Synthesis, biological screening and molecular docking studies of some ethylated sulfonamides having 1,4-Benzodioxane moiety

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Abstract: The presented study comprises the synthesis of a new series of ethylated sulfonamides in which 1,4-benzodioxane moietyhas been incorporated. The reaction of 1,4-benzodioxane-6-amine (1) with ethane sulfonyl chloride (2) yielded *N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethanesulfonamide (3), which further on treatment with various alkyl/aralkyl halides, 4a-r, in *N,N*□-dimethylformamide (DMF) and in the presence of lithium hydride (LiH) acting as a weak base and catalyst;yielded derivativesof*N*-alkyl/aralkyl substituted *N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethanesulfonamides (5a-r). The characterization of these derivatives was carried out by different spectroscopic techniques like infra red, proton-NMR and mass spectrometry; then screened against various enzymes i.e. acetylcholinesterase, butyrylcholinesterase, lipoxygenase and α-glucosidase enzymes and five different bacterial strains. The synthesized compounds were found to be good inhibitors of lipoxygenase but moderate inhibitors of AChE, BChE and α-glucosidase; whereas compounds 3, 5a, 5f, 5n and 5r were found good antibacterial compounds. The interaction between inhibitors and target enzymes (cholinestrases and lipoxygenase) was computationally observed which correlated with the experimental results.

Keywords: Antibacterial activity; Enzyme inhibition; Ethylated sulfonamides; Lipoxygenase and molecular docking.

INTRODUCTION

For an extensive period of time interesting work has been done on the chemistry of heterocyclic compounds. Amongst the so far studied heterocycles, cyclic sulfonamides have originated as an inimitable moiety which is recognized to demonstrate various pharmacological activities. The sulfonamides belong to distinctive class of compounds that constitute at least five different classes of pharmacologically active agents (Supuran *et al*., 1999). In 1935 sulfonilamide was identified by Domagk and his colleaguesas the active metabolite of the red azo dye known as prontosil. Prontosildoes not possess any activity *in vitro*; however it metabolizes *in vivo* to give the active agent sulfanilamide; where it can interfere with the process of bacterial DNA synthesis and act as potent antibacterial agent (Patrick, 2001; Thomas G, 2007).

Apart from the commercial application as antibacterial/antibiotic agents, various sulfonamides are also known to inhibit several enzymes like CA, CP and cyclooxygenase (Supuran *et al*., 2003). Moreover, they have potential therapeutic applications in cancer chemotherapy, diuretics, hypoglyceamia (Supuran *et al*., 2004) and inhibit HIV reverse transcriptase (Zhao *et al*., 2008) and cell entry (Lu *et al*., 2009). The basic sulfonamide group-SO_2NH- is present in various biologically active compounds including antimicrobial drugs, antithyroid and antitumor agents (Remko and Lieth, 2004). Due to the structural similarity to *p*-aminobenzoiac acid (PABA) that is needed by bacteria for the synthesis of FA and its growth, they inhibit the conversion of PABA into folic acid and ultimately synthesis of purine and DNA is blocked (El-Sayed *et al*., 2011; Garcia-Galan *et al*., 2008).

Compounds containing 1,4-benzodioxane ring systems demonstrated important biological activities like antihepatotoxic (Ahmed *et al*., 2003; Khan *et al*., 2006; Ahmed *et al*., 2011), α-adrenergic blocking agent (Chapleo *et al*., 1983), anti-inflammatory and D_2 antagonist/5-HT1A partial agonistic activity (Vazquez *et al*., 1997). Amides (generally) synthesized from piperidides of benzodioxane-6-carboxylic and piperonylic acids are identified to potentiate the activity of memory development, treating Alzheimer's disease and other neurodegenerative diseases (Arai *et al*., 1994). 1,4-Benzodioxane ring system is important constituent of Silymarin (commonly known as milk thistle) isolated from seeds of *Silybum marianum*; it is a potent

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antihepatotoxic agent against a variety of toxicants (Ahmed *et al*., 2003; Khan *et al*., 2006).

Acetyl cholinesterase (AChE) and Butyrylcholinesterase (BChE) are listed among the family of enzymes, which are included in serine hydrolases. They exhibited varied specificities for their substrates and inhibitors owing to their dissimilarities in AA residues at their active sites (Cygler *et al*., 1993; Tougu, 2001). These enzymes catalyze the hydrolysis of the neurotransmitter AC, thus result in termination of nerve impulse. The inhibitors of these enzymes lift up the quantity of acetylcholine in neuromuscular junctions (Bertaccini, 1982; Gauthie, 2001). In lipoxygenase type-1 (LOX) the iron is present in the divalent state and is oxidized to the catalytically active Fe^{3+} during arachidonic acid metabolism (Clapp *et al*., 1985;Kemal *et al*., 1987). Leukotrienes are biologically significant & active mediators; and help against various inflammations (Steinhilber, 1999; Alitonou *et al*., 2006).

In continuation of preceding research efforts (Abbasi *et al*., 2014; Irshad *et al*., 2014), the current research work is based on the synthesis of ethyl sulfonated derivatives bearing 1,4-benzodioxane nucleus with the target to have influential enzyme inhibition and antibacterial potential, which might lead to the development of new therapeutics. Therefore, studies were undertaken to screen the synthesized compounds against different bacterial strains and to ascertain inhibitory potential against acetyl and butyrylcholinestrase and lipoxygenase enzymes followed by their computational docking studies.

MATERIALS AND METHODS

General

With Griffin and George M.P apparatus; melting points of all the synthesized compounds were traced out by open capillary tube. Purity was checked out by TLC plates (G-25-UV_{254}) prepared with pre-coated Si-gel, by using various solvent systems particularly C_2H_5-acetate and *n*-C_6H_{14}. UV lamp was used to detect the spots of compounds at 254 nm while ceric-SO_4 was used to develop spots on heating. The IR spectra were recorded on a Jasco-320-A spectrophotometer, after forming KBr pellet of each sample, outcomes are specified as wave number in cm^{-1}. Bruker spectrometer was used for NMR spectra marking in CD_3OD at 300 & 400 MHz. TMS was taken as internal reference standard; chemical shifts were calculated in ppm and hertz was used to define coupling constant. On a JMS-HX-110 spectrometer having data system; EIMS of synthesized compounds were documentated. The chemicals used herein, were purchased from Merck, Alfa Aesar and Sigma Aldrich. The solvents utilized in the project were of analytical grade.

Synthesis

Synthesis of N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethanesulfonamide (3)

10.0mmol/1.25mL of 2,3-dihydrobenzo[1,4]dioxin-6-amine (1) was dissolved in 25mL de-ionized H_2O in a 100 mL round bottom flask and adjust pH between 9-10 by adding10 % aqueous Na_2CO_3 solution. Then equimolar amount (10.0mmol; 0.95mL) of ethane sulfonyl chloride (2) was added to the reaction medium gradually in afew mins. For 4-5 hrs the reaction contents were stirred together with supervision by TLC giving single spot. On reaction completion dil. HCl (about 2.0mL) was gradually added and strongly hand shaked to lower the pH to 2-3. The solid product ppts thus formed on keeping the flask contents for some time were filtered, rinsed by de-ionized H_2O and desiccated to get the solid compound 3 in good yield. Recrystallization was done in methanol.

General procedure for the synthesis of N-alkyl/aralkyl *substituted derivatives of N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethanesulfonamide* (5a-r)

8.0mmol; 0.2g of *N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethanesulfonamide (3) was homogeneously dissolved in *N,N*□-dimethylformamide (DMF; 10mL) placed in a round bottom flask (50mL) and activated by LiH (5.0 mmol). For 25-30 min the contents were stirred and then the calculated equimolar amount of electrophiles 4a-r (alkyl and aralkyl halides) were added to reaction mixture and further stirred for 4-5 hours. The reaction progress was checked *via* TLC till single spot. Ice cold distilled along with aqueous Na_2CO_3 solution (5mL) was added and flask was manually shaken slowly. The target products 5a-r were obtained by filtration or extracted with chloroform (depending upon their states or nature).

Biological studies

Cholinesterase assays

The AChE & BChE inhibition activities were carried out according to the method of Ellman *et al*., (1961) with slight modifications.

Lipoxygenase assay

Lipoxygenase (LOX) activity was evaluated by the earlier stated method (Tappel, 1953; Evans, 1987; Baylac and Racine, 2003) with some changes.

α-Glucosidase assay

The activity of synthesized compounds against *α*-glucosidase was executed by the method of Chapdelaine *et al*., (1978).

Antibacterial assay

The antimicrobial activity was determined following the principle that increased absorbance of broth medium is directly related to log phase of growth and was performed in sterile 96-wells micro plates under aseptic conditions (Kaspady *et al*., 2009; Yang *et al*., 2006).

