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Enzyme Inhibition and *In Silico* Studies of New Synthetic N-Substituted-(4-Bromophenyl)-4-Ethoxybenzenesulfonamides

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Abstract

A series of new N-substituted-(4-bromophenyl)-4-ethoxybenzenesulfonamides (5a-o) were synthesized and evaluated for enzyme inhibition potential. The task was accomplished by the reaction of 4-bromobenzenesulfonyl chloride

a. with 4-ethoxyaniline

b. to get the intermediate 4-bromophenyl-4-ethoxybenzenesulfonamide in the first step.

The compound 3 on further reaction with different electrophiles (4a-o) yielded the target compounds 5a-o, which were characterized with the help of FTIR, ^{1}H-, ^{13}C-NMR spectroscopic and EI-MS & HR-EI-MS spectrometric data. These sulfonamides (5a-o) were evaluated for their acetylcholinesterase (AChE) and α-glucosidase inhibitory potential. Compounds 5l, 5n, 5g, 5j and 5h exhibited excellent potential against AChE with IC_{50} values of 52.63 ± 0.14, 82.75 ± 0.16, 92.13 ± 0.15, 92.52 ± 0.16 and 98.72 ± 0.12 µM, respectively. Compounds 5h, 5j, 5c, 5d and 5l were found potent inhibitors of α-glucosidase with IC_{50} values of 57.38 ± 0.19, 123.36 ± 0.19, 123.42 ± 0.19, 124.35 ± 0.15 and 124.74 ± 0.18 µM, respectively. The activity results were also substantiated by *in silico* studies.

Keywords: Sulfonamide; Enzyme inhibition; Acetylcholinesterase inhibition; α-Glucosidase inhibition; Molecular docking studies

Introduction

The sulfonamides (-SO_2NH-) are proven as fascinating compounds as a main core for different bioactivities and can be more like a string of distinguished pearls. Sulfonamides are called sulfa drugs, which were the first antibacterial agents to be used systemically and paved the way for the antibiotic revolution in medicine. The first medicine of this class was Prontosil discovered as effective treatment of a range of bacterial infections. It had strong protective action against infections caused by Streptococci, including blood infections, childbed fever, and erysipelas [1]. Sulfonamides are structurally like p-aminobenzoic acid (PABA), a cofactor that is needed by bacteria for the synthesis of folic acid. Various medicines for the treatment of different diseases having the -SO_2NH- function are available in the market like sulfafurazole as children antibiotic, gliquidone as an antidiabetic for diabetes mellitus type-2, furosemide as a diuretic, zonisamide to treat epilepsy and Parkinson, mafenide as an antibiotic to treat skin infections, and dasabuvir to kill hepatitis C virus (Figure 1).

Clinically, sulfonamides are used to treat several urinary tract and gastrointestinal infections [2]. This group of compounds acts as antitumor agents by inhibiting the carbonic anhydrase.

Sulfonamides antibiotics inhibit the conversion of PABA into folic acid and thus ultimately inhibit the synthesis of purines and DNA [3,4]. Besides, antibacterial properties, sulfonamides also have other activities, like carbonic anhydrase inhibitors [5,6], anticancer, anti-inflammatory and analgesic agents [7], *β3*-adrenergic receptor agonists [8], PC-1 inhibitors [9], antifungal [10] and antiviral agents [11]. Keeping in view the broad-spectrum biological activities of sulfonamides, we designed a straightforward and efficient method to synthesize high yield sulfonamides and believe that this route will further be used for the synthesis of biologically active compounds.

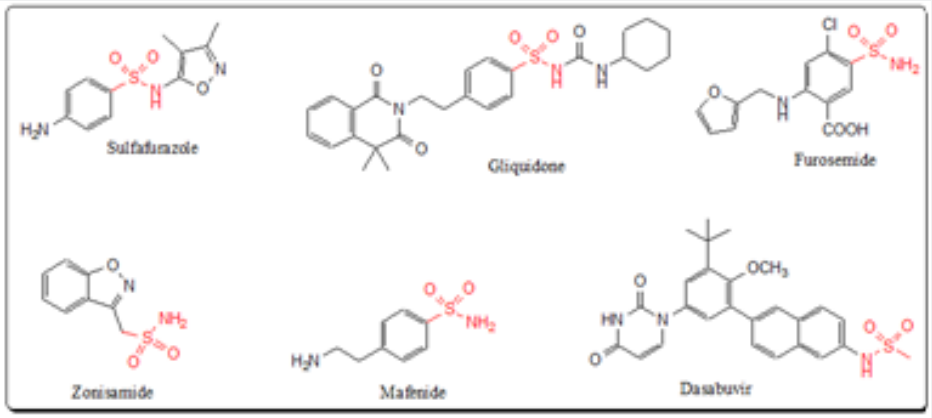

Figure 1: Some available drugs containing sulfonamide functionality in their structures.

Acetylcholinesterase (AChE, EC 3.1.1.7) is an enzyme that catalyzes the breakdown of acetylcholine esters that function as neurotransmitters. AChE is found mainly at neuromuscular junctions and in chemical synapses of the cholinergic type, where its activity serves to terminate synaptic transmission. It belongs to carboxylesterase family of enzymes that are primary target of inhibition by organophosphorus compounds such as nerve agents and pesticides [12], α-Glucosidase (EC, 3.2.1.20) inhibitors are used for the treatment of diabetes mellitus type-2 by inhibiting the digestion of carbohydrates. Conversely, carbohydrates are not converted into simple sugars by the enzyme present on cells lining the intestine. Hence, immediate post-prandial increase is restricted and sudden rise in blood sugar levels does not occur [13].

Structure-based drug design for an enzyme target has been facilitated with crystal structures which enable the computational searches to identify 'lead' compounds for refinement. There are feasible large-scale computational approaches which include analysis of off-target activity and combining suitable pharmacophores for enzyme combinations. However, such compounds still must be synthesized and tested experimentally to confirm the predicted inhibitory effects against each of the targets [14]. The aim of the present study was to synthesize alkyl/aralkyl substituted-N-(4-ethoxyphenyl)-4-bromobenzenesulfonamides (5a-o) and investigate them for their enzyme inhibitory activities against AChE and α-glucosidase in search for the 'lead' compounds against these enzymes of therapeutical importance. Synthesis of the intermediate and target compounds were carried out according to the protocol as shown in Figure 2.

