Synthesis, Structural Characterization and Biological Screening of Various Sulfa Drugs Derived from 2-Anisidine

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Summary: In the present study, a series of *N*-alkyl substituted sulfa drugs (sulfonamides) has been synthesized. The reaction of 2-anisidine (**1**) with 4-methylbenzenesulfonyl chloride (**2**) yielded *N*-(2-methoxyphenyl)-4-methylbenzenesulfonamide (**3**), which on bromination with bromine in the presence of glacial acetic acid gave *N*-(4,5-dibromo-2-methoxyphenyl)-4-methylbenzenesulfonamide (**6**). These two products **3** and **6** on further treatment with different alkyl halides in the presence of lithium hydride yielded fourteen new *N*-substituted sulfonamides. These compounds were characterized by their EI-MS and ^{1}H-NMR spectra and screened against acetylcholinesterase, butyrlcholinesterase and chymotrypsin enzymes. The results revealed that *N*-propyl-*N*-(2-methoxyphenyl)-4-methylbenzenesulfonamide (**5c**), *N*-(4,5-dibromo-2-methoxyphenyl)-4-methylbenzenesulfonamide (**6**) and *N*-methyl-*N*-(4,5-dibromo-2-methoxyphenyl)-4-methylbenzenesulfonamide (**7e**) exhibited good inhibitory potential against acetylcholinesterase, butyrlcholinesterase and chymotrypsin respectively.

Introduction

Sulfa drugs (sulfonamides) have been widely used in medicinal chemistry due to their broad spectrum of antimicrobial activity and non-interference with host body mechanism [1]. Derivatives of sulfonamides have specific pharmacological properties and according to that they are used as anti-carbonic anhydrase and hypoglycemic agents [2]. Aromatic sulfonamides have large hydrophobic area [3] and occur in many naturally active compounds, such as insulin-releasing sulfonamides, antithyroid agents, and antitumor drugs [2, 4]. Sulfonamides cause the inhibition of folic acid formation. Due to inhibition of folic acid formation, bacteria can not synthesize tetrahydrofolate and then the biosynthesis of thymine disturbs which causes inhibition of nucleic acid [5]. In human cell, folic acid synthesis does not occur due to absence of folate synthetase. Human may gain the folic acid from diet, hence sulfonamides not affect them [1]. Although, the excessive use of other antibiotics has ceased the utility of different sulfonamides, yet these are still being used due to their broad spectrum activity against various microbes [6]. They are also used as protecting groups of -OH and -NH in organic synthesis [7].

Acetyl cholinesterase (AChE, EC 3.1.1.7) and butyryl cholinesterase (BChE, EC 3.1.1.8) comprise a family of enzymes which include serine hydrolases. The different specificities for substrates and inhibitors for these enzymes are due to the differences in amino acid residues of the active sites of AChE and BChE. The enzyme system is responsible for the termination of acetylcholine at cholinergic synapses. These are key components of cholinergic brain synapses and neuromuscular junctions. The major function of AChE and BChE is to catalyze the hydrolysis of the neurotransmitter acetylcholine and termination of the nerve impulse in cholinergic synapses [8, 9]. Chymotrypsin belongs to the class serine proteases. This class of enzymes is involved in many pathological diseases such as destruction of extracellular matrix of articular cartilage, bone in arthritic joints, tumor invasion and inflammatory infections [10, 11].

Glucuronidase has pharmacological importance due to development of specific inhibitors. In diseases like rheumatoid arthritis, release of enzyme into inflammatory joints synovial fluid, triggers the symptoms of the diseases [12]. D-saccharic acid 1,4-lactone and D-glucurate help in the prevention of pulmonary tumourigenesis, inhibiting the activity of their enzyme *ß*-glucuronidase [13]. For metastasis, the enzyme causes the breakdown of membrane of cancer cells thus they easily proliferate [14].