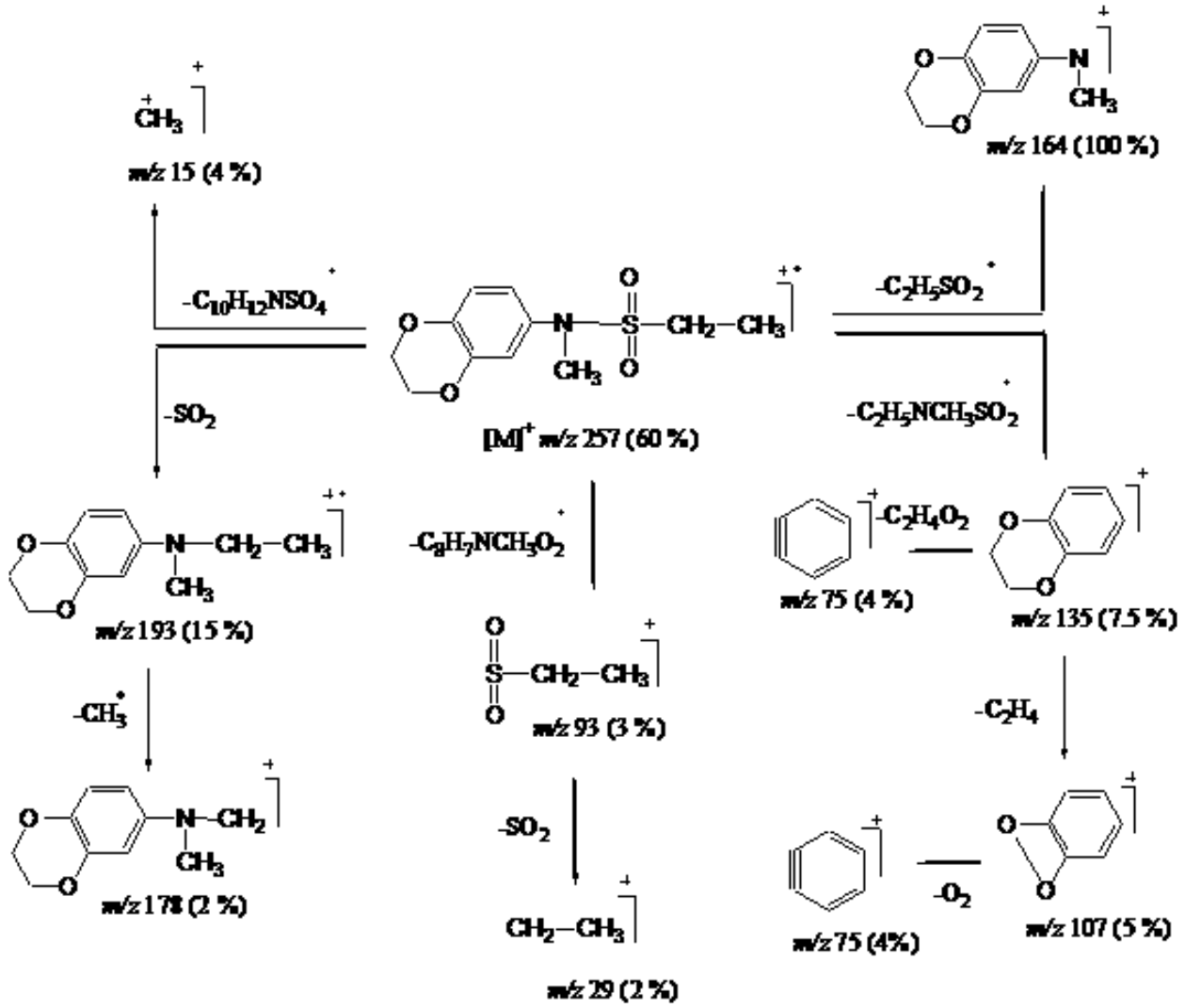


Fig. 1: Mass fragmentation pattern of *N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)-*N*-methylethanesulfonamide (5a).

STATISTICAL ANALYSIS

All the experiments were performed in triplicates and Microsoft Excel 2010 was used for statistical analysis. The values are reported as mean ± sem.

Computational methodology

The protein molecule included in our study (acetyl, butyrylcholinestrace and lipoxygenase) were retrieved from Protein Data Bank. Water molecules were removed and the 3D protonation of the protein molecule was carried out. The energy minimization algorithm of MOE tool was used to lower down the energy of protein molecules; for this purpose the energy minimization parameters were used including gradient, Force Field, Solvation and Chiral Constraint etc. When the RMS gradient drops lower than 0.05, then energy minimization was stopped. Thus, for docking studies the structure with minimization energy was used as the template.

Molecular docking

MOE docking program was used to analyze the binding of the lig and molecule with the protein molecule after the correct conformation was found to obtain the structure with minimum energy. The default parmeters of MOE-Dock program were used for the molecular docking of the ligands. At the end of docking, the competitive conformations were analyzed for hydrogen bonding/π-π interactions (Wadood *et al*., 2013).

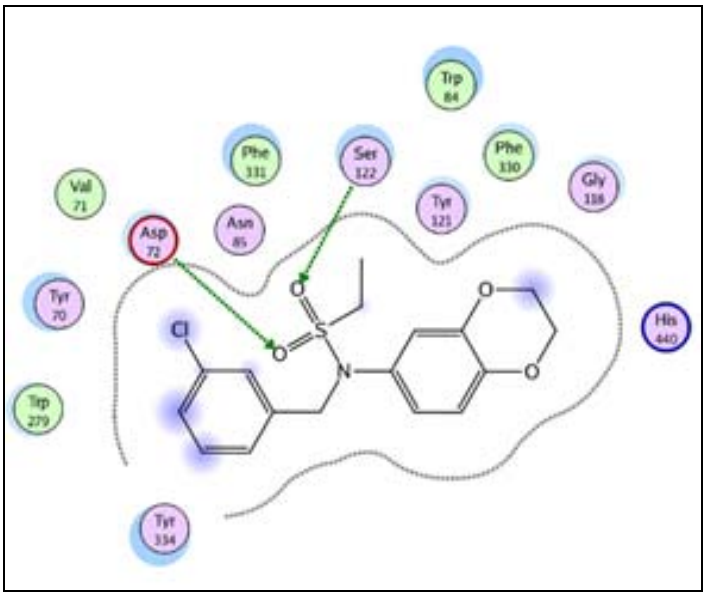


Fig. 2: The 2D interaction analysis of docked compound 5m against AChE.

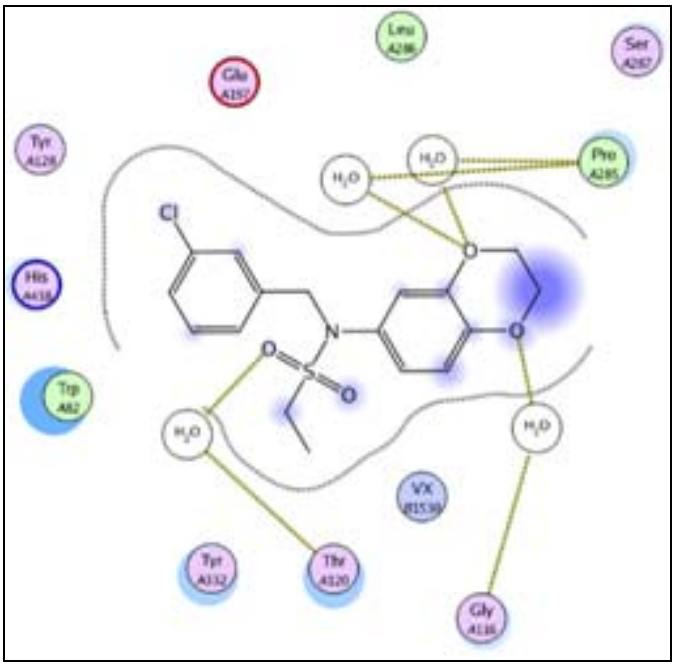


Fig. 3: The 2D interaction analysis of compound 5m against BChE.

Spectral characterization of the synthesized compounds

N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethanesulfonamide (3)

Dark gray amorphous solid; yield: 93%; M.P. 89°C; molecular formula: $C_{10}H_{13}NO_4S$; molecular weight: 243 $gmol^{-1}$; IR (KBr, cm^{-1})ν_{max}: 3467, 3388 (N-H stretching), 3072 (C-H stretching of aromatic ring), 2928 (-CH_2-stretching), 1627 (C=C stretching of aromatic ring), 1374 (-SO_2 stretching), 1158 (C-O-C stretching of ether); ^{1}H-NMR (CD_3)$_2$SO, 400 MHz): δ (ppm) 9.42(s, 1H, N-H),6.80 (d, *J*=8.4 Hz, 1H, H-8),6.72 (d, *J*=2.4 Hz, 1H, H-5), 6.67 (dd, *J*=2.4, 8.8 Hz, 1H, H-7), 4.18-4.21 (m, 4H, CH_2-2 &CH_2-3), 2.98 (q, *J*=7.2 Hz, 2H, CH_2-1'), 1.16 (t, *J* =7.2 Hz, 3H, CH_3-2□); EI-MS: *m/z* 243 $[M]^+$, 179 $[M-SO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 93 $[C_2H_5SO_2]^+$, 75 $[C_6H_3]^+$, 29 $[C_2H_5]^+$.

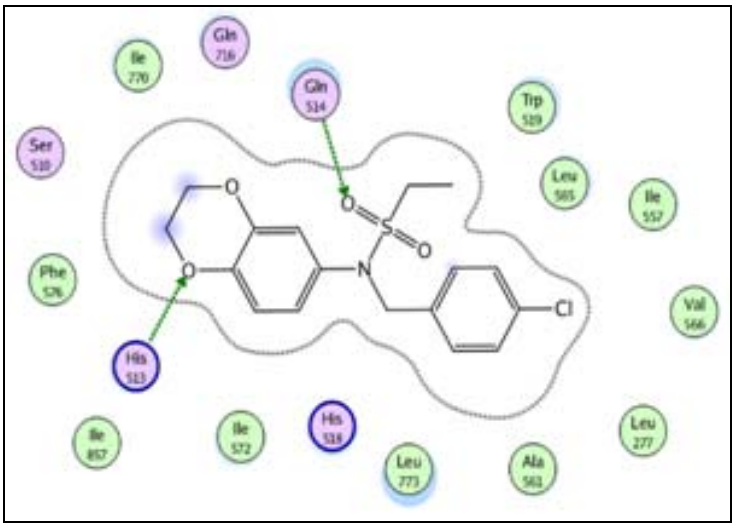


Fig. 4: The 2D interaction analysis of compound **5n**against LOX.

