, alkaloids, steroids, glycosides, saponins etc., and the extracts showed curative in epilepsy, jaundice and hepatitis[1-3]. The leaf alcoholic extract showed anticonvulsant effect on Pentylenetetrazole induced seizures and maximal electroshock induced seizures in mice[3]. The ether fraction from the flowers shown activity against Ehrlich Ascites Carcinoma (EAC) in mice[4] and also inhibited gram-positive and gram-negative microorganisms[5]. The plant's stem bark extracts showed to inhibit rheumatic pain[6] and inflammation. In animal model it showed abortifacient and hence it can be an effective antifertility[7]. In view of the its varied pharmacological activity and few phytochemical reports phytochemical and biological activity screening of stem bark of *A. salviifolium* was taken as part of our continuing phytochemistry work[8,9].

MATERIALS AND METHODS

1. Collection of plant material

The stem bark was collected from CSIR-CIMAP-Research Centre, Hyderabad, AP, India. The plant was taxonomically identified and authenticated by Dr. Venkat Ramana, Assistant Professor, Department of Botany, Nizam College, Osmania University, AP, India. A voucher specimen (CIMAP-AL/1/14) was deposited at CIMAP, Research Centre, Hyderabad.

2. Extraction and Isolation:

The stem bark (1.5 kg) was shade dried, powdered and successively extracted with hexane (5 L, 12 h), ethyl acetate (5 L, 18) and methanol (5 L, 24 h) using hot percolation method. Later the extracts were filtered and distilled under reduced pressure to obtain desired concentration and the percentage yield was calculated accordingly. The chromatographic separation of hexane extract (18 g) over silica gel 100- 200 mesh yielded a white coloured compound **1** (30 mg) in 20 % ethyl acetate in hexane. Similarly, chromatographic separation of ethyl acetate extract (42 g) obtained compound **2** (65 mg) in pure form in 80 % ethyl acetate in hexane. Likewise, methanol extract (80 g) was also subjected to column chromatography to get compound **3** (35 mg) in 5% methanol in chloroform and compound **4** (45 mg) in 10 % methanol in chloroform.

3. Estimation of Total phenolic content and Total Flavonoid content:

a. Total phenolic assay:

The total phenolic content of *A. salviifolium stem bark* in hexane, ethyl acetate and methanol extracts were determined by Folin-Ciocalteu's assay[10]. An aliquot (1 ml) of extracts or standard solution of Gallic acid (50, 100, 150, 200 and 250 mg/lit) was added to 25 ml volumetric flask, containing 9 ml of distilled deionised water. Reagent blank using distilled deionised water was prepared. 1 ml of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7 % sodium carbonate solution was added to the mixture. The solution was diluted to 25 ml with distilled deionised water. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 750 nm with UV-Visible spectrophotometer. The phenolic content expressed as mg Gallic Acid Equivalents (GAE) per 100 grams fresh weight. All samples were analysed in duplicates and the results were shown in Table 1.

b. Total flavonoid assay:

Total flavonoid content was measured by the aluminium chloride colorimetric assay[10]. An aliquot of extracts or standard solution of Quercitin (150, 200, 300, 400 and 500 mg/lit) was added to 10 ml volumetric flask, containing 4 ml of distilled deionised water. To the flask was added 0.3 ml of 5 % sodium nitrite ($NaNO_2$). After 5 min, 0.3 ml of

10 % aluminium chloride was added. At 6th min, 2 ml of 1 M sodium hydroxide was added and total volume was made up to 10 ml with distilled deionised water. The solution was mixed well and the absorbance (in UV-VIS spectrophotometer) was measured against prepared reagent blank at 510 nm. Flavonoid content expressed as mg Quercitin equivalents/100 g fresh mass (Table 1).

4. Antioxidant activity by DPPH radical scavenging method:

The ability of the extracts to scavenge DPPH radical was determined by using DPPH radical scavenging method[11]. Sample stock solutions (1.0 mg/ml) were diluted to final concentrations of 250, 125, 50, 25, 10 and 5 µg/ml in methanol. 1 ml of a 0.3 mM DPPH methanol solution was added to 2.5 ml solution of the extract or standard and allowed to react at room temperature for 30 min. The absorbance of the mixture was measured at 518 nm and converted to percentage antioxidant activity (AA %) using the formula: AA% = 100 – [(Abs sample – Abs blank) × 100]/Abs control Methanol (1.0 ml) plus extract solution (2.5 ml) was used as a blank. 1 ml of 0.3 mM DPPH plus methanol (2.5 ml) was used as a negative control (Table 1).