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In the undertaken investigation, a series of sulfonamides was synthesized by *N*-alkylation of *N*-(2-methoxyphenyl)-4-methylbenzenesulfonamide (**3)** using different alkylating agents. The parent compound *N*-(2-methoxyphenyl)-4-methylbenzenesulfonamide (**3**) was prepared by reacting toluenesulfonyl chloride (**2**) with *o*-anisidine (**1**) at ambient temperature in excellent yield. Further bromination [15] of **3**, yielded *N*-(4,5-dibromo-2-methoxyphenyl)-4-methylbenzenesulfonamide (**6**). Simple stirring in basic media gave the desired parent precursor that was further processed to obtain different derivatives of *N*-alkyl substituted sulfonamides.

Literature survey related to structures of sulfonamides exposed that minor modification in the structure of compound can results change in qualitative and quantitative activity that propped up us to carry out the synthesis of various sulfa drugs (sulfonamides) derivatives derived from 2-anisidine and evaluate their enzyme inhibitory potential.

Results and Discussion

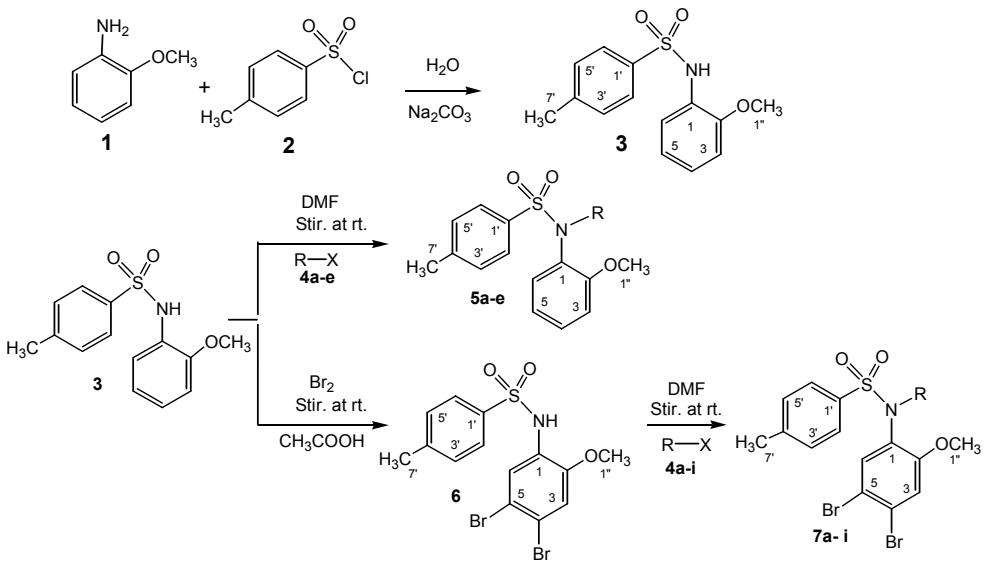
The designed sulfa drugs derived from of 2-anisidine (**1**) were synthesized according to scheme-1. Starting from 2-anisidine (**1**) and 4-methylphenylsulfonyl chloride (**2**), the parent compound **3** was synthesized as an off-white crystalline solid. Its molecular formula, ($C_{14}H_{15}NO_3S$) was established by counting the number of protons in its ^{1}H-NMR spectrum and by its molecular ion peak in EIMS at *m/z* 277. In the EIMS two fragment ion peaks were observed at *m/z* 262 and 246 which represented the loss of methyl and methoxy groups respectively in the molecule. Similarly a distinct peak at *m/z* 213 for a fragment ion after the removal of $–SO_2$ group was also observed. In the aromatic region of its ^{1}H-NMR spectrum, two *ortho* coupled doublet at at δ 7.62 (d, J = 8.5 Hz, 2H, H-2' and H-6') and 7.16 (d, J = 8.0 Hz, 2H, H-3' and H-5') together with a signal for methyl group at 2.33 (s, -CH_3-7') depicted the tolyl (4-methylphenyl) group in the molecule. The signals appearing at 7.50 (dd, J = 1.5, 8.0 Hz, 1H, H-6), 7.00 (ddd, J = 1.5, 8.0, 8.0 Hz, 1H, H-4), 6.86 (ddd, J = 1.5, 8.0, 8.0 Hz, 1H, H-5), and 6.71 (dd, J = 1.0, 8.0 Hz, 1H, H-3) were characteristics of other di-ortho substituted aromatic ring having one substituent as methoxyl group which was obvious by a singlet signal at 3.62 (s, 3H, -OCH_3-1"). On the basis of these evidences the structure of **3** was assigned as *N*-(2-methoxyphenyl)-4-methylbenzenesulfonamide. Similarly on the basis of structural evidences from EI-MS, and ^{1}H-NMR, as described in experimental section, the structures of other derivatives (scheme-1) were elucidated.