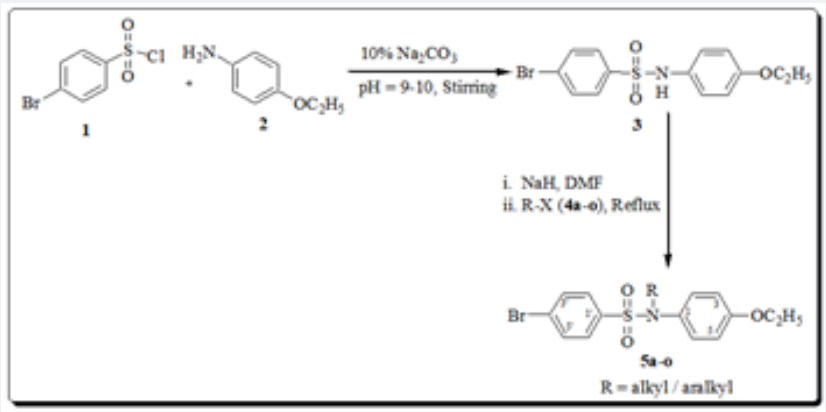

Figure 2: Protocol for the synthesis of compounds 5a-o.

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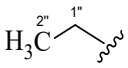
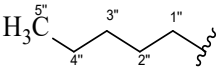
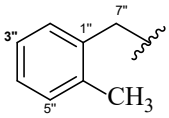
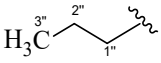
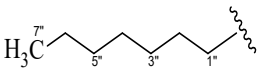
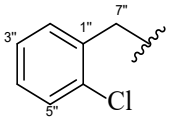
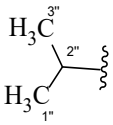
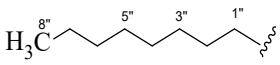
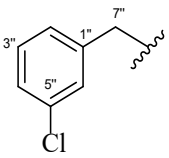
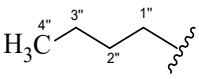
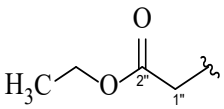
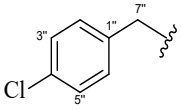
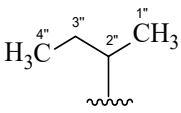
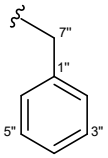
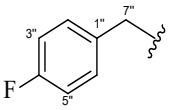
Compound 5a was synthesized as off-white amorphous solid. The molecular formula was found to be $C_{16}H_{18}BrNO_3S$ since HR-EI-MS showed molecular ion $[M]^+$ peak at *m/z* 383.0199 (calculated for $C_{16}H_{18}BrNO_3S$; 383.0190). The IR spectrum exhibited the presence of aromatic and aliphatic hydrogens (3039, 2968cm^{-1}), unsaturation (1625-1576 cm^{-1}), sulfonyl (1341cm^{-1}) and ethoxy (1258cm^{-1}) moieties in the molecule. The aliphatic region of the ^{1}H-NMR spectrum of 5a showed signals for *N*-ethyl and *O*-ethyl moieties at δ 1.05 (3H, t, J = 7.0 Hz, H-2''), 1.39 (3H, t, J = 7.0 Hz, CH_3-CH_2-O), 3.53 (2H, q, J = 7.0 Hz, H-1'') and 3.99 (2H, q, J = 7.0 Hz, CH_3-CH_2-O). The aromatic region of the spectrum showed two pairs of ortho-coupled doublets at δ 6.79 (2H, d, J = 8.5 Hz, H-2,6), 6.89 (2H, d, J = 8.5 Hz, H-3,5), 7.44 (2H, d, J = 8.5 Hz, H-2′,6′) and 7.56 (2H, d, J = 8.5 Hz, H-3′,5′), which were assigned to two p-substituted phenyl rings attached to the sulfonamide functionality.

The ^{13}C-NMR both broad band (BB) and distortion less enhancement by polarization transfer (DEPT) spectra of compound 5a showed altogether twelve carbon signals for sixteen carbons corroborated the presence of two methyl, two methylene, four methine and four quaternary carbons. The presence of an ethoxy and nitrogen substituted ethyl group was confirmed due to signals at δ 15.8, 63.7 & 15.0, 45.9, respectively. The confirmation of two 1,4-disubstituted benzene rings was done due to the resonances at δ 130.6 (C-1), 129.2 (C-2,6), 115.8 (C-3,5), 158.6 (C-5) & 137.6 (C-1′), 130.1 (C-2′,6′), 132.0 (C-3′,5′), 127.5 (C-5′), respectively. Based on this evidence, the structure of 5a was established as 4-bromo-N-(4-ethoxyphenyl)-N-ethylbenzene sulfonamide. Other compounds, 5b-o, were also characterized by using IR, EI-MS, HR-EI-MS, ^{1}H-NMR, and ^{13}C-NMR. Their details are described in the experimental section.

Table 1: Alkyl/aralkyl substituted groups (5a-o).

Compd.	R	Compd.	R	Compd.	R
5a		5f		5k	
5b		5g		5l	
5c		5h		5m	
5d		5i		5n	
5e		5j		5o	

Enzyme Inhibition Studies

AChE and α-glucosidase inhibition activity

The biological screening of these newly synthesized sulfonamides was carried out against AChE and α-glucosidase enzymes. The results showed that these compounds exhibited good inhibitory potential against AChE and α-glucosidase (Table 2). Amongst the tested compounds, 5l, 5n, 5g, 5j, 5h exhibited excellent AChE inhibition with IC_{50} values of 52.63 ± 0.14, 82.75 ± 0.16, 92.13 ± 0.15, 92.52 ± 0.16 and 98.72 ± 0.12 µM, respectively. The compounds, 5h, 5j, 5c, 5d, 5l showed potent inhibitory activity against α-glucosidase with IC_{50} values of 57.38 ± 0.19, 123.36 ± 0.19, 123.42 ± 0.19, 124.35 ± 0.15 and 124.74 ± 0.18µM, respectively (Table 2).

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Table 2: AChE and α-glucosidase inhibitory activities of compounds 5a-o.