N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)-N-methylethanesulfonamide(5a)

Yellowish brown liquid; yield: 75%; molecular formula: $C_{11}H_{15}NO_4S$; molecular weight: 257 $gmol^{-1}$; IR (KBr, cm^{-1}) ν_{max}: 3068 (C-H stretching of aromatic ring), 2927 (-CH_2- stretching), 1628 (C=C stretching of aromatic ring), 1392 (-SO_2 stretching), 1143 (C-O-C stretching of ether); ^{1}H-NMR $(CD_3)_2SO$, 400 MHz): δ (ppm) 6.76 (d, *J*=2.0 Hz, 1H, H-5), 6.72 (dd, *J*=2.0, 8.4 Hz, 1H, H-7), 6.69 (d, *J*=8.8 Hz, 1H, H-8), 4.16-4.19 (m, 4H, CH_2-2 &CH_2-3), 3.10 (q, *J*=7.6 Hz, 2H, CH_2-1'), 2.74 (s, 3H, CH_3-1"), 1.19 (t, *J*=7.2 Hz, 3H, CH_3-2'); EIMS: *m/z* 257 $[M]^+$, 193 $[M-SO_2]^+$, 178 $[C_6H_3C_2H_4O_2NCH_2CH_3]^+$, 164 $[C_6H_3C_2H_4O_2NCH_3]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 75 $[C_6H_3]^+$, 15 $[CH_3]^+$.

N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)-N-ethylethanesulfonamide(5b)

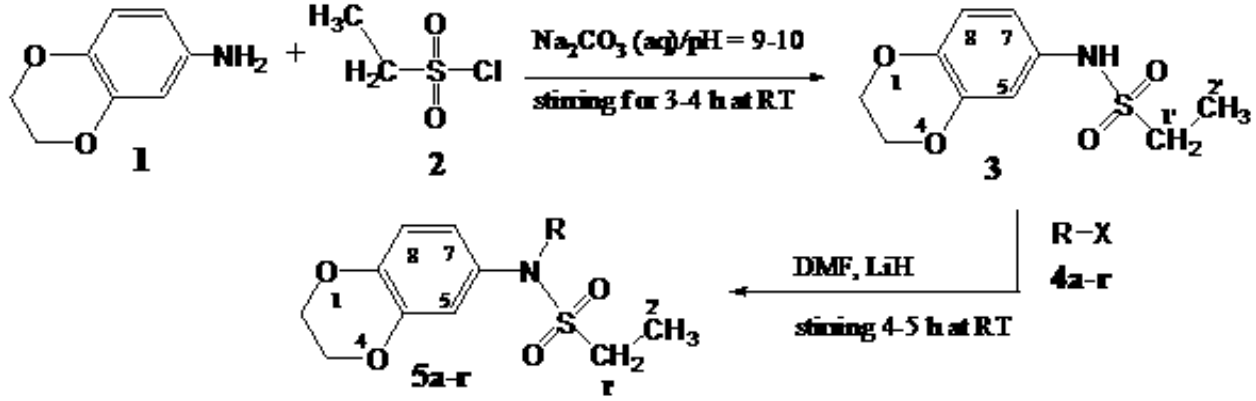
Light brown solid; yield: 79%; M.P. 111°C; molecular formula: $C_{12}H_{17}NO_4S$; molecular weight: 271 $gmol^{-1}$; IR (KBr, cm^{-1}) ν_{max}: 3028 (C-H stretching of aromatic ring), 2897 (-CH_2 stretching), 1618 (C=C stretching of aromatic ring), 1374 (-SO_2 stretching), 1174 (C-O-C stretching of ether); ^{1}H-NMR $(CD_3)_2SO$, 400 MHz): δ (ppm) 6.78 (d, *J* =2.4 Hz, 1H, H-5), 6.74 (dd, *J*=2.4, 8.4 Hz, 1H, H-7), 6.68 (d, *J*=8.0 Hz, 1H, H-8), 4.21 (s, 4H, CH_2-2 &CH_2-3), 3.14 (q, *J*=7.2 Hz, 2H, CH_2-1'), 3.10 (q, *J*=7.2 Hz, 2H, CH_2-1"), 1.21 (t, *J*=7.6 Hz, 3H, CH_3-2'), 1.15 (t, *J*=7.6 Hz, 3H, CH_3-2"); EIMS: *m/z* 271 $[M]^+$, 207 $[M-SO_2]^+$, 192 $[C_6H_3C_2H_4O_2NCH_2C_2H_5]^+$, 178 $[C_6H_3C_2H_4O_2NC_2H_5]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 75 $[C_6H_3]^+$, 29 $[C_2H_5]^+$.

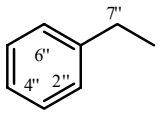
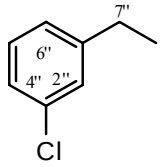
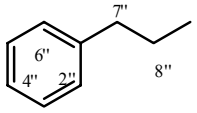
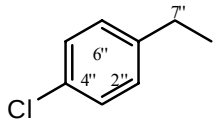
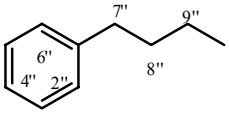
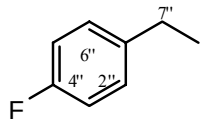
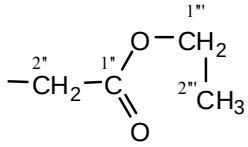
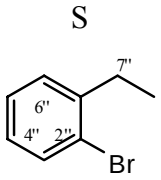
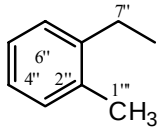
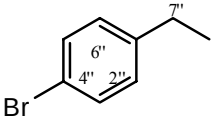
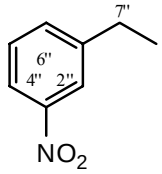
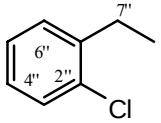
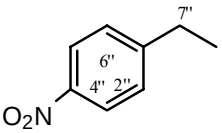
N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)-N-propylethanesulfonamide (5c)

Brownish black liquid; yield: 72 %; molecular formula: $C_{13}H_{19}NO_4S$; molecular weight: 285 $gmol^{-1}$; IR (KBr, cm^{-1}) ν_{max}: 3067 (C-H stretching of aromatic ring), 2912 (-CH_2 stretching), 1613 (C=C stretching of aromatic ring), 1347 (-SO_2 stretching), 1168 (C-O-C stretching of ether); ^{1}H-NMR $(CD_3)_2SO$, 400 MHz): δ (ppm) 6.83 (d, *J*=2.4 Hz, 1H, H-5), 6.79 (dd, *J*=2.4, 8.0 Hz, 1H, H-7), 6.73 (d, *J* =8.0 Hz, 1H, H-8), 4.19 (s, 4H, CH_2-2 &CH_2-3), 3.28 (t, *J*=7.2 Hz, 2H, CH_2-1"), 3.12 (q, *J*=7.2 Hz, 2H, CH_2-1'), 1.91 (sext, *J* = 7.2 Hz, 2H, CH_2-2"), 1.25 (t, *J*=7.6 Hz, 3H, CH_3-2'), 1.06 (t, *J*=7.2 Hz, 3H, CH_3-3"); EIMS: *m/z* 285 $[M]^+$, 221 $[M-SO_2]^+$, 206 $[C_6H_3C_2H_4O_2NCH_2C_3H_7]^+$, 192 $[C_6H_3C_2H_4O_2NC_3H_7]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 75 $[C_6H_3]^+$, 43 $[C_3H_7]^+$, 29 $[C_2H_5]^+$.

Ethyl 2-(N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethylsulfonamido)acetate(5d)

Brown sticky solid; yield: 71%; molecular formula: $C_{14}H_{19}NO_6S$; molecular weight: 329 $gmol^{-1}$; IR (KBr, cm^{-1}) ν_{max}: 3058 (C-H stretching of aromatic ring), 2892 (-CH_2 stretching), 1663 (C=C stretching of aromatic ring), 1347 (-SO_2 stretching), 1153 (C-O-C stretching of ether); ^{1}H-NMR $(CD_3)_2SO$, 400 MHz): δ (ppm) 6.85 (d, *J*=2.0 Hz, 1H, H-5), 6.80 (dd, *J*=2.0, 8.4 Hz, 1H, H-7), 6.75 (d, *J*=8.4 Hz, 1H, H-8), 4.26 (q, *J*=7.2 Hz, 2H, CH_2-1'"), 4.18 (s, 4H, CH_2-2 &CH_2-3), 4.06 (s, 2H, CH_2-2"), 3.14 (q, *J* =7.6 Hz, 2H, CH_2-1'), 1.29 (t, *J*=7.2 Hz, 3H, CH_3-2□"), 1.28 (t, *J*=7.2 Hz, 3H, CH_3-2'); EIMS: *m/z* 329 $[M]^+$, 265 $[M-SO_2]^+$, 250 $[C_6H_3C_2H_4O_2NCH_2C_4H_7CO_2]^+$, 236 $[C_6H_3C_2H_4O_2NC_4H_7CO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 87 $[C_4H_7CO_2]^+$, 75 $[C_6H_3]^+$, 45 $[OC_2H_5]^+$.