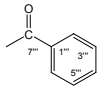
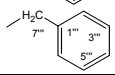
The screening of these sulfonamide derivatives against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), glucuronidase (GUS) and α-chymotrypsin enzymes revealed that **5c**, **6**, **7d**, **7h**, **7g** showed inhibitory potential against AChE and among these only **5c** showed moderate inhibitory effect against this enzyme with IC_{50} value 137.41 ± 0.99 μmoles, relative to eserine, a reference standard having IC_{50} value of 0.04 ± 0.001 μmoles. The compound **5c** was also active against BChE and GUS with IC_{50} values of 127.14 ± 0.98 and 238.32 ± 0.88 μmoles however, against these two enzymes, **6** exhibited relatively greater inhibitory potential with IC_{50} values of 17.25 ± 0.99 and 97.87 ± 0.99 μmoles respectively. Eserine, with IC_{50} value of 0.85 ± 0.001 μmoles, was used as a reference standard for BChE and SAL, with IC_{50} value 0.8 ± 0.002 μmoles, was used as a reference standard for GUS enzyme. Against α-chymotrypsin, **5d**, **7b**, **7d** and **7e** showed the inhibitory effect and among these only **7e** explored a moderate inhibitory potential with IC_{50} value 123.81±0.74 μmoles, relative to chymostatin, a reference standard having IC_{50} value 8.24±0.11 μmoles.

Experimental

General

^{1}H-NMR spectra were recorded in $CDCl_3$ on a Burker Aspect AM-500 MHz spectrometer. Chemical shifts are given in ppm. Mass spectra (EIMS) were measured on Finnigan MAT-112 instrument. The melting points were recorded on a Griffin and George melting point apparatus and were uncorrected. TLC was performed on pre-coated silica gel G-25-UV_{254} plates. Detection was carried out at 254 nm, and by ceric sulphate reagent. Purity was checked on TLC with different solvent systems using ethyl acetate and *n*-hexane giving single spot.



Compd. No.	-R	Compd. No.	-R
5a	—CH_2–CH_2–CH_2–CH_2–CH_3 (1‴ 2‴ 3‴ 4‴ 5‴)	**7c**	—CH_2–CH_2–CH_3 (1‴ 2‴ 3‴)
5b	—CH_2–CH_2–CH_2–CH_3 (1‴ 2‴ 3‴ 4‴)	**7d**	—CH_2–CH_3 (1‴ 2‴)
5c	—CH_2–CH_2–CH_3 (1‴ 2‴ 3‴)	**7e**	—CH_3 (1‴)
5d	—CH_2–CH_3 (1‴ 2‴)	**7f**	—CH_2–CH=C(H_a)(H_b) (1‴ 2‴ 3‴)
5e	—CH_3 (1‴)	**7g**	—CH_2–CH_2–Br (1‴ 2‴)
7a	—CH_2–CH_2–CH_2–CH_2–CH_3 (1‴ 2‴ 3‴ 4‴ 5‴)	**7h**	
7b	—CH_2–CH_2–CH_2–CH_3 (1‴ 2‴ 3‴ 4‴)	**7i**	