Comp.	AChE		Yeast α-Glucosidase	
	Inhibition (%) at 0.5mM	IC_{50} (μM)	Inhibition (%) at 0.5mM	IC_{50} (μM)
5a	21.54 ± 0.14	-	56.53 ± 0.29	447.24 ± 0.18
5b	64.38 ± 0.19	416.54 ± 0.16	79.43 ± 0.23	273.78 ± 0.16
5c	75.45 ± 0.21	365.32 ± 0.15	85.15 ± 0.27	123.42 ± 0.19
5d	12.36 ± 0.12	-	85.23 ± 0.28	124.35 ± 0.15
5e	78.67 ± 0.21	302.25 ± 0.17	81.75 ± 0.28	253.52 ± 0.16
5f	86.23 ± 0.18	157.45 ± 0.14	51.63 ± 0.26	<500
5g	89.47 ± 0.19	92.13 ± 0.15	82.58 ± 0.23	153.54 ± 0.17
5h	89.26 ± 0.17	98.72 ± 0.12	87.34 ± 0.25	57.38 ± 0.19
5i	37.53 ± 0.15	-	68.27 ± 0.23	312.42 ± 0.18
5j	89.38 ± 0.21	92.52 ± 0.16	85.22 ± 0.28	123.36 ± 0.19
5k	89.75 ± 0.23	112.84 ± 0.18	84.83 ± 0.25	148.53 ± 0.16
5l	91.46 ± 0.19	52.63 ± 0.14	82.52 ± 0.26	124.74 ± 0.18
5m	43.29 ± 0.16	-	23.41 ± 0.23	-
5n	90.54 ± 0.21	82.75 ± 0.16	84.39 ± 0.27	142.52 ± 0.18
5o	89.72 ± 0.19	142.63 ± 0.15	82.24 ± 0.28	172.38 ± 0.19
Eserine	91.27 ± 1.17	0.04 ± 0.001	-	-
Acarbose	-	-	65.73 ± 1.93	375.82 ± 1.76

The sulfonamides with alkyl groups on nitrogen atom showed AChE inhibitory activity. Amongst these compounds, 5g bearing n-heptyl and 5h bearing n-octyl groups showed good inhibition against AChE with IC_{50} values of 92.13±0.15 and 98.72±0.12μM, respectively. However, it is found that there was a decrease in the activity with the decrease in carbon chain length on nitrogen atom, that is, increase in lipophilicity enhanced the activity. Amongst the benzyl substituted sulfonamides, compounds having o-chlorobenzyl substitution on the nitrogen atom pronounced the activity (5i, IC_{50} 52.63±0.14μM) whereas the compounds with p-chlorobenzyl (5n) and only benzyl substitution (5j) on nitrogen atom were found to be the significant AChE inhibitors (IC_{50} 82.75±0.16 and 92.52±0.16μM, respectively). The compound with m-chlorobenzyl substitution on nitrogen atom showed the least AChE inhibitory activity.

As for as the α-glucosidase activity is concerned, the sulfonamides having n-octyl group (5h) attached to nitrogen offered potent inhibition (IC_{50} 57.38 ± 0.19 μM) whereas the activity decreased with the decrease in carbon chain length (Table 2) except the compound 5d with n-butyl group on nitrogen (IC_{50} 124.35 ± 0.15 μM). These observations lead to the conclusion that increases in lipophilicity on nitrogen increased the anti-α-glucosidase activity. Amongst the benzyl substituted sulfonamides, compounds having o-and p-chlorobenzyl substitution on nitrogen atom were found as good inhibitors of α-glucosidase (IC_{50} 124.74 ± 0.18; 142.52 ± 0.18 μM, respectively). The isomer 5m with m-chlorobenzyl group was the least inhibitor indicating that the substitution at m-position retarded the inhibition whereas the benzyl group without any substitution (5j) showed significant α-glucosidase inhibition (IC_{50} 123.36 ± 0.19 μM).

In silico Studies

AChE docking studies

For docking studies against AChE enzyme, the most active compound 5l was selected. The crystal structure of hAChE (PDB id: 4M0E, 2Å) was downloaded from the PDB, the docking studies were performed according to our previously reported protocol [20] using Lead IT docking software [19]. Compound 5l was found to bind in the same region of the active site as that of co-crystallized inhibitor. Figure 3 shows docked conformation of 5l. The bromo phenyl ring was making a π-anion interaction with Asp74, a π-π T-shaped interaction was observed between the same bromo phenyl ring with Tyr124 and Asp74. Another π-π T-shaped interaction was seen between chloro phenyl ring and Phe338. A π-alkyl interaction was observed between the chloro group of same phenyl ring and Tyr337. Two more π-alkyl interactions were observed for the ethoxy side chain with Val294 and Tyr341. A π-π stacked interaction was observed between the phenyl ring containing ethoxy substituent and Tyr341. Hydrogen bond was predicted between one of the sulfonamide oxygen atoms with Ty341. Another hydrogen bond was observed with the oxygen atom of the ethoxy group and Phe295.

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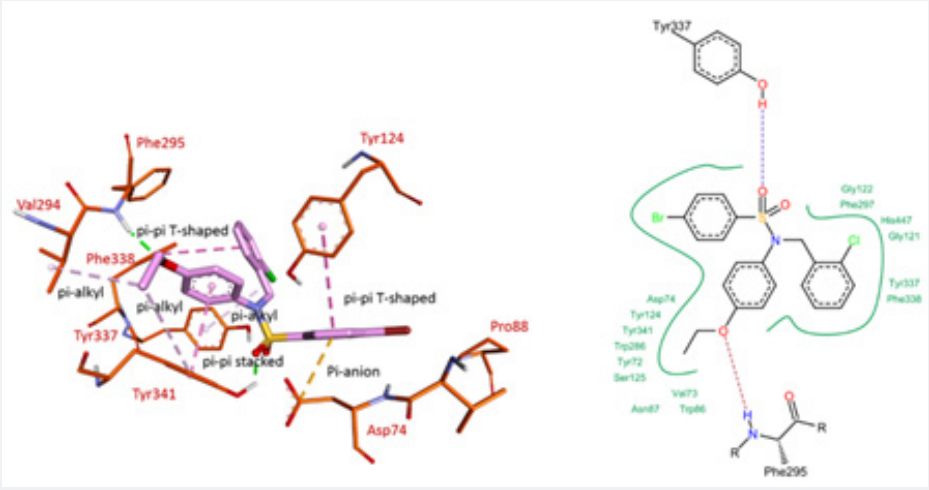

Figure 3: Binding site interactions of 5l inside active site of hAChE.

α-Glucosidase docking studies

To get a better understanding of binding site interactions of the most active α-glucosidase inhibitor, compound 5h was docked into homology-built model of yeast α-glucosidase according to our previously reported protocol [15]. The compound was found to bind in the active site of the enzyme. Several bonded and non-bonded interactions were observed that were responsible for the inhibitory activity of the compound. The N-alkyl side chain was making π-alkyl interactions with amino acids Ala278, Leu218 and His245. Similarly, the ethoxy side chain was also making π-alkyl interactions with Phe177 and Tyr71. The bromo phenyl ring was making π-alkyl interactions with Arg439 and Arg312, it was also making a π-anion interaction with Asp408. The oxygen atom of ethoxy side chain was making hydrogen bond with Arg439 (Figure 4).