Comp.	R	Comp.	R	Comp.	R
5a	—CH3	5g		5m	
5b	—CH2—CH3	5h		5n	
5c	—CH2—CH2—CH3	5i		5o	
5d		5j		5p	
5e	—CH2—CH2—Br	5k		5q	
5f	—CH2—CH2—Cl	5l		5r	

Scheme 1: Synthesis of *N*-substituted derivatives of *N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethanesulfonamide (5a-r).

Table 1: Enzyme inhibition studies of *N*-substituted derivatives of *N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethane sulfonamide (5a-r).

Compound	AChE		BChE		LOX		*a*-Glucosidase	
	Inhibition (%)	IC_{50} (µM)	Inhibition (%)	IC_{50} (µM)	Inhibition (%)	IC_{50} (µM)	Inhibition (%)	IC_{50} (µM)
3	8.85±0.16	-	12.78±0.19	-	92.5±0.25	294.51±0.44	1.35±0.16	-
5a	91.15±0.16	219.52±0.54	68.86±0.17	400.11±0.89	98.24±1.25	178.98±0.23	94.35±0.26	210.12±0.23
5b	12.86±0.12	-	23.71±0.27	-	34.21±0.15	-	32.16±0.18	-
5c	89.64±0.17	250.45±0.34	18.18±0.12	-	86.91±0.42	351.11±0.03	69.79±0.03	312.41±0.02
5d	82.91±0.42	345.17±0.71	14.27±0.12	-	74.15±0.86	400.12±0.74	64.46±0.02	369.11±0.11
5e	84.92±1.25	216.34±0.24	76.91±0.19	278.16±0.54	91.41±0.21	298.67±0.38	90.14±1.26	175.93±0.97
5f	96.14±0.12	256.98±0.22	71.11±0.26	367.47±0.77	84.67±0.49	314.54±0.14	91.56±1.17	167.04±0.12
5g	11.80±0.11	-	24.72±0.22	-	38.21±0.17	-	31.18±0.11	-
5h	9.51±0.08	-	41.48±0.59	<500	26.81±0.16	-	9.65±0.08	-
5i	15.30±0.13	-	36.25±0.29	<500	37.91±0.11	-	82.65±0.13	242.91±0.11
5j	24.26±0.21	-	50.06±0.46	<500	93.11±0.11	257.9±0.31	75.53±0.21	231.42±0.19
5k	27.46±0.26	-	32.61±0.39	<500	73.45±0.82	363.5±0.61	94.35±0.26	220.29±0.22
5l	5.74±0.08	-	19.26±0.11	-	96.77±0.12	289.51±0.44	56.06±0.08	<500
5m	98.92±1.25	189.66±0.98	78.08±0.98	218.79±0.87	37.21±0.38	-	89.24±1.25	174.45±0.98
5n	10.57±0.13	-	10.97±0.06	-	96.15±0.16	229.2±0.24	38.81±0.13	-
5o	21.89±0.19	-	26.08±0.26	-	51.29±0.68	500	68.76±0.17	402.21±0.13
5p	12.84±0.11	-	28.41±0.21	-	28.71±0.21	-	25.06±0.19	-
5q	78.41±0.14	367.98±0.75	66.26±0.15	412.10±0.91		298.88±0.52	96.12±0.14	217.89±0.21
5r	85.87±1.23	215.14±0.27	72.43±0.25	312.11±0.63	83.32±0.41	310.76±0.56	95.35±0.28	211.19±0.34
Control	91.29±1.17[a]	0.04±0.0001[a]	82.82±1.09[a]	0.85±0.0001[a]	93.79±1.27[b]	22.4±1.3[b]	92.23±0.14[c]	38.25±0.12[c]

AChE = Acetyl cholinesterase BchE = Butyrylcholinesterase LOX = Lipoxygenase a = Eserine b = Baicalein c = Acarbose

N-(2-bromoethyl)-N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethanesulfonamide (5e)

Grayish brown solid; yield: 75%; M.P. 125°C; molecular formula: $C_{12}H_{16}BrNO_4S$; molecular weight: 349 $gmol^{-1}$; IR (KBr, cm^{-1}) ν_{max}: 3022 (C-H stretching of aromatic ring), 2907 (-CH_2 stretching), 1624 (C=C stretching of aromatic ring), 1352 (-SO_2 stretching), 1123 (C-O-C stretching of ether), 640 (C-Br stretching); ^{1}H-NMR $(CD_3)_2SO$, 400 MHz): δ (ppm) 6.89 (d, *J*=2.4 Hz, 1H, H-5), 6.81 (dd, *J*=2.0, 8.0 Hz, 1H, H-7), 6.76 (d, *J*=8.0 Hz, 1H, H-8), 4.20 (s, 4H, CH_2-2 &CH_2-3), 3.68 (t, *J*=7.2 Hz, 2H, CH_2-1''), 3.74 (t, *J*=7.2 Hz, 2H, CH_2-2''), 3.13 (q, *J*= 7.2 Hz, 2H, CH_2-1'), 1.24 (t, *J*=7.2 Hz, 3H, CH_3-2'); EIMS: *m/z* 351 $[M+2]^+$, 349 $[M]^+$, 285 $[M\text{-}SO_2]^+$, 270 $[C_6H_3C_2H_4O_2NCH_2C_2H_4Br]^+$, 256 $[C_6H_3C_2H_4O_2NC_2H_4Br]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_2H_4Br]^+$, 75 $[C_6H_3]^+$.

N-(2-chloroethyl)-N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethanesulfonamide (5f)

Yellowish gray sticky solid; yield: 69%; molecular formula: $C_{12}H_{16}ClNO_4S$; molecular weight: 305.5 $gmol^{-1}$; IR (KBr, cm^{-1}) ν_{max}: 3157 (C-H stretching of aromatic ring), 2912 (-CH_2 stretching), 1613 (C=C stretching of aromatic ring), 1347 (-SO_2 stretching), 1168 (C-O-C stretching of ether), 594 (C-Cl stretching); ^{1}H-NMR $(CD_3)_2SO$, 400 MHz): δ (ppm) 6.78 (d, *J*=2.4 Hz, 1H, H-5), 6.72 (dd, *J*=2.4, 8.0 Hz, 1H, H-7), 6.69 (d, *J* = 8.0 Hz, 1H, H-8), 4.17 (s, 4H, CH_2-2 &CH_2-3), 3.93 (t, *J*=7.2 Hz, 2H, CH_2-2''), 3.61 (t, *J*=7.2 Hz, 2H, CH_2-1''), 3.14 (q, *J*= 7.2 Hz, 2H, CH_2-1'), 1.27 (t, *J*=7.6 Hz, 3H, CH_3-2'); EIMS: *m/z* 307 $[M+2]^+$, 305 $[M]^+$, 241 $[M\text{-}SO_2]^+$, 226 $[C_6H_3C_2H_4O_2NCH_2C_2H_4Cl]^+$, 212 $[C_6H_3C_2H_4O_2NC_2H_4Cl]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 75 $[C_6H_3]^+$, 63 $[C_2H_4Cl]^+$.

Table 2: Antibacterial activity of *N*-substituted derivatives of *N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethanesulfonamide (5a-r).

Compound	*S. typhi (-)*		*E. coli (-)*		*P. aeruginosa (-)*		*B. subtilis (+)*		*S. aureus (+)*	
	% age inhibition	MIC	% age inhibition	MIC	% age inhibition	MIC	% age inhibition	MIC	% age inhibition	MIC
3	58.01±1.96	14.93±4.47	51.29±4.29	19.65±4.40	44.31±4.19	-	63.82±2.53	16.65±2.54	80.38±2.15	11.12±3.79
5a	60.22±1.84	14.47±3.68	38.67±1.08	-	36.56±4.76	-	60.97±0.65	16.74±3.25	78.54±2.22	11.79±1.38
5b	62.52±1.92	14.11±3.24	58.25±4.05	15.67±4.53	54.88±3.00	18.33±2.23	56.12±3.18	16.99±3.68	73.04±1.99	12.14±2.61
5c	57.79±0.44	13.90±4.12	45.00±3.00	-	50.50±3.62	19.85±1.14	60.00±0.11	17.05±4.63	74.67±2.99	12.18±2.38
5d	60.22±1.84	14.36±2.43	63.99±3.38	13.51±4.12		-	50.01±1.70	19.91±1.88	64.32±3.47	13.78±1.13
5e	61.37±1.24	12.45±1.48	60.26±4.18	14.56±2.45		-	69.16±3.93	15.67±2.74	67.88±2.19	14.99±4.01
5f	61.12±1.32	12.22±2.05	60.08±3.43	14.44±2.63	64.22±2.93	13.56±3.34	72.42±1.21	12.78±2.28	72.14±4.04	12.11±4.08
5g	52.50±3.09	17.21±1.05	52.21±3.96	19.41±3.13	28.87±3.88	-	59.89±4.62	16.81±3.44	62.28±1.14	12.53±4.31
5h	51.25±1.10	18.64±1.50	40.75±4.17	-	39.31±3.19	-	52.04±3.70	19.51±4.06	62.28±1.14	14.53±4.06
5i	41.32±1.32	-	24.38±4.46	-	4.13± 4.25	-	30.70±3.75	-	36.27±4.13	-
5j	34.34±4.04	-	53.67±4.43	20.32±4.60	29.88±4.00	-	51.40±3.33	19.67±4.25	58.16±1.57	15.21±1.44
5k	38.90±2.13	-	34.75±2.08	-	35.58±2.98	-	54.46±1.99	17.87±4.56	66.65±1.46	13.83±4.56
5l	40.70±2.76	-	6.79± 5.00	-	48.25±4.50	-	39.95±1.27	14.26±3.31	61.08±4.89	-
5m	39.93±4.69	-	36.38±4.63	-	16.38±2.54	-	44.63±5.00	13.07±2.13	62.03±0.25	-
5n	58.75±1.69	14.36±2.43	63.92±3.50	13.50±4.03	46.00±1.00	-	50.27±0.16	19.91±1.88	70.13±4.06	13.78±1.13
5o	52.50±1.59	16.88±2.54	58.25±4.05	15.22±4.43	30.13±3.88	-	55.32±1.34	18.50±3.19	69.87±2.41	13.61±5.00
5p	52.13±3.46	17.64±1.61	49.96±4.54	-	27.44±5.00	-	54.09±3.60	18.89±4.56	68.35±4.62	14.86±3.00
5q	54.30±1.51	15.77±2.51	61.51±3.16	14.21±2.11	-	-	59.45±2.39	17.56±4.48	63.18±1.74	12.45±2.53
5r	55.78±1.76	15.12±2.18	62.13±4.12	13.67±2.78	50.11±3.69	19.78±1.68	57.92±2.71	16.34±3.11	69.04±1.99	12.71±1.48
Ciprofloxacin	91.65±1.04	9.22±1.36	90.98±1.43	8.79±2.00	90.46±1.99	8.93±2.42	89.90±0.79	9.43±1.87	92.04±1.44	9.04±1.50