Scheme-1: Synthesis of sulfonamides **5a-e** and dibromosulfonamides **7a-i.**

Procedure for the Synthesis of Sulfonamide in Aqueous Medium

A mixture of 4-methylbenzenesulfonyl chloride (10.0 mmol; 1.3 mL) and 2-anisidine (10.0mmol; 2.6 mL) was suspended in 45 mL water. The pH of the suspension was adjusted and was maintained at 9.0 by adding basic aqueous solution of Na_2CO_3 at room temperature. The reaction mixture was stirred and monitored with TLC for completion of reaction. After completion of reaction, concentrated HCl was added gradually to adjust the pH to 2.0. The precipitates were collected by filtration, washed with distilled water and dried to afford the title compound **3**. This product was dissolved in methanol and re-crystallized by slow evaporation of the solvent, to generate colorless needle like crystals of *N*-(2-methoxyphenyl)-4 methylbenzenesulfonamide. Yield was 95%.

Bromination of N-(2-methoxyphenyl)-4-methylbenzenesulfonamide **(3)**

2g of compound **3** was dissolved in 17 mL of glacial acetic acid. The bromine liquid (2.3 mL) was added gradually in the reaction mixture for the bromination of **3**. The reaction mixture was stirred at room temperature for at least 2 hours and the completion of reaction was monitored by TLC. The product was filtered, washed with distilled water and dried to afford light yellow precipitates of *N*-(2-methoxyphenyl)-4 methylbenzenesulfonamide (**6**). Yield was 93%.

General Procedure for the Synthesis of N-alkyl Substituted Sulfonamide in DMF

The calculated amount of compound **3** or **6** (0.1 mmol) was taken in a round bottomed flask (50 mL) and DMF (10 mL) was added to dissolve it followed by the addition of lithium hydride (0.1 mmol) to the mixture therein. The blend was stirred for 30 minutes at ambient temperature and then slowly added the respective alkyl halide to the mixture and it was further stirred for three hours. The progress of reaction was monitored *via* TLC till single spot. The product was precipitated by adding water. It was filtered, washed with water and crystallized from aquous methanol.

Structural Characterization

N-(2-Methoxyphenyl)-4-methylbenzenesulfonamide **(3)**

Off-white crystalline solid, yield 93%. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.62 (d, J = 8.5 Hz, 2H, H-2' and H-6'), 7.50 (dd, J = 1.5, 8.0 Hz, 1H, H-6), 7.16 (d, J = 8.0, 2H, H-3' and H-5') ,7.00 (ddd, J = 1.5, 8.0, 8.0 Hz, 1H, H-4), 6.86 (ddd, J = 1.5, 8.0, 8.0 Hz, 1H, H-5), 6.71 (dd, J = 1.0, 8.0 Hz, 1H, H-3), 3.62 (s, 3H, -OCH_3-1"), 2.33 (s, 3H, CH_3-7'). EIMS: *m/z* 277 $[M]^+$, 262 $[M\text{-}CH_3]^+$, 246 $[M\text{-}OCH_3]^+$, 213 $[M\text{-}SO_2]^+$, 155 $[CH_3\text{-}C_6H_4SO_2]^+$.

N-Pentyl-N-(2-methoxyphenyl)-4-methylbenzene-sulfonamide **(5a)**

White amorphous solid, yield 91%. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.47 (d, J = 8.5 Hz, 2H, H-2' and H-6'), 7.22 (ddd, J= 1.5, 8.0, 8.0 Hz, 1H, H-4), 7.15 (d, J = 8.0, 2H, H-3', H-5') 7.15 (m ,1H ,H-3), 3.32 (s, 3H, -OCH3-1''), 6.85 (ddd, J = 1.5, 7.5 , 7.5 Hz, 1H, H-5), 6.71 (dd, J = 1.0, 8.5 Hz, 1H, H-6), 3.45 (m, 2H, H-1'"), 2.34 (s, 3H, CH_3-7'), 1.67 (m, 2H, H-2'"), 1.33 (m, 2H, H-3'"), 1.33 (m, 2H, H-4'"), 0.84 (t , J = 7.0, 3H, H-5'"). EIMS: *m/z* 347 $[M]^+$, 332 $[M\text{-}CH_3]^+$, 316 $[M\text{-}OCH_3]^+$, 283 $[M\text{-}SO_2]^+$, 155 $[CH_3\text{-}C_6H_4SO_2]^+$.