5. Antimicrobial Activity:

The bacterial cultures of Gram-positive (*Staphylococcus aureus)* and Gram-negative (*Chromobacterium violaceum*) were obtained from the culture collection centre, MTCC, IMTECH, Chandigarh. Both the microorganisms were pre-cultured in nutrient broth overnight in a rotary shaker at 37 °C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically. The antibacterial screening of the extracts were carried out by determining the zone of inhibition using disc diffusion method[12]. The sterile discs (6mm diameter) were dipped in *n*-hexane, ethyl acetate and methanolic extracts for five minutes. The discs were dried and placed on Nutrient Agar Medium plates which were previously inoculated with bacteria and incubated at 37 ºC for 24 hours. The discs dipped in distilled water and Methanol was used as negative controls. The zone of inhibition was recorded and shown in Table 2.

RESULTS AND DISCUSSION

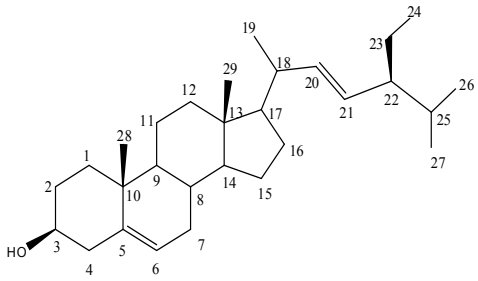
1. Chemistry:

Based on the spectroscopic experiments (^{1}H, ^{13}C NMR, 2D NMR and MASS) and previously reported literature, the structures of isolated compounds **1**, **2**, **3** and **4** were confirmed as Stigmasterol[13], Stigmasterol-3-0-β-D-glucoside[13], Rutin and α-D-Glucopyranoside-β-D-Fructofuranosyl[14] respectively. Compounds **2**, **3** and **4** are being isolated for the first time from this source. Compound **3** was obtained as yellow amorphous powder from the methanol extract. It gave neutral ferric chloride test and Molisch test positive indicating it to be a flavonoid glycoside. The compound′s molecular formula was established as $C_{28}H_{32}O_{16}$ by ESI-MS data (*m/z* 624). IR spectra (KBr, υ (cm^{-1})) showed important absorptions at 3421.97 (-OH), 2984.09, 1600 (C=O), 1559.06, 1457.32, 1361.72, 1205.41, 1124.9. 75 MHz ^{13}C-NMR spectrum, measured in pyridine-d_5, displayed 28 carbons, the terminal sugar was determined as a rhamnose by lowfield chemical shift of glucose C-6″ methylene (δ 67. 9). The carbon signals at δ 100.7 and δ 102. 2 showed glucosyl C-1″ and rhamnosyl C-1‴ respectively. C-3 signal at δ 133.2 proved that the glucosyl moiety is attached at the third position with C-1″ glucosyl signal being at δ 100.7. Based on the above data and also comparing with previously reported literature[15], the structure of the compound **3** was confirmed to be Rutin[15]. Co-TLC Comparison of **3** with a reference compound also confirmed the structure assigned. Compound **3**: ^{1}H NMR (chemical shift δ in ppm, coupling constant *J* in Hz) 6.21 (1H, d, *J*=2, C6-H), 6.40 (1H, d, *J*=2, C8-H), 7.54 (1H, d, *J*=2.1, C2′-H), 6.86 (1H, d *J*=9, C5′-H), 7.56 (1H, dd, *J*=9, C6′-H), 12.62 (1H, s, C5-OH), 5.35 (1H, d, *J*=7.4, H-1″-G), 5.15 (1H,

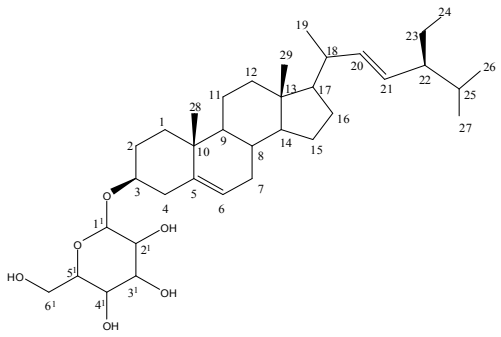
d, *J*=1.9, H-1‴), 1.00 (3H, d, *J*=6.1, CH3); ^{13}C NMR (chemical shift δ in ppm) 156.3 (C-2), 133.2 (C-3), 177.3 (C-4), 156.5 (C-5), 98.6 (C-6), 164.0 (C-7), 93.5 (C-8), 161.1 (C-9), 103.8 (C-10), 121.5 (C-1′), 116.2 (C-2′), 144.6 (C-3′), 148.3 (C-4′) 116.2 (C-5′), 121.1 (C-6′), 100.7 (C-1″), 74.9 (C-2″), 76.3 (C-3″), 71.7 (C-4″), 75.8 (C-5″), 67.9 (C-6″), 102.2 (C-1‴), 70.8 (C-2‴), 70.2 (C-3‴), 70.4 (C-4‴), 68.2 (C-5‴), 17.7 (C-6‴). [″ and ‴ represent signals from rhamnose and glucose moieties, respectively].