Note: Minimum inhibitory concentration (MIC) was measured with suitable dilutions (5-30mM/ well) and results were calculated using EZ-FitPerrella Scientific Inc. Amherst USA software.

N-benzyl-N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethanesulfonamide (5g)

Light brown solid; yield: 78%; M.P. 133°C; molecular formula: $C_{17}H_{19}NO_4S$; molecular weight: 333 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3071 (C-H stretching of aromatic ring), 2918 (-CH_2 stretching), 1619 (C=C stretching of aromatic ring), 1344 (-SO_2 stretching), 1161 (C-O-C stretching of ether); ^{1}H-NMR $(CD_3)_2SO$, 400 MHz): δ (ppm) 7.20-7.27 (m, 5H, H-2" to H-6"), 6.85 (d, *J*=2.0 Hz, 1H, H-5), 6.81 (dd, *J*=2.4, 8.4 Hz, 1H, H-7), 6.76 (d, *J*=8.8 Hz, 1H, H-8), 4.80 (s, 2H, CH_2-7"), 4.18 (s, 4H, CH_2-2 &CH_2-3), 3.17 (q, *J*=7.2 Hz, 2H, CH_2-1'), 1.26 (t, *J*=7.2 Hz, 3H, CH_3-2□); EIMS: *m/z* 333 $[M]^+$, 269 $[M\text{-}SO_2]^+$, 254 $[C_6H_3C_2H_4O_2N(CH_2)_2C_6H_5]^+$, 240 $[C_6H_3C_2H_4O_2NCH_2C_6H_5]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 75 $[C_6H_3]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)-N-phenethylethanesulfonamide (5h)

Chocolate brown semisolid; yield: 74%; molecular formula: $C_{18}H_{21}NO_4S$; molecular weight: 347 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3055 (C-H stretching of aromatic ring), 2943 (-CH_2 stretching), 1622 (C=C stretching of aromatic ring), 1322 (-SO_2 stretching), 1134 (C-O-C stretching of ether); ^{1}H-NMR $(CD_3)_2SO$, 400 MHz): δ (ppm) 7.18-7.35 (m, 5H, H-2" to H-6"), 6.80 (d, *J*=8.4 Hz, 1H, H-8), 6.72 (d, *J*=2.4 Hz, 1H, H-5), 6.67 (dd, *J*=2.4, 8.4 Hz, 1H, H-7), 4.17-4.24 (m, 4H, CH_2-2 &CH_2-3), 3.58 (t, *J*=7.6 Hz, 2H, CH_2-8"), 3.07 (t, *J* = 7.2 Hz, 2H, CH_2-7"), 2.98 (q, *J* = 7.6 Hz, 2H, CH_2-1'), 1.16 (t, *J*=7.2 Hz, 3H, CH_3-2"); EIMS: *m/z* 347 $[M]^+$, 283 $[M\text{-}SO_2]^+$, 268 $[C_6H_3C_2H_4O_2N(CH_2)_3C_6H_5]^+$, 254 $[C_6H_3C_2H_4O_2N(CH_2)_2C_6H_5]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 105 $[(CH_2)_2C_6H_5]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 75 $[C_6H_3]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)-N-(3-phenylpropyl)ethanesulfonamide(5i)

Grayish brown solid; yield: 84%; M.P. 82°C; molecular formula: $C_{19}H_{23}NO_4S$; molecular weight: 361 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3056 (C-H stretching of aromatic ring), 2919 (-CH_2- stretching), 1621 (C=C stretching of aromatic ring), 1356 (-SO_2 stretching), 1169 (C-O-C stretching of ether); ^{1}H-NMR $(CD_3)_2SO$, 400 MHz): δ (ppm) 7.69 (d, *J*=2.7 Hz, 2H,H-2" &H-6"), 6.83-6.89 (m, 3H, H-3" to H-5"), 6.80 (d, *J*=8.8 Hz, 1H, H-8), 6.72 (d, *J* = 2.4 Hz, 1H, H-5), 6.67 (dd, *J*=2.4, 8.4 Hz, 1H, H-7), 4.19-4.20 (m, 4H, CH_2-2 &CH_2-3), 3.51 (t, *J*=7.2 Hz, 2H, CH_2-9"), 3.05 (q, *J*=7.6 Hz, 2H, CH_2-1'), 1.28 (brt, *J*=7.2 Hz, 2H, CH_2-7"), 1.18 (quint, *J*=7.2 Hz, 2H, CH_2-8"), 0.08 (t, *J*=7.2 Hz, 3H, CH_3-2'); EIMS: *m/z* 361 $[M]^+$, 297 $[M\text{-}SO_2]^+$, 282 $[C_6H_3C_2H_4O_2N(CH_2)_3C_6H_5]^+$, 268 $[C_6H_3C_2H_4O_2N(CH_2)_4C_6H_5]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 119 $[(CH_2)_3C_6H_5]^+$, 107 $[C_6H_3O_2]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 75 $[C_6H_3]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

N-(2-bromobenzyl)-N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethanesulfonamide (5j)

Dark brown semisolid; yield: 73%; molecular formula: $C_{17}H_{18}BrNO_4S$; molecular weight: 411 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3187 (C-H stretching of aromatic ring), 2934 (-CH_2- stretching), 1639 (C=C stretching of aromatic ring), 1351 (-SO_2 stretching), 1169 (C-O-C stretching of ether), 596 (C-Br stretching); ^{1}H-NMR $(CD_3)_2SO$, 300 MHz): δ (ppm) 7.62 (dd, *J*=1.8, 7.5 Hz, 1H, H-3□□), 7.53 (t, *J*=7.2 Hz, 1H, H-4□□), 7.50 (dd, *J*=1.8, 7.8 Hz, 1H, H-6□□), 7.16 (t, *J*=7.2 Hz, 1H, H-5□□), 6.95 (d, *J*=2.4 Hz, 1H, H-5), 6.91 (dd, *J*=2.4, 8.7 Hz, 1H, H-7), 6.79 (d, *J*=8.4 Hz, 1H, H-8), 4.88 (s, 2H, CH_2-7"), 4.18 (s, 4H, CH_2-2 &CH_2-3), 3.21 (q, *J*=7.2 Hz, 2H, CH_2-1'), 1.26 (t, *J*=7.5 Hz, 3H, CH_3-2□); EIMS: *m/z* 413 $[M+2]^+$, 411 $[M]^+$, 347 $[M\text{-}SO_2]^+$, 332 $[C_6H_3C_2H_4O_2N(CH_2)_2C_6H_4Br]^+$, 318 $[C_6H_3C_2H_4O_2NCH_2C_6H_4Br]^+$, 169 $[C_7H_6Br]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 90 $[C_7H_6]^+$, 75 $[C_6H_3]^+$, 64 $[C_5H_4]^+$.

N-(4-bromobenzyl)-N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethanesulfonamide (5k)

Light brown solid; yield: 82%; M.P. 87°C, molecular formula: $C_{17}H_{18}BrNO_4S$; molecular weight: 411 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3197 (C-H stretching of aromatic ring), 2954 (-CH_2- stretching), 1668 (C=C stretching of aromatic ring), 1349 (-SO_2 stretching), 1173 (C-O-C stretching of ether), 610 (C-Br stretching); ^{1}H-NMR $(CD_3)_2SO$, 400 MHz): δ (ppm) 7.48 (d, *J*=8.0 Hz, 2H, H-3" & H-5"), 7.20 (d, *J*=8.0 Hz, 2H, H-2" & H-6"), 6.86 (d, *J*=2.0 Hz, 1H, H-5), 6.79 (dd, *J*=2.0, 8.0 Hz, 1H, H-7), 6.77 (d, *J*=8.8 Hz, 1H, H-8), 4.78 (s, 2H, CH_2-7"), 4.18 (s, 4H, CH_2-2 &CH_2-3), 3.18 (q, *J*=7.2 Hz, 2H, CH_2-1'), 1.26 (t, *J*=7.6 Hz, 3H, CH_3-2'); EIMS: *m/z* 413 $[M+2]^+$, 411 $[M]^+$, 347 $[M\text{-}SO_2]^+$, 332 $[C_6H_3C_2H_4O_2N(CH_2)_2C_6H_4Br]^+$, 318 $[C_6H_3C_2H_4O_2NCH_2C_6H_4Br]^+$, 169 $[C_7H_6Br]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 90 $[C_7H_6]^+$, 75 $[C_6H_3]^+$, 64 $[C_5H_4]^+$.