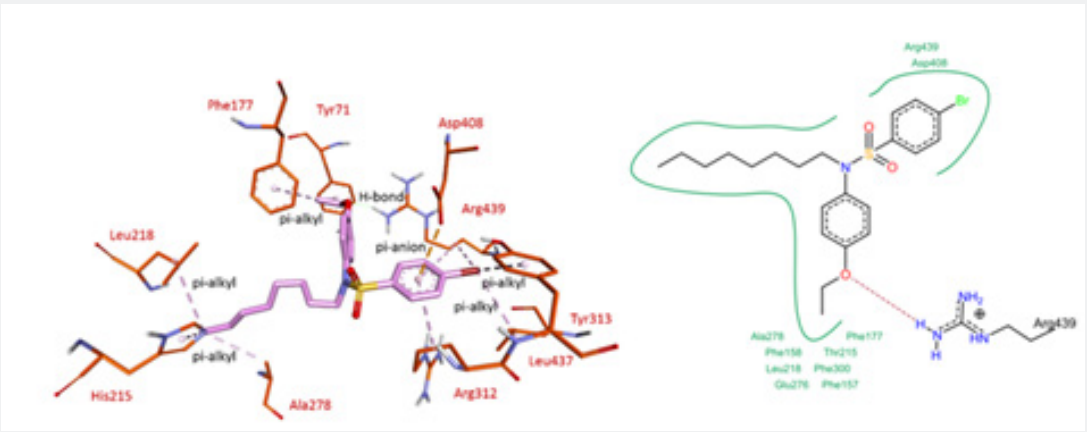

Figure 4: Binding site interactions of 5h inside active site of α-glucosidase.

ADME properties of compounds (5a-o)

The ADME (absorption, distribution, metabolism, excretion) are pharmacological properties of molecules were calculated by Med Chem Designer and data is given in Table 3. Permeability and solubility of the drugs are the two basic requirements for a drug to have good pharmacokinetic properties. In Lipinski's rules, molecular weights of compounds, log P, number of hydrogen bond acceptors and hydrogen bond donors are associated with the permeability and solubility properties of molecules. The determination of polar surface area and molecular flexibility is associated with the oral bioavailability of drugs [18].

From the table it is observed that the compounds having higher logD and logP values and lower number of hydrogen bonds predict higher bioavailability of drugs. S+logP and MlogP are octanol-water distribution coefficients and molecules with values below 5 predict them to have drug-like properties *in silico*.

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TPSA is the topological polar surface area. A molecule with TPSA value of exceeding 140Å^2, predicts the decreased bioavailability of molecule. In the present studies, all molecules showed excellent TPSA values between 46.61 and 72.91Å^2. Thus, all these molecules possess desirable drug-like TPSA property.

Table 3: ADME properties of compounds 5a-j.

Comp	MlogP	S+logP	S+logD	MW	MNO	TPSA	HBDH
5a	3.465	4.343	4.343	384.299	4	46.61	0
5b	3.7	4.62	4.62	398.326	4	46.61	0
5c	3.7	4.705	4.705	398.326	4	46.61	0
5d	3.93	5.056	5.056	412.353	4	46.61	0
5e	3.93	4.927	4.927	412.353	4	46.61	0
5f	4.155	5.486	5.486	426.38	4	46.61	0
5g	4.592	6.486	6.486	454.434	4	46.61	0
5h	4.805	6.982	6.982	468.461	4	46.61	0
5i	2.7	3.871	3.871	442.336	6	72.91	0
5j	4.37	5.2	5.2	446.37	4	46.61	0
5k	4.583	5.686	5.686	460.397	4	46.61	0
5l	4.583	5.752	5.752	480.815	4	46.61	0
5m	4.583	5.747	5.747	480.815	4	46.61	0
5n	4.583	5.824	5.824	480.815	4	46.61	0
5o	4.477	5.458	5.458	464.361	4	46.61	0

S+logP and MlogP are octanol-water distribution coefficients (It should be <5.0).
S+logD is pH dependent octanol-water distribution coefficient.
HBDH indicates number of hydrogen bond donors (It should be < 5 H-bond donors)
MNO value indicates total number of hydrogen bond acceptor (sum of N & O atoms). It should be <10 H-bond acceptors.
Mol Wt is molecular weight (It should be 180-480 Daltons).
TPSA is the topological polar surface area expressed in square angstroms (It should be <140Å^2).

Experimental

General experimental procedures

All the chemicals and solvents were of analytical grade purchased from local supplier of Sigma Aldrich and Alfa Aesar. Melting points were measured by Gallen Kamp electrothermal apparatus. The purity of synthesized compounds was confirmed by using silica coated TLC plates F_{256} 20×20 cm. ^{1}H NMR spectra were recorded on 500 MHz Bruker spectrometers while ^{13}C NMR spectra were taken at 125 MHz using the same instrument. The chemical shift value δ was taken on ppm scale and TMS was used as internal reference standard. Jasco-320-A spectrophotometer was used to record IR spectra as KBr pellets. EI-MS and HR-EI-MS spectra were recorded on JMS-HX-110 spectrometer.

Synthesis of sulfonamide (3)

4-Bromobenzenesulfonyl chloride (1; 0.02mol; 6g) was added with p-ethoxy aniline (2; 0.02mol; 3mL) in 250 mL round bottom flask together with 50mL water. The pH of the reaction mixture was adjusted at 10.0 by adding aqueous solution of Na_2CO_3 at room temperature. The reaction mixture was stirred continuously, and completion of the reaction was monitored by TLC. On completion of reaction, concentrated HCl was added drop wise to the mixture to adjust the pH to 2.0 to precipitate the product. The precipitates were filtered, washed with cold distilled water, and were crystallized in ethanol to get the off-white crystals of 4-bromo-N-(4-ethoxyphenyl) benzene sulfonamide (3) with 87% yield. Yield 87%; Off white crystals; mp: 118-119˚C; IR (KBr, cm^{-1}) ν_{max}: 3322 (N-H), 3037 (Ar-H), 2940 (C-H), 1629-1568 (Ar C=C), 1376 (S=O), 1237 (C-O). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.40 (t, J = 6.5 Hz, 3H, CH_3-CH_2-O), 3.98 (q, J = 6.5 Hz, 2H, CH_3-CH_2-O), 6.78 (d, J = 8.5 Hz, 2H, H-2,6), 6.87 (d, J = 8.5 Hz, 2H, H-3,5), 7.43 (d, J = 8.5 Hz, 2H, H-2′,6′), 7.57 (d, J = 8.5 Hz, 2H, H-3′,5′). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 14.8 (CH_3), 63.7 (CH_2), 114.7 (C-3,5), 127.9 (C-4′), 129.2 (C-2,6), 130.1 (C-2′,6′), 130.6 (C-1), 132.3 (C-3′,5′), 137.6 (C-1′), 158.6 (C-4). HR-EI-MS (*m/z*) [M]$^+$: 354.9889, calculated for $C_{14}H_{14}BrNO_3S$; 354.9877.