Figure.1.

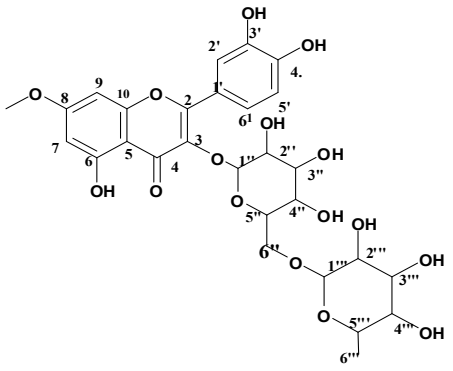
Structures of isolated compounds 1-4.



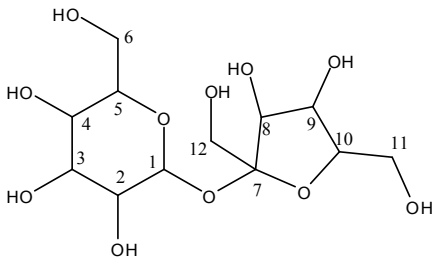
1. Stigmasterol



2. Stigmasterol -3-0-β-D-glucoside



3. Rutin



4. α -D-Glucopyranoside-β-D-Fructofuranosyl.

2. Biological activity:

Total phenolic, flavonoid assay and antioxidant activity:

Total phenolic and flavonoid assays revealed the methanol extract (88.47 mg GAE and 395.8 mg QE) as rich in these contents. The ethylacetate extract (IC_{50} 65.7 µg/ml) showed significant anti-oxidant activity using DPPH method. Hexane extract showed better anti-bacterial activity against *Streptococcus aureus* while methanol extract was better against *Chromobacterium violaceum.*

Table 1.

Total phenolic content, Total flavonoid content and *In vitro* antioxidant activity of stem bark extracts of *A. salivifolium*

No.	Extract	DPPH free radical scavenging activity (IC_{50} in µg/ml)	Total Phenolic content[a] (GA* equivalents)	Total Flavonoid content[a] (Q* equivalents)
1	Hexane	455± 0.003	15.526	65.37
2	Ethyl acetate	65.7±0.001	81.948	270.3
3	Methanol	49.7±0.001	88.47	395.8
4	Ascorbic acid	30.1±0.001	---	---

[a]GA*- Gallic acid; Q*-Quercetin

3. Antimicrobial activity:

Table 2.

Zone of inhibition against *S. aureus* and *C. violaceum* shown by extracts of stem bark of *A. Salivfolium*

S. No	Extract	Inhibition zone in mm							
		Gram positive bacteria (*S. aureus*)				Gram negative bacteria (*C. violaceum*)			
		Concentration (µg)							
		200	400	600	800	200	400	600	800
1	Hexane	10	30	40	40	20	1	1	1
2	Ethyl acetate	30	20	20	20	1	1	10	20
3	Methanol	1	1	5	8	1	1	10	30

SUMMARY AND CONCLUSION:

Extraction and isolation of powdered stem bark of *A. salviifolium* resulted in the separation of compounds 1-4 whose structures were established basing on IR, 1D/2D NMR and Mass spectrometry as stigmasterol, Stigmasterol -3-0-β-D-glucoside, Rutin and α- D-glucopyranoside-β-fructofuranosyl respectively. The compounds **2**, **3** and **4** are being reported for the first time from the stem bark of *A. salviifolium.* Total phenolic and flavonoid assay revealed methanol as rich in content and antioxidant activity was performed using DPPH method showed ethylacetate extract as the most active one with IC_{50} value 65.7 µg/ml. Also, the anti-microbial activity was performed using disc diffusion method showed hexane extract as most potent inhibitor of gram positive bacteria, *S. aureus* and methanol extract as most potent inhibitor of gram negative bacteria, *C. violaceum*.

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