N-(2-chlorobenzyl)-N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethanesulfonamide (5l)

Light brown solid; yield: 79%; M.P. 93°C; molecular formula: $C_{17}H_{18}ClNO_4S$; molecular weight: 367.5 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3154 (C-H stretching of aromatic ring), 2924 (-CH_2 stretching), 1672 (C=C stretching of aromatic ring), 1351 (-SO_2 stretching), 1156 (C-O-C stretching of ether), 597 (C-Cl stretching); ^{1}H-NMR $(CD_3)_2SO$, 400 MHz): δ (ppm) 7.60 (dd, *J*=2.4, 7.2 Hz, 1H, H-3"), 7.41 (dd, *J*=2.0, 7.2 Hz, 1H, H-6"), 7.18-7.23 (m, 2H, H-4" &H-5"), 6.93 (d, *J*=2.0 Hz, 1H, H-5), 6.89 (dd, *J*=2.0, 8.4 Hz, 1H, H-7), 6.75 (d, *J*=8.4 Hz, 1H, H-8), 4.83 (s, 2H, CH_2-7"), 4.18 (s, 4H, CH_2-2 &CH_2-3), 3.18 (q, *J*=7.6 Hz, 2H, CH_2-1'), 1.27 (t, *J*=7.2 Hz, 3H, CH_3-2'); EIMS: *m/z* 369 $[M+2]^+$, 367 $[M]^+$, 303 $[M\text{-}SO_2]^+$, 288 $[C_6H_3C_2H_4O_2N(CH_2)_2C_6H_4Cl]^+$, 274 $[C_6H_3C_2H_4O_2NCH_2C_6H_4Cl]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 125 $[C_7H_6Cl]^+$, 107 $[C_6H_3O_2]^+$, 90 $[C_7H_6]^+$, 75 $[C_6H_3]^+$, 64 $[C_5H_4]^+$.

N-(3-chlorobenzyl)-N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethanesulfonamide (5m)

Grayish brown solid; yield: 83%; M.P. 91°C; molecular formula: $C_{17}H_{18}ClNO_4S$; molecular weight: 367.5 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3173 (C-H stretching of aromatic ring), 2923 (-CH_2- stretching), 1672 (C=C stretching of aromatic ring), 1352 (-SO_2 stretching), 1159 (C-O-C stretching of ether), 584 (C-Cl stretching); ^{1}H-NMR (CD_3)$_2$SO, 400 MHz): δ (ppm) 7.32 (brs, 1H, H-2"), 7.20-7.30 (m, 2H, H-4" to H-6"), 6.87 (d, *J*=2.4 Hz, 1H, H-5), 6.80 (dd, *J*=2.0, 8.4 Hz, 1H, H-7), 6.65 (d, *J*=8.8 Hz, 1H, H-8), 4.81 (s, 2H, CH_2-7"), 4.19 (s, 4H, CH_2-2 &CH_2-3), 3.19 (q, *J*=7.2 Hz, 2H, CH_2-1'), 1.26 (t, *J*=7.6 Hz, 3H, CH_3-2'); EIMS: *m/z* 369 $[M+2]^+$, 367 $[M]^+$, 303 $[M-SO_2]^+$, 288 $[C_6H_3C_2H_4O_2N(CH_2)_2C_6H_4Cl]^+$, 274 $[C_6H_3C_2H_4O_2NCH_2C_6H_4Cl]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 125 $[C_7H_6Cl]^+$, 107 $[C_6H_3O_2]^+$, 90 $[C_7H_6]^+$, 75 $[C_6H_3]^+$, 64 $[C_5H_4]^+$.

N-(4-chlorobenzyl)-N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethanesulfonamide (5n)

Grayish brown solid; yield: 80%; M.P. 114°C; molecular formula: $C_{17}H_{18}ClNO_4S$; molecular weight: 367.5 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3164 (C-H stretching of aromatic ring), 2918 (-CH_2- stretching), 1669 (C=C stretching of aromatic ring), 1348 (-SO_2 stretching), 1161 (C-O-C stretching of ether), 592 (C-Cl stretching); ^{1}H-NMR (CD_3)$_2$SO, 300 MHz): δ (ppm) 7.35 (d, *J*=8.0 Hz, 2H, H-3" & H-5"), 7.26 (d, *J*=8.0 Hz, 2H, H-2" & H-6"), 6.86 (d, *J*=1.8 Hz, 1H, H-5), 6.78 (dd, *J*=2.1, 8.1 Hz, 1H, H-7), 6.68 (d, *J*=8.4 Hz, 1H, H-8), 4.79 (s, 2H, CH_2-7"), 4.18 (s, 4H, CH_2-2 &CH_2-3), 3.18 (q, *J*=7.2 Hz, 2H, CH_2-1'), 1.26 (t, *J*=7.6 Hz, 3H, CH_3-2'); EIMS: *m/z* 369 $[M+2]^+$, 367 $[M]^+$, 303 $[M-SO_2]^+$, 288 $[C_6H_3C_2H_4O_2N(CH_2)_2C_6H_4Cl]^+$, 274 $[C_6H_3C_2H_4O_2NCH_2C_6H_4Cl]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 125 $[C_7H_6Cl]^+$, 107 $[C_6H_3O_2]^+$, 90 $[C_7H_6]^+$, 75 $[C_6H_3]^+$, 64 $[C_5H_4]^+$.

N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)-N-(4-fluorobenzyl)ethanesulfonamide (5o)

Whitish brown solid; yield: 84%; M.P. 137°C; molecular formula: $C_{17}H_{18}FNO_4S$; molecular weight: 351 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3152 (C-H stretching of aromatic ring), 2944 (-CH_2- stretching), 1656(C=C stretching of aromatic ring), 1365 (-SO_2 stretching), 1177 (C-O-C stretching of ether); ^{1}H-NMR (CD_3)$_2$SO, 300 MHz): δ (ppm) 7.28 (d, *J*=8.0 Hz, 2H, H-3" & H-5"), 7.23 (d, *J*= 8.0 Hz, 2H, H-2" & H-6"), 6.85 (d, *J*=2.4 Hz, 1H, H-5), 6.72 (dd, *J*=2.1, 8.4 Hz, 1H, H-7), 6.67 (d, *J*=8.7 Hz, 1H, H-8), 4.78 (s, 2H, CH_2-7"), 4.18 (s, 4H, CH_2-2 &CH_2-3), 3.18 (q, *J*=7.2 Hz, 2H, CH_2-1'), 1.26 (t, *J*=7.2 Hz, 3H, CH_3-2'); EIMS: *m/z* 353 $[M+2]^+$, 351 $[M]^+$, 287 $[M-SO_2]^+$, 272 $[C_6H_3C_2H_4O_2N(CH_2)_2C_6H_4F]^+$, 258 $[C_6H_3C_2H_4O_2NCH_2C_6H_4F]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 109 $[C_7H_6F]^+$, 107 $[C_6H_3O_2]^+$, 90 $[C_7H_6]^+$, 75 $[C_6H_3]^+$, 64 $[C_5H_4]^+$.

N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)-N-(2-methylbenzyl)ethanesulfonamide(5p)

Light brown solid; yield: 79%; M.P. 115°C; molecular formula: $C_{18}H_{21}NO_4S$; molecular weight: 347 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3072 (C-H stretching of aromatic ring), 2910 (-CH_2- stretching), 1625 (C=C stretching of aromatic ring), 1348 (-SO_2 stretching), 1172 (C-O-C stretching of ether); ^{1}H-NMR (CD_3)$_2$SO, 300 MHz): δ (ppm) 7.07-7.20 (m, 4H, H-3" to H-6"), 6.88 (d, *J*=2.1 Hz, 1H, H-5), 6.84 (dd, *J*=2.1, 8.1 Hz, 1H, H-7), 6.76 (d, *J* = 8.4 Hz, 1H, H-8), 4.82 (s, 2H, CH_2-7"), 4.17 (s, 4H, CH_2-2 &CH_2-3), 3.16 (q, *J*=7.5 Hz, 2H, CH_2-1'), 2.24 (s, 3H, CH_3-1'"), 1.26 (t, *J*=7.2 Hz, 3H, CH_3-2□); EIMS: *m/z* 347 $[M]^+$, 283 $[M-SO_2]^+$, 268 $[C_6H_3C_2H_4O_2N(CH_2)_2C_6H_4CH_3]^+$, 254 $[C_6H_3C_2H_4O_2NCH_2C_6H_4CH_3]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 105 $[C_7H_6CH_3]^+$, 90 $[C_7H_6]^+$, 75 $[C_6H_3]^+$, 64 $[C_5H_4]^+$.