N-Butyl-N-(2-methoxyphenyl)-4-methylbenzene-sulfonamide **(5b)**

Mustard needle like crystals, yield 87%. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.50 (d, J = 8.0 Hz, 2H, H-2' and H-6'), 7.25 (ddd, J = 1.5, 8.0, 8.0 Hz, 1H, H-4), 7.18(d, J = 8.0, 2H, H-3', H-5') 7.19 (m ,1H, H-3), 3.36 (s, 3H, -OCH3-1"), 6.89 (ddd, J = 1.5, 7.5 , 8.0 Hz, 1H, H-5), 6.76 (dd, J = 1.0, 8.5 Hz, 1H, H-6), 3.50 (m, 2H, H-1'"), 2.37 (s, 3H, CH_3-7'), 1.83 (m, 2H, H-2'"), 1.36 (m, 2H, H-3'"), 0.98 (t , J = 7.5, 3H, H-4'"). EIMS: *m/z* 333 $[M]^+$, 318 $[M\text{-}CH_3]^+$, 302 $[M\text{-}OCH_3]^+$, 269 $[M\text{-}SO_2]^+$, 155 $[CH_3\text{-}C_6H_4SO_2]^+$.

N-Propyl-N-(2-methoxyphenyl)-4-methylbenzene-sulfonamide **(5c)**

Off-white amorphous powder, yield 84%. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.50 (d, J = 8.5 Hz, 2H, H-2' and H-6'), 7.26 (ddd, J = 1.5, 8.0, 8.5 Hz, 1H, H-4), 7.22 (m, 1H, H-6), 7.19 (m ,1H, H-3), 3.36 (s, 3H, -OCH_3-1"), 6.89 (ddd, J = 1.5, 7.5 , 8.5 Hz, 1H, H-5), 6.75 (d, J = 8.0, 2H, H-3', H-5') 3.45 (brt, J = 7.5, 2H, H-1'"), 2.38 (s, 3H, CH3-7'), 1.37 (m, 2H, H-2'"), 0.81 (t , J = 7.5, 3H ,H-3'"). EIMS: *m/z* 319 $[M]^+$, 304 $[M\text{-}CH_3]^+$, 288 $[M\text{-}OCH_3]^+$, 255 $[M\text{-}SO_2]^+$, 155 $[CH_3\text{-}C_6H_4SO_2]^+$.

N-Ethyl-N-(2-methoxyphenyl)-4-methylbenzene-sulfonamide **(5d)**

White amorphous powder, yield 87%. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.56 (d, J = 8.0 Hz, 2H, H-2' and H-6'), 7.27 (ddd, J = 1.5, 7.5, 7.5 Hz, 1H, H-4), 7.24 (m, 1H, H-6), 7.19 (m ,1H, H-3), 6.90 (ddd, J = 1.0, 7.5 , 7.5 Hz, 1H, H-5), 6.77 (d, J = 8.0, 2H, H-3', H-5') 3.60 (m, 2H, H-1'"), 3.39 (s, 3H, -OCH_3-1"), 2.38 (s, 3H, CH3-7'), 1.02 (t , J = 7.0, 3H, H-2'"). EIMS: *m/z* 305 $[M]^+$, 290 $[M\text{-}CH_3]^+$, 274 $[M\text{-}OCH_3]^+$, 241 $[M\text{-}SO_2]^+$, 155 $[CH_3\text{-}C_6H_4SO_2]^+$.