Synthesis of N-alkyl/aralkyl substituted sulphonamides (5a-o)

The calculated amount of 3 (0.1mmol) was taken in 50mL round bottomed flask and 10.0mL of N, N-dimethyl formamide (DMF) was added followed by the addition of sodium hydride (0.01mmol). The mixture was stirred for 30 minutes at room temperature with onward addition of electrophiles alkyl/aralkyl halides (4a-o) (Table 1) separately to the reaction mixture

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which was further stirred for three hours. The reaction progress was monitored by TLC till the appearance of single spot on chromatogram. The targeted products (5a-o) were precipitated by adding cold water followed by filtration, washing with cold water and crystallization in methanol.

Spectral characterization of the compounds 5a-o

4-Bromo-N-(4-ethoxyphenyl)-N-ethylbenzenesulfonamide (5a)

Yield 63%; mp: 118-119˚C; IR (KBr) ν_{max}: 3039, 2968, 1625-1576, 1351, 1258 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.05 (t, J = 7.0 Hz, 3H, H-2′′), 1.39 (t, J = 7.0 Hz, 3H, CH_3-CH_2-O), 3.53 (q, J = 7.0 Hz, 2H, H-1′′), 3.99 (q, J = 7.0 Hz, 2H, CH_3-CH_2-O), 6.79 (d, J = 8.5 Hz, 2H, H-2,6), 6.89 (d, J = 8.5 Hz, 2H, H-3,5), 7.44 (d, J = 8.5 Hz, 2H, H-2′,6′), 7.56 (d, J = 8.5 Hz, 2H, H-3′,5′). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 14.0 (C-2′′), 14.8 (CH_3), 45.9 (C-1′′), 63.7 (CH_2), 114.8 (C-3,5), 127.4 (C-4′), 129.2 (C-2,6), 130.1 (C-2′,6′), 130.6 (C-1), 132.0 (C-3′,5′), 137.6 (C-1′), 158.6 (C-4). HR-EI-MS (*m/z*): 383.0199 $[M]^+$ calculated for $C_{16}H_{18}BrNO_3S$; 383.0190.

4-Bromo-N-(4-ethoxyphenyl)-N-propylbenzenesulfonamide (5b)

Yield 70%; mp: 118-120˚C; IR (KBr) ν_{max}: 3035, 2960, 1620-1587, 1355, 1250 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.87 (t, J = 6.5 Hz, 3H, H-3′′), 1.39 (t, J = 7.0 Hz, 3H, CH_3-CH_2-O), 1.43 (m, 2H, H-2′′), 3.42 (t, J = 7.0 Hz, 2H, H-1′′), 4.00 (q, J = 7.0 Hz, 2H, CH_3-CH_2-O), 6.77 (d, J = 8.0 Hz, 2H, H-2,6), 6.90 (d, J = 8.0 Hz, 2H, H-3,5), 7.42 (d, J = 8.5 Hz, 2H, H-2′,6′), 7.56 (d, J = 8.5 Hz, 2H, H-3′,5′). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 10.9 (C-3′′), 14.8 (CH_3), 21.4 (C-2′′), 52.5 (C-1′′), 63.6 (CH_2), 114.8 (C-3,5), 127.4 (C-4′), 129.2 (C-2,6), 130.0 (C-2′,6′), 130.9 (C-1), 132.0 (C-3′,5′), 137.5 (C-1′), 158.6 (C-4). HR-EI-MS (*m/z*): 397.0361 $[M]^+$ calculated for $C_{17}H_{20}BrNO_3S$; 397.0347.

4-Bromo-N-(4-ethoxyphenyl)-N-isopropylbenzenesulfonamide (5c)

Yield 69%; mp: 118-119˚C; IR (KBr) ν_{max}: 3037, 2962, 1618-1595, 1357, 1255 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.01 (d, J = 6.5 Hz, 6H, H-1′′,3′′), 1.39 (t, J = 6.5 Hz, 3H, CH_3-CH_2-O), 4.00 (q, J = 6.5 Hz, 2H, CH_3-CH_2-O), 4.57 (m, ^1H, H-1′′), 6.79 (d, J = 8.5 Hz, 2H, H-2,6), 6.88 (d, J = 8.5 Hz, 2H, H-3,5), 7.56 (br s, 4H, H-2′,3′,5′,6′). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 14.8 (CH_3), 22.1 (C-1′′,3′′), 51.3 (C-2′′), 63.7 (CH_2), 114.6 (C-3,5), 126.6 (C-4′), 127.1 (C-1), 128.9 (C-2,6), 132.0 (C-2′,6′), 133.4 (C-3′,5′), 140.6 (C-1′), 159.1 (C-4). HR-EI-MS (*m/z*): 397.0361 $[M]^+$ calculated for $C_{17}H_{20}BrNO_3S$; 397.0347.

4-Bromo-N-(n-butyl)-N-(4-ethoxyphenyl) benzenesulfonamide (5d)

Yield 60%; mp: 124-125˚C; IR (KBr) ν_{max}: 3035, 2960, 1617-1596, 1357, 1255 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.84 (t, J = 7.0 Hz, 3H, H-4′′), 1.29 (m, 2H, H-3′′), 1.36 (m, 2H, H-2′′), 1.39 (t, J = 7.0 Hz, 3H, CH_3-CH_2-O), 3.45 (t, J = 7.0 Hz, 2H, H-1′′), 3.99 (q, J = 7.0 Hz, 2H, CH_3-CH_2-O), 6.78 (d, J = 8.5 Hz, 2H, H-3,5), 6.88 (d, J = 8.5 Hz, 2H, H-2,6), 7.42 (d, J = 8.5 Hz, 2H, H-2′,6′), 7.56 (d, J = 8.5 Hz, 2H, H-3′,5′). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 13.6 (C-4′′), 14.8 (CH_3), 19.6 (C-3′′), 30.2 (C-2′′), 50.6 (C-1′′), 63.7 (CH_2), 114.8 (C-3,5), 127.4 (C-4′), 129.2 (C-2,6), 129.9 (C-2′,6′), 130.9 (C-1), 132.0 (C-3′,5′), 137.4 (C-1′), 158.6 (C-4). HR-EI-MS (*m/z*): 411.0517 $[M]^+$ calculated for $C_{18}H_{22}BrNO_3S$; 413.0503.