N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)-N-(3-nitrobenzyl)ethanesulfonamide (5q)

Light gray solid; yield: 75%; M.P. 119°C; molecular formula: $C_{17}H_{18}N_2O_6S$; molecular weight: 378 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3072 (C-H stretching of aromatic ring), 2910 (-CH_2- stretching), 1625 (C=C stretching of aromatic ring), 1348 (-SO_2 stretching), 1172 (C-O-C stretching of ether); ^{1}H-NMR (CD_3)$_2$SO, 400 MHz): δ (ppm) 8.32 (brs, 1H, H-2"), 8.15 (d, *J*=8.0 Hz, H-4'), 7.47-7.53 (m, 2H, H-5" to H-6"), 6.86 (d, *J*=2.0 Hz, 1H, H-5), 6.79 (dd, *J*=2.0, 8.4 Hz, 1H, H-7), 6.65 (d, *J*=8.4 Hz, 1H, H-8), 4.86 (s, 2H, CH_2-7"), 4.17 (s, 4H, CH_2-2 &CH_2-3), 3.17 (q, *J*=7.2 Hz, 2H, CH_2-1'), 1.24 (t, *J*=7.2 Hz, 3H, CH_3-2'); EIMS: *m/z* 378 $[M]^+$, 314 $[M-SO_2]^+$, 299 $[C_6H_3C_2H_4O_2N(CH_2)_2C_6H_4NO_2]^+$, 285 $[C_6H_3C_2H_4O_2NCH_2C_6H_4NO_2]^+$, 136 $[C_7H_6NO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 106 $[C_6H_4CH_2O]^+$, 78 $[C_5H_4CH_2]^+$, 75 $[C_6H_3]^+$, 64 $[C_5H_4]^+$.

N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)-N-(4-nitrobenzyl)ethanesulfonamide(5r)

Yellowish brown solid; yield: 78%; M.P. 111°C; molecular formula: $C_{17}H_{18}N_2O_6S$; molecular weight: 378 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3122 (C-H stretching of aromatic ring), 2937 (-CH_2- stretching), 1663 (C=C stretching of aromatic ring), 1381 (-SO_2 stretching), 1127 (C-O-C stretching of ether); ^{1}H-NMR (CD_3)$_2$SO, 400 MHz): δ (ppm) 8.18 (d, *J*=8.8 Hz, 2H, H-3" & H-5"), 7.65 (d, *J*=8.8 Hz, 2H, H-2" & H-6"), 6.87 (d, *J*=2.0 Hz, 1H, H-5), 6.74 (dd, *J*=2.4, 8.0 Hz, 1H, H-7), 6.62 (d, *J*= 8.0 Hz, 1H, H-8), 4.89 (s, 2H, CH_2-7"), 4.18 (s, 4H, CH_2-2 &CH_2-3), 3.18 (q, *J*=7.2 Hz, 2H, CH_2-1'), 1.26 (t, *J*=7.2 Hz, 3H, CH_3-2'); EIMS: *m/z* 378 $[M]^+$, 314 $[M-SO_2]^+$, 299 $[C_6H_3C_2H_4O_2N(CH_2)_2C_6H_4NO_2]^+$, 285 $[C_6H_3C_2H_4O_2NCH_2C_6H_4NO_2]^+$, 136 $[C_7H_6NO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 106 $[C_6H_4CH_2O]^+$, 78 $[C_5H_4CH_2]^+$, 75 $[C_6H_3]^+$, 64 $[C_5H_4]^+$.

RESULTS

The sulfamoyl and alkyl/aryl functionalities have been successfully introduced through a series of different methods by the protocol sketched in Scheme-1. First the new parent compound *N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethane sulfonamide (3) was synthesized by the reaction of 1,4-benzodioxane-6-amine (1) with ethane sulfonyl chloride (2).Reaction of sulfonamide 3 with various alkyl/aralkyl halides, 4a-r in DMF and lithium hydride (LiH) yielded *N*-alkyl/aralkyl substituted *N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethane sulfonamides (5a-r). The reaction was completed in 4 to 5h by stirring. Depending upon the nature of the synthesized products filtration or solvent extraction methods was utilized. IR, 1H-NMR and mass spectrometry techniques were used to confirm the structure of all the synthesized compounds as illustrated in experimental section.

Enzyme inhibition studies

All the procedures with reaction conditions are mentioned in experimental section. The synthesized molecules were evaluated for the anti-enzymatic (table 1) and antibacterial activities (table 2) and found to be valuable molecules against different bacterial strains as evident from their MIC values (table 2).

DISCUSSION

Chemistry

The compound 5a was obtained as yellowish brown liquid in 75 % yield. Its molecular formula $C_{11}H_{15}NO_4S$ was checked out by molecular ion peak at *m/z* 257 in EI-MS and by integration curve of protons in ^{1}H-NMR spectrum. The structure of a compound was also supported by different absorption bands of IR spectrum mainly at 3068 $cm^{-1}$for C-H (stretching of aromatic ring), 1392 $cm^{-1}$for SO_2 (stretching) and at 1143 cm^{-1} for C-O-C (stretching of ether) affirming the *N*-substituent, ethyl sulfonyl group and benzodioxane ring respectively. EI-MS spectrum showed that molecular ion $M^{+\bullet}$ losses $C_2H_5NSO_2CH_3^{\bullet}$ to form $C_6H_3C_2H_4O_2^+$ which appeared at *m/z* 135; which further losses C_2H_4 molecule to give $C_6H_3O_2^+$ at *m/z* 107, by the loss of $C_2H_5SO_2^{\bullet}$ from $M^{+\bullet}$ an important fragment$C_6H_3C_2H_4O_2NCH_3^+$ appeared at *m/z* 164; other prominent peaks appeared at *m/z* 193 for $C_6H_3C_2H_4O_2NCH_2(CH_3)_2^+$ and at *m/z* 178 for $C_6H_3C_2H_4O_2NCH_2CH_3^+$ cation. For expediency the mass fragmentation pattern of compound 5a have also been sketched in fig. 1. The ^{1}H-NMR spectrum indicated four signals for 1,4-benzodioxane ring attached at sulfamoyl group at δ6.76 (d, *J*=2.0 Hz, 1H, H-5), 6.72 (dd, *J*=2.0, 8.4 Hz, 1H, H-7) and 6.69 (d, *J*=8.8 Hz, 1H, H-8) for aromatic protons and a multiplet for aliphatic protons at δ4.16-4.19 exhibiting the presence of two methylene groups. The five protons of ethyl group were attributed by quadrate at δ3.10 (*J*=7.6 Hz, 2H, CH_2-1') and triplet at δ1.19 (*J*=7.2 Hz, 3H, CH_3-2□); whereas the presence of *N*-substituted methyl group was confirmed by a singlet at δ 2.74 (s, 3H, CH_3-1"). All this accumulative discussion, corroborated the structure of 5aas *N-(2,3-Dihydrobenzo[1,4]dioxin-6-yl)-N-methylethanesul fonamide*. The structures of other synthesized compounds were confirmed on the basis of spectral data described in experimental section.

Enzyme inhibition assay

The precursor sulfonamide 3 and its *N*-substituted derivatives 5a-r were evaluated against acetyl cholinesterase, butyrylcholinesterase, lipoxygenase and α-glucosidase enzymes in order to check their inhibitory potential. In AChE assay compound 5m exhibited a promising activity potential with IC_{50} value of 189.66±0.98μM, whereas compounds 5b, 5g-l and 5n-p did not show any noticeable activity, while the rest displayed moderate activity as evaluated against control Eserine (0.04±0.001). Here in BChE assay compounds 5b-d, 5g, 5l and 5n-p were found to be inactive, whereas rest of them exhibited moderate to weak activity potential, only (again) compound 5m with IC_{50} value of 218.79±0.87μM showed good activity potential; while comparing them against standard Eserine (0.85±0.001) as a control. Against LOX, 5a with IC_{50} value of (178.98±0.17) displayed (somehow) promisingly good inhibitory potential, while rest of them executed moderate potential as compared to standard Baicalein (22.4±1.3). In α-glucosidase assay compound 5e (175.93±0.11), 5f (167.04±0.12) and 5m (174.45±0.98) showed good activity; Where as most of the other exhibited weak activity potential while comparing them against standard Acarbose (38.25±0.001) as control. The reason for abetter activity of the cited compounds like 5a, 5e, 5f and 5m might be the presence of *N*-substituted methyl, bromo ethyl, chloro ethyl &*m*-chloro benzyl groups respectively. It is generally concluded that the aliphatic *N*-substitution results in better inhibition in comparison to the aromatic ones. The results are tabulated in table 1.

Antibacterial activity (in vitro)

The screening of the synthesized series against various Gram-negative and Gram-positive bacteria displayed that most of them executed moderate to good bioactivity. Only a few compounds remained inactive such as 5i &5m against the three bacterial strains i.e. *S. typhi, B. subtilis* and *S. aureus*, while most of the molecules (particularly 3, 5a-h and 5n-r) showed good activity. Against *S. typhi, B. subtilis* and *P$_s$. aeruginosa* compound 5f with MIC value 12.22±2.05μM, 12.78±2.28μM and13.56±3.34 was found to bea potent antibacterial agent amongst all the series entries, relative to ciprofloxacin with MIC 9.22±1.36μM, 9.43±1.87μM and 8.93±2.42μM respectively; whereas compound 5a was found to be the most active antibacterial candidate against *S. aureus* displaying MIC value of 11.79±1.38μM relative to standard MIC value

9.04±1.50µM. The most active molecule against *E. coli*was 5n with MIC value of 13.50±4.03 µM relative to ciprofloxacin with MIC values 8.79±2.00µM. The prominent bioactivity of 5a and 5f molecule is due to the presence of methyl and 2-chloro ethyl groups respectively. The results are presented in table 2.