N-Methyl-N-(2-methoxyphenyl)-4-methylbenzene sulfonamide **(5e)**

Rust greasy powder, yield 84%. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.55 (d, J = 8.0 Hz, 2H, H-2' and H-6'), 7.26 (m, 1H, H-6), 7.24 (m, 1H, H-4), 7.21 (m ,1H, H-3), 6.89 (ddd, J = 1.0, 7.5 , 7.5 Hz, 1H, H-5), 6.77 (d, J = 8.0, 2H, H-3', H-5') 3.41 (s, 3H, -OCH_3-1"), 3.18 (s, 3H, H-1'"), 2.39 (s, 3H, CH3-7'). EIMS: *m/z* 291 $[M]^+$, 276 $[M\text{-}CH_3]^+$, 260 $[M\text{-}OCH_3]^+$, 227 $[M\text{-}SO_2]^+$, 155 $[CH_3\text{-}C_6H_4SO_2]^+$.

N-(4,5-Dibromo-2-methoxyphenyl)-4-methylbenzene sulfonamide **(6)**

Light orange colored powder. Yield 93%. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.65 (d, J = 8.0 Hz, 2H, H-2' and H-6'), 7.22(d, J = 8.0, 2H, H-3' and H-5') 2.36 (s, 3H, CH3-7'), 7.74 (s, 1H, H-6), 6.94 (s, 1H, H-3), 3.65 (s, 3H, -OCH_3-1"). EIMS: *m/z* 435 $[M]^+$, 420 $[M\text{-}CH_3]^+$, 404 $[M\text{-}OCH_3]^+$, 371 $[M\text{-}SO_2]^+$, 155 $[CH_3\text{-}C_6H_4SO_2]^+$.

N-Pentyl-N-(4,5-dibromo-2-methoxyphenyl)-4-methylbenzenesulfonamide **(7a)**

White needle like crystals. yield 81%. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.53 (d, J = 8.5 Hz, 2H, H-2' and H-6'), 7.23(d, J =8.0, 2H, H-3' and H-5') 2.39 (s, 3H, CH_3-7'), 7.41 (s, 1H, H-6), 7.02 (s, 1H, H-3), 3.39 (s, 3H, -OCH_3-1''), 3.45 (m, 2H, H-1'''), 1.21 (m, 2H, H-2'''), 1.35 (m, 2H, H-3'''), 1.23 (m, 2H, H-4'''), 0.82 (t , J = 7.0, 3H, H-5'''). EIMS: *m/z* 505 $[M]^+$, 490 $[M\text{-}CH_3]^+$, 474 $[M\text{-}OCH_3]^+$, 441 $[M\text{-}SO_2]^+$, 155 $[CH_3\text{-}C_6H_4SO_2]^+$.

N-Butyl-N-(4,5-dibromo-2-methoxyphenyl)-4-methylbenzenesulfonamide **(7b)**

Mustard needle like crystals, yield 88%.^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.53 (d, J = 8.5 Hz, 2H, H-2' and H-6'), 7.22 (d, J = 8.5, 2H, H-3' and H-5'), 2.39 (s, 3H, CH_3-7'), 7.41 (s, 1H, H-6), 7.08 (s, 1H, H-3), 3.38 (s, 3H, -OCH_3-1''), 3.46 (m, 2H, H-1'''), 1.34 (quintet, J =8.0 2H, H-2'''), 1.26 (quintet, J =7.0, 2H, H-3'''), 0.83 (t , J = 7.5, 3H, H-4'''). EIMS: *m/z* 491 $[M]^+$, 476 $[M\text{-}CH_3]^+$, 460 $[M\text{-}OCH_3]^+$, 427 $[M\text{-}SO_2]^+$, 155 $[CH_3\text{-}C_6H_4SO_2]^+$.