4-Bromo-N-(sec-butyl)-N-(4-ethoxyphenyl) benzenesulfonamide (5e)

Yield 65%; mp: 120-124˚C; IR (KBr) ν_{max}: 3039, 2969, 1617-1599, 1350, 1261 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.92 (t, J = 7.0 Hz, 3H, H-4′′), 0.99 (d, J = 6.5 Hz, 3H, H-1′′), 1.20, 1.34 (m, 2H, H-3′′), 1.39 (t, J = 7.0 Hz, 3H, CH_3-CH_2-O), 4.00 (q, J = 7.0 Hz, 2H, CH_3-CH_2-O), 4.25 (m, ^1H, H-2′′), 6.79 (d, J = 8.5 Hz, 2H, H-2,6), 6.88 (d, J = 8.5 Hz, 2H, H-3,5), 7.54 (m, 4H, H-2,3,5,6). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 11.5 (C-4′′), 14.8 (CH_3), 19.9 (C-1′′), 28.6 (C-3′′), 57.5 (C-2′′), 63.6 (CH_2), 114.5 (C-3,5), 126.8 (C-4′), 127.0 (C-1), 128.9 (C-2,6), 132.0 (C-2′,6′), 133.3 (C-3′,5′), 140.6 (C-1′), 159.1 (C-4). HR-EI-MS (*m/z*): 411.0517 $[M]^+$ calculated for $C_{18}H_{22}BrNO_3S$; 413.0503.

4-Bromo-N-(4-ethoxyphenyl)-N-pentylbenzenesulfonamide (5f)

Yield 68%; mp: 120-122˚C; IR (KBr) ν_{max}: 3055, 2965, 1619-1597, 1351, 1260 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.82 (t, J = 7.0 Hz, 3H, H-5′′), 1.21-1.36 (m, 6H, H-2′′-4′′), 1.39 (t, J = 7.0 Hz, 3H, CH_3-CH_2-O), 3.44 (t, J = 7.0 Hz, 2H, H-1′′), 3.99 (q, J = 7.0 Hz, 2H, CH_3-CH_2-O), 6.78 (d, J = 8.5 Hz, 2H, H-2,6), 6.88 (d, J = 8.5 Hz, 2H, H-3,5), 7.42 (d, J = 8.5 Hz, 2H, H-2′,6′), 7.55 (d, J = 8.5 Hz, 2H, H-3′,5′). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 14.0 (C-5′′), 14.8 (CH_3), 22.2 (C-4′′), 27.8 (C-3′′), 28.5 (C-2′′), 50.8 (C-1′′), 63.7 (CH_2), 114.7 (C-2,5), 127.3 (C-4′), 129.2 (C-2,6), 129.9 (C-2′,6′), 130.9 (C-1), 132.0 (C-3′,5′), 137.5 (C-1′), 158.6 (C-4). HR-EI-MS (*m/z*): 425.0675 $[M]^+$ calculated for $C_{19}H_{24}BrClNO_3S$; 425.0660.

4-Bromo-N-(4-ethoxyphenyl)-N-heptylbenzenesulfonamide (5g)

Yield 66%; mp: 120-123˚C; IR (KBr) ν_{max}: 3051, 2962, 1621-1595, 1353, 1252 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.82 (t, J = 6.8 Hz, 3H, H-7′′), 1.18-1.36 (m, 10H, H-2′′-6′′), 1.39 (t, J = 7.0 Hz, 3H, CH_3-CH_2-O), 3.44 (t, J = 6.8 Hz, 2H, H-1′′), 3.98 (q, J = 7.0 Hz, 2H, CH_3-CH_2-O), 6.76 (d, J = 8.4 Hz, 2H, H-2,6), 6.88 (d, J = 8.4 Hz, 2H, H-3,5), 7.41 (d, J = 8.4 Hz, 2H, H-2′,6′), 7.55 (d, J = 8.4 Hz, 2H, H-3′,5′). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 14.1 (C-7′′), 14.8 (CH_3), 22.6 (C-6′′), 26.3 (C-5′′), 28.2 (C-4′′), 28.8 (C-3′′), 50.9 (C-2′′), 63.7 (CH_2), 114.7 (C-3,5), 127.4 (C-4′), 129.2 (C-2,6), 129.9 (C-2′,6′), 130.9 (C-1′), 132.0 (C-3′,5′), 137.5 (C-1′), 158.6 (C-4). HR-EI-MS (*m/z*): 453.0987 $[M]^+$ calculated for $C_{21}H_{28}BrClNO_3S$; 453.0976.

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4-Bromo-N-(4-ethoxyphenyl)-N-octylbenzenesulfonamide (5h)

Yield 71%; mp: 121-123˚C; IR (KBr) ν_{max}: 3052, 2960, 1617-1599, 1350, 1252 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.84 (t, J = 7.0 Hz, 3H, H-8′′), 1.18-1.36 (m, 12H, H-2′′-7′′), 1.40 (t, J = 7.0 Hz, 3H, CH_3-CH_2-O), 3.44 (t, J = 7.0 Hz, 2H, H-1′′), 3.99 (q, J = 7.0 Hz, 2H, CH_3-CH_2-O), 6.78 (d, J = 7.5 Hz, 2H, H-2,6), 6.88 (d, J = 7.5 Hz, 2H, H-3,5), 7.42 (d, J = 7.5 Hz, 2H, H-2′,6′), 7.56 (d, J = 7.5 Hz, 2H, H-3′,5′). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 14.1 (C-8′′), 14.8 (CH_3), 22.6 (C-7′′), 31.7 (C-6′′), 29.0 (C-5′′), 28.1 (C-4′′), 26.3 (C-3′′), 29.1 (C-2′′), 50.9 (C-1′′), 63.7 (CH_2), 114.7 (C-3,5), 127.4 (C-4′), 129.2 (C-2,6), 129.9 (C-2′,6′), 130.9 (C-1), 132.0 (C-3′,5′), 137.5 (C-1′), 158.5 (C-4). HR-EI-MS (*m/z*): 467.1144 $[M]^+$ calculated for $C_{22}H_{30}BrNO_3S$; 467.1132.