Computational analysis

Interaction analysis of compound 5m against AChE: In case of compound 5m, two residues from binding site of AChE protein contributed in the interaction. The amino acid residue Asp72 and Ser122 interacts with the oxygen of sulfonamide moiety (fig. 2). The interaction analysis showed that the sulfonamide moiety is important feature in the binding of ligand with protein.

Interaction analysis of compound 5m against BChE: The interaction analysis of compound 5m in case of BChE showed that three residues from binding site of the protein make interaction with two important moieties of the compound. Thr120 interacts with sulfonamide moiety through water molecule. Gly116 and Pro285 were linked to benzodioxine moiety *via* water molecule (fig. 3).

Interaction analysis of compound 5n against LOX: The interactions analysis of compound 5n showed that two active site residues are contributing in the interaction between ligand and the protein. His513 interacts with the oxygen of benzodioxine moiety, whereas Gln514 interacts with the sulfonamide moiety (fig. 4).

CONCLUSIONS

The presented series of compounds was synthesized in good yields in order to initiate some new antibacterial agents. The proposed structures were corroborated by the spectroscopic techniques. The IC_{50} values of these molecules rendered them better inhibitors of lipoxygenase enzyme and moderately less inhibitors of cholinesterase enzymes; compound 5m was found comparatively good inhibitor ofcholinesterase enzyme. All the molecules exhibited moderate to good activity against *B. subtilis* except 5i (remained inactive) while molecule 5f was the most active antibacterial agent among this series. The synthesized molecules might be helpful for the pharmaceutical industries in drug development program.

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REFERENCES

Abbasi MA, Saeed A, Aziz-ur-Rehman, Khan KM, Ashraf M and Ejaz SA (2014). Synthesis of brominated 2-phenetidine derivatives as valuable inhibitors of cholinesterase for the treatment of Alzheimer's disease. *Iran. J. Pharm. Res.,* **13**: 87-94.

Ahmed A, Khan SA and Alam T (2003). Synthesis and antihepatotoxic activity of some heterocyclic compounds containing the 1,4-dioxane ring system. *Pharmazie.,* **58**: 173-176.

Ahmed B, Habibullah and Khan S (2011). Synthesis and antihepatotoxic activity of 2-(substituted-phenyl)-5-(2,3-dihydro-1,4-benzodioxane-2-yl)-1,3,4-oxadiazole derivatives. *J. Enz. Inhib. Med. Chem.,* **26**: 216-221.

Alitonou GA, Avlessi F, Sohounhloue DK, Agnaniet H, Bessiere JM and Menut C (2006). Investigations on the essential oils *Cymbopogon giganteus* from benin for its potential use as an anti-inflammatory agent. *Int. J. Aromatherap*., **16**: 37-41.

Arai A, Kessler M and Xiao P (1994). A currently active drug that modulates AMPA receptors gate currents. *Brain Research,* **638**: 343-346.

Baylac S and Racine P (2003). Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. *Int. J. Aromather*., **13**: 138-142.

Bertaccini G and Substance P (1982). *In*: Handbook of experimental pharmacology. *Berlin, Springer,* **59**(II): 85-105.

Chapdelaine P, Tremblay RR and Dube JY (1978). *p*-Nitrophenol-alpha-D-glucopyranoside as substrate for measurement of maltase activity in human semen. *Clin. Chem*., **24**: 208-211.

Chapleo CB, Myers PL, Butler CM, Doxey JC, Roach AG and Smith CF (1983). Alpha-adrenoreceptor reagents, synthesis of some 1,4-benzodioxans as selective presynaptic alpha 2-adrenoreceptor antagonists and potential antidepressants. *J. Med. Chem.,* 26: 823-831.

Clapp HC, Banerjee A and Rotenberg SA (1985). Inhibition of soybean lipoxygenase 1 by *n*-alkylhydroxylamines. *J. Biochem.,* **24**: 1826-1830.

Cygler M, Schrag JD, Sussman J, Harel LM, Silman I and Gentry MK (1993). Relationship between sequence conservation and three-dimensional structure in a large family of esterases, lipases and related proteins. *Protein Sci*., **2**: 366-382.

Ellman GL, Courtney KD, Andres V and Featherstone RM (1961). A new and rapid calorimetric determination of acetylcholinesterase activity. *Biochem.Pharmacol.*,7: 88-90.

El-Sayed NS, El-Bendary RE, El-Ashry SM and El-Kerdawy MM (2011). Synthetic profiles to different thiadiazolopyrimidines. *Eur. J. Med. Chem*., **46**: 3714-3720.

Evans AT (1987). Action of cannabis constituents on enzymes of arachidonate, mechanism: Anti-

inflammatory potential. *Biochem. Pharmacol*., 36: 2035-2037

Garcia-Galan MJ, Diaz-Cruz MS and Bercelo D (2008). Identification and determination of metabolites and degradation products of sulfonamide antibiotics. *Trends Anal. Chem*., **27**: 1008-1022.

Gauthier S (2001). Cholinergic adverse effects of cholinesterase inhibitors of Alzheimers disease. *Drug & Aging*, **18**: 853-862.

Irshad M, Abbasi MA, Aziz-ur-Rehman, Rasool S, Siddiqui SZ, Ahmad I, Ashraf M, Lodhi MA and Jamal SB (2014). *O*- and *N*-Substituted derivatives of planetol as valuable bioactive compounds. *Asian J. Chem.,* **26**: 1151-1160.

Kaspady M, Narayanaswamy VK, Raju M and Rao GK (2009). Synthesis, antibacterial activity of 2,4-disubstituted oxazoles and thiazoles as bioisosteres. *Lett. Drug Des. Discov.,* **6**: 21-28.

Kemal C, Louis-Flemberg P, Krupinski-Olsen R and Shorter AL (1987). Reproductive inactivation of soybean lipoxygenase activity. *J. Biochem.,* **26**: 7064-7072.

Khan SA, Ahmed B and Alam T (2006). Synthesis and antihepatotoxic activity of some new chalcones containing 1,4-dioxane ring system. *Pak. J. Pharm. Sci*., **19**: 290-294.

Lu RJ, Tucker JA, Pickens J, Ma YA, Zinevitch T, Kirichenko O, Konoplev V, Kuznetsova S, Sviridov S, Brahmachary E, Khasanov A, Mikel C, Yang Y, Liu CH, Wang J, Freel S, Fisher S, Sullivan A, Zhou JY, Stanfield-Oakley S, Baker B, Sailstad J, Greenberg M, Bolognesi D, Bray B, Koszalka B, Jeffs P, Jeffries C, Chucholowski A and Sexton C (2009). Heterobiaryl human immunodeficiency virus entry inhibitors. *J. Med. Chem.,* **52**: 4481-4487.

Patrick G (2001). An Introduction to Medicinal Chemistry. 2nd ed. Oxford University Press., New York, p.13.

Remko M and Lieth CWV (2004). Theoretical study of gas phase acidity, pKa, lipophilicity and solubility of some biological active sulfonamides. *Bioorg. Med. Chem*., **12**: 5395-5403.

Steinhilber D (1999). 5-Lipoxygenase: A target for anti-inflammatory drugs revisited. *Curr. Med. Chem.,* **6**: 71-85.

Supuran CT, Casini A and Scozzafava A (2003). Protease inhibitors of the sulfonamide type: Anticancer, anti-inflammatory and anti-viral agents. *Med. Res. Rev.,* **23**: 535-358.

Supuran CT, Innocenti A, Mastrolorenzo A and Scozzafava A (2004). Antiviral sulfonamide derivatives. *Mini-Rev. Med. Chem.,* **4**: 189-200.

Supuran CT, Scozzafava A, Menabuoni L, Mincione F, Briganti F and Mincione G (1999). Carbonic anhydrase inhibitors synthesis & ocular pharmacology of a new class of water soluble, topically ineffective intraocular pressure lowering sulfonamides incorporating picolinoyl moieties. *Eur. J. Pharm. Sci*., **8**: 317-328.

Tappel AL (1953). The mechanism of oxidation of unsaturated fatty acids catalyzed by hematin compounds. *Arch. Biochem. Biophys*., **44**: 378-395.

Thomas G (2007). An Introduction to Medicinal Chemistry. 2nd ed. University of Portsmouth, John Wiley and sons., England, p.54.

Tougu V (2001). Acetylcholinesterase: Mechanism of catalysis and inhibition. *Curr. Med. Chem.,* **1**: 155-170.

Vazquez MT, Rosell G and Pujol MD (1997). Synthesis and anti-inflammatory activity of rac-2-(2,3-dihydro-1,4-benzodioxin)propionic acid and its *R* and *S* enantiomers. *Eur. J. Med. Chem.,* **32**: 529-534.

Wadood A, Riaz M, Jamal SB, Shah M and Lodhi MA (2013). Molecular docking study of P4-Benzoxaborolesubstituted ligands as inhibitors of HCV NS3/4A protease. *Bioinformation*, **9**: 309-314.

Yang CR, Ying Z, Jacob, MR, Khan SI, Zhang YJ and Li XC (2006). Antifungal activity of C-27 steroidal saponins. *Antimicrob. Agents Chemother*., **50**: 1710-1714.

Zhao ZJ, Wolkenberg SE, Lu MQ, Munshi V, Moyer G, Feng, MZ, Carella AV, Ecto LT, Gabryelski LJ, Lai MT, Prasad SG, Yan Y, McGaughey GB, Miller MD, Lindsley CW, Hartman GD, Vacca JP and Williams TM (2008). Novel indole-3-sulfonamides as potent HIV non-nucleoside reverse transcriptase inhibitors. *Bioorg. Med. Chem. Lett*., **18**: 554-559.