Ethyl N-((4-bromophenyl) sulfonyl)-N-(4-ethoxyphenyl) glycinate (5i)

Yield 53%; mp: 123-124˚C; IR (KBr) ν_{max}: 3037, 2962, 1739, 1618-1595, 1357, 1255 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.20 (t, J = 7.0 Hz, 3H, CH_3-CH_2-O), 1.38 (t, J = 7.0 Hz, 3H, CH_3-CH_2-O), 3.97 (q, J = 7.0 Hz, 2H, CH_3-CH_2-O), 4.13 (q, J = 7.0 Hz, 2H, CH_3-CH_2-O), 4.35 (s, 2H, N-CH_2), 6.75 (d, J = 8.5 Hz, 2H, H-2,6), 7.07 (d, J = 8.5 Hz, 2H, H-3,5), 7.51 (d, J = 8.5 Hz, 2H, H-2′,6′), 7.56 (d, J = 8.5 Hz, 2H, H-3′,5′). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3), 14.8 (CH_3), 53.2 (C-2′′), 61.5 (CH_2), 63.7 (CH_2), 114.9 (C-3,5), 127.8 (C-4′), 129.4 (C-2,6), 130.5 (C-2′,6′), 131.6 (C-1), 131.9 (C-3′,5′), 138.2 (C-1′), 159.0 (C-4), 168.9 (C=O). HR-EI-MS (*m/z*): 441.0261 $[M]^+$ calculated for $C_{18}H_{20}BrNO_5S$; 443.0245.

N-Benzyl-4-bromo-N-(4-ethoxyphenyl) benzenesulfonamide (5j)

Yield 69%; mp: 118-120˚C; IR (KBr) ν_{max}: 3039, 2969, 1617-1599, 1350, 1261 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.35 (t, J = 7.0 Hz, 3H, CH_3-CH_2-O), 3.92 (q, J = 7.0 Hz, 2H, CH_3-CH_2-O), 4.65 (s, 2H, H-7′′), 6.68 (d, J = 8.5 Hz, 2H, H-2,6), 6.81 (d, J = 8.5 Hz, 2H, H-3,5), 7.17-7.24 (m, 5H, H-2′′-6′′), 7.49 (d, J = 8.5 Hz, 2H, H-2′,6′), 7.60 (d, J = 8.5 Hz, 2H, H-3′,5′). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 14.8 (CH_3), 55.2 (C-7′′), 63.6 (CH_2), 114.7 (C-3,5), 127.6 (C-4′) 127.7 (C-4′′), 128.4 (C-3′′,5′′), 128.7 (C-2,6), 129.2 (C-2′′,6′′), 130.2 (C-2′,6′), 130.8 (C-1), 132.2 (C-3′,5′), 135.7 (C-1′′), 137.8 (C-1′), 158.5 (C-4). HR-EI-MS (*m/z*): 445.0359 $[M]^+$ calculated for $C_{21}H_{19}BrClNO_3S$; 445.0347.

4-Bromo-N-(4-ethoxyphenyl)-N-(2-methylbenzyl) benzenesulfonamide (5k)

Yield 72%; mp: 120-122°C; IR (KBr) ν_{max}: 3039, 2969, 1617-1599, 1350, 1261 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.34 (t, J = 6.5 Hz, 3H, CH_3-CH_2-O), 2.30 (s, 3H, CH_3), 3.91 (q, J = 6.5 Hz, 2H, CH_3-CH_2-O), 4.67 (s, 2H, H-7′′), 6.65 (d, J = 8.5 Hz, 2H, H-2,6), 6.76 (d, J = 8.5 Hz, 2H, H-3,5), 6.97-7.06 (m, 4H, H-3′′-6′′), 7.50 (d, J = 8.0 Hz, 2H, H-2′,6′), 7.61 (d, J = 8.0 Hz, 2H, H-3′,5′). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 14.7 (CH_3), 19.2 (CH_3), 52.9 (C-7′′), 63.5 (CH_2), 114.6 (C-3,5), 125.8 (C-4′′), 127.7 (C-4′), 127.9 (C-5′′), 129.4 (C-2,6), 130.0 (C-2′,6′), 130.2 (C-3′′), 130.4 (C-6′′), 130.6 (C-1), 132.1 (C-3′,5′), 133.1 (C-1), 137.1 (C-1′), 137.4 (C-2′′), 158.5 (C-4). HR-EI-MS (*m/z*): 459.0515 $[M]^+$ calculated for $C_{22}H_{22}BrNO_3S$; 459.0503.

4-Bromo-N-(2-chlorobenzyl)-N-(4-ethoxyphenyl) benzenesulfonamide (5l)

Yield 73%; mp: 121-123˚C; IR (KBr) ν_{max}: 3039, 2969, 1617-1599, 1350, 1261 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.35 (t, J = 6.5 Hz, 3H, CH_3-CH_2-O), 3.93 (q, J = 6.5 Hz, 2H, CH_3-CH_2-O), 4.82 (s, 2H, H-7′′), 6.69 (d, J = 8.5 Hz, 2H, H-2,6), 6.90 (d, J = 8.5 Hz, 2H, H-3,5), 7.10-7.24 (m, 3H, H-4′′-6′′), 7.46 (d, J = 8.5 Hz, H-2′,6′), 7.52 (d, J = 8.0 Hz, ^1H, H-3′′), 7.61 (d, J = 8.5 Hz, 2H, H-3′,5′). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 14.8 (CH_3), 52.1 (C-7′′), 63.6 (CH_2), 114.7 (C-3,5), 127.0 (C-4′′), 127.8 (C-4′), 128.9 (C-5′′), 129.3 (C-2,6), 129.4 (C-3′′), 130.0 (C-2′,6′), 130.5 (C-6′′), 130.9 (C-1), 132.2 (C-3′,5′), 133.4 (C-2), 133.5 (C-1′′), 137.3 (C-1′), 158.6 (C-4). HR-EI-MS (*m/z*): 478.9959 $[M]^+$ calculated for $C_{21}H_{19}BrClNO_3S$; 478.9947.

4-Bromo-N-(3-chlorobenzyl)-N-(4-ethoxyphenyl) benzenesulfonamide (5m)

Yield 72%; mp: 120-122°C; IR (KBr) ν_{max}: 3039, 2969, 1617-1599, 1350, 1261 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.35 (t, J = 7.0 Hz, 3H, CH_3-CH_2-O), 3.94 (q, J = 7.0 Hz, 2H, CH_3-CH_2-O), 4.62 (s, 2H, H-7′′), 6.70 (d, J = 8.5 Hz, 2H, H-2,6), 6.81 (d, J = 8.5 Hz, 2H, H-3,5), 7.13-7.19 (m, 4H, H-2′′,4′′-6′′), 7.48 (d, J = 8.5 Hz, 2H, H-2′,6′), 7.60 (d, J = 8.5 Hz, 2H, H-3′,5′). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 14.8 (CH_3), 54.7 (C-7′′), 63.6 (CH_2), 114.8 (C-3,5), 126.8 (C-4′′), 127.8 (C-4′), 128.0 (C-6′′), 127.8 (C-2′′), 128.6 (C-2′′), 129.2 (C-2,6), 129.7 (C-5′′), 130.1 (C-2′,6′), 130.6 (C-1), 132.2 (C-3′,5′), 134.3 (C-3′′), 137.4 (C-1′′), 138.0 (C-1′), 158.7 (C-4). HR-EI-MS (*m/z*): 478.9959 $[M]^+$ calculated for $C_{21}H_{19}BrClNO_3S$; 478.9947.

4-Bromo-N-(4-chlorobenzyl)-N-(4-ethoxyphenyl) benzenesulfonamide (5n)

Yield 70%; mp: 120-122˚C; IR (KBr) ν_{max}: 3039, 2969, 1617-1599, 1350, 1261 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.35 (t, J = 7.0 Hz, 3H, CH_3-CH_2-O), 3.93 (q, J = 7.0 Hz, 2H, CH_3-CH_2-O), 4.61 (s, 2H, H-7′′), 6.69 (d, J = 8.5 Hz, 2H, H-2,6), 6.78 (d, J = 8.5 Hz, 2H, H-3,5), 7.12 (d, J = 8.0 Hz, 2H, H-2′′,6′′), 7.18 (d, J = 8.0 Hz, 2H, H-3′′,5′′), 7.48 (d, J = 8.5 Hz, 2H, H-2′,6′), 7.60 (d, J = 8.5 Hz, 2H, H-3′,5′). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 14.8 (CH_3), 54.5 (C-7′′), 63.6 (CH_2), 114.8 (C-3,5), 127.8 (C-4′), 128.7 (C-2,6), 129.2 (C-2′,6′), 130.0 (C-2′′,6′′), 130.1 (C-3′′,5′′), 130.5 (C-1), 132.2 (C-3′,5′), 133.6 (C-4′′), 134.4 (C-1′′), 137.6 (C-1′), 158.6 (C-4). HR-EI-MS (*m/z*): 478.9959 $[M]^+$ calculated for $C_{21}H_{19}BrClNO_3S$; 478.9947.

4-Bromo-N-(4-flourobenzyl)-N-(4-ethoxyphenyl) benzenesulfonamide (5o)

Yield 66%; mp: 120-121˚C; IR (KBr) ν_{max}: 3039, 2969, 1617-1599, 1350, 1261 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.35 (t, J = 7.0 Hz, 3H, CH_3-CH_2-O), 3.93 (q, J = 7.0 Hz, 2H, CH_3-CH_2-O),

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4.61 (s, 2H, H-7′′), 6.69 (d, J = 8.5 Hz, 2H, H-2,6), 6.78 (d, J = 8.5 Hz, 2H, H-3,5), 6.89 (d, J = 8.5 Hz, 2H, H-2′′,6′′), 7.15 (d, J = 8.5 Hz, 2H, H-3′′,5′′), 7.48 (d, J = 8.5 Hz, 2H, H-2′,6′), 7.60 (d, J = 8.5 Hz, 2H, H-3′,5′). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 14.8 (CH_3), 54.5 (C-7′′), 63.6 (CH_2), 114.7 (C-3,5), 115.3 (C-3′′,5′′), 127.7 (C-4′), 129.2 (C-2,6), 130.2 (C-2′,6′), 130.4 (C-2′′,6′′), 130.5 (C-1), 131.6 (C-1′′), 132.2 (C-3′,5′), 137.7 (C-1′), 158.6 (C-4), 163.3 (C-4′′). HR-EI-MS (*m/z*): 463.0265 $[M]^+$ calculated for $C_{21}H_{19}BrFNO_3S$; 463.0253.

Enzyme Inhibition Assays

Acetylcholinesterase inhibition assay

AChE inhibition activity was performed by the reported method with some modifications [17]. Reaction mixture of 100 µL contained 60 µL 50 mM phosphate buffer of pH 7.7, 10 µL (0.5 mM/well) test compound and 10 µL (0.005 unit/well) of electric eel enzyme (Sigma Inc). Contents were pre-incubated at 37°C for 10 min and pre-read at 405nm. The reaction was initiated by the addition of 10µL of 0.5mM/well substrate, acetylthiocholine iodide, followed by the addition of 10 µL DTNB (0.5 mM/well). Incubation was continued for further 30 minutes and absorbance was measured using Synergy HTX (BioTek, USA) 96-well plate reader. Eserine (0.5 mM/well) was used as a positive control. The percent inhibition was calculated by the help of following equation. The active compounds were serially diluted and assayed against the enzyme. The data obtained was used to calculate IC_{50} values, that is, the concentration at which the enzyme activity is inhibited by 50%.

$$\text{Inhibition}(\%) = \frac{\text{Control-Test}}{\text{Control}} \times 100 \quad (1)$$

α-Glucosidase inhibition assay

The αglucosidase inhibition assay was performed according to the reported method with some modification [15]. Total volume of the reaction mixture of 100 µL contained 70µL of 50 mM phosphate buffer with pH 6.8, 10µL (0.5 mM) test compound, followed by the addition of 10 µL (0.057 units) yeast enzyme (Sigma Inc.). The contents were mixed, pre-incubated for 10 min at 37°C and pre-read at 400nm. The reaction was initiated by the addition of 10µL of 0.5mM substrate (p-nitrophenyl glucopyranoside). After 30 min of incubation at 37 °C, absorbance was measured at 400nm using Synergy HTX microplate reader. Acarbose was used as positive control. All experiments were carried out in triplicates. The percentage inhibition and IC_{50} values were determined as mentioned above for AChE.

Conclusion

The targeted N-substituted-(4-bromophenyl)-4-ethoxybenzenesulfonamides (5a-o) were synthesized in good yields and these molecules possessed broad range spectrum against AChE (5l, 5n, 5g, 5j, 5h; IC_{50} values 52.63 ± 0.14, 82.75 ± 0.16, 92.13 ± 0.15, 92.52 ± 0.16, 98.72 ± 0.12µM, respectively) and α-glucosidase (5h, 5j, 5c, 5d , 5l; IC_{50} values 57.38 ± 0.19, 123.36 ± 0.19, 123.42 ± 0.19, 124.35 ± 0.15, 124.74 ± 0.18 µM, respectively). Therefore, these studies conclude that the newly synthesized molecules might serve as promising drug candidates for further structural optimizations and drug designing studies.

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