N-Propyl -N-(4,5-dibromo-2-methoxyphenyl)-4-methylbenzenesulfonamide **(7c)**

Rust needle like crystals, yield 83%. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.53 (d, J = 8.5 Hz, 2H, H-2' and H-6'), 7.23(d, J = 8.5, 2H, H-3' and H-5'), 2.34 (s, 3H, CH3-7'), 7.41 (s, 1H, H-6), 7.02 (s, 1H, H-3), 3.34 (s, 3H, -OCH_3-1''), 3.46 (m, 2H, H-1'''), 1.36 (m, 2H, H-2'''), 0.833 (t , J = 7.0, 3H, H-3'''). EIMS: *m/z* 477 $[M]^+$, 462 $[M\text{-}CH_3]^+$, 446 $[M\text{-}OCH_3]^+$, 413 $[M\text{-}SO_2]^+$, 155 $[CH_3\text{-}C_6H_4SO_2]^+$.

N-Ethyl-N-(4,5-dibromo-2-methoxyphenyl)-4-methylbenzenesulfonamide **(7d)**

Rust cubic crystals, yield 89%. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.50 (d, J = 8.5 Hz, 2H, H-2' and H-6'), 7.25(d, J = 8.0, 2H, H-3'and H-5') 2.39 (s, 3H, CH_3-7'), 7.41 (s, 1H, H-6), 7.03 (s, 1H, H-3), 3.41 (s, 3H, -OCH_3-1''), 3.56 (m , 2H, H-1'''), 1.01(t, J =7.5 Hz, H-2'''). EIMS: *m/z* 463$[M]^+$, 448 $[M\text{-}CH_3]^+$, 432$[M\text{-}OCH_3]^+$,399$[M\text{-}SO_2]^+$, 308$[M\text{-}CH_3\text{-}C_6H_4SO_2]$, 155$[CH_3\text{-}C_6H_4SO_2]^+$.

N-Methyl-N-(4,5-dibromo-2-methoxyphenyl)-4-methylbenzenesulfonamide **(7e)**

Mustard needle like crystals, yield 79%.^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.50 (d, J = 8.5 Hz, 2H, H-2' and H-6'), 7.25(d, J = 8.0, 2H, H-3' and H-5'), 2.41 (s, 3H,CH_3-7'), 7.47 (s, 1H, H-6), 7.02 (s, 1H, H-3), 3.41 (s, 3H, -OCH_3-1''), 3.11 (s, 3H , H-1'''). EIMS: *m/z* 449 $[M]^+$, 434 $[M\text{-}CH_3]^+$, 418$[M\text{-}OCH_3]^+$,385 $[M\text{-}SO_2]^+$, 155 $[CH_3\text{-}C_6H_4SO_2]^+$.

N-(Prop-2-en-1-yl)-N-(4,5-dibromo-2-methoxyphenyl)-4-methylbenzenesulfonamide **(7f)**

Rust sticky liquid, yield 68%.^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.55 (d, J = 8.0 Hz, 2H, H-2' and H-6'), 7.24 (d, J = 8.0, 2H, H-3' and H-5') 2.41 (s, 3H, CH_3-7'), 7.41 (s, 1H, H-6), 7.00 (s, 1H, H-3), 3.41 (s, 3H,-OCH3-1''), 4.11 (brd, J = 4.0, 2H, H-1'''), 5.73 (m, 1H, H-2'''), 5.67 (dd , J = 3.0, 6.5 Hz, 1H, H_a), 5.02 (dd , J= 4.5, 11.0 Hz, 1H, H_b). EIMS: *m/z* 475$[M]^+$, 460 $[M\text{-}CH_3]^+$, 444$[M\text{-}OCH_3]^+$,411$[M\text{-}SO_2]^+$, 155$[CH_3\text{-}C_6H_4SO_2]^+$.

N-(2-Bromoethyl)-N-(4,5-dibromo-2-methoxyphenyl)-4-methylbenzenesulfonamide **(7g)**

Mustard needle like crystals. yield 78%.^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.52 (d, J = 8.5 Hz, 2H, H-2' and H-6'), 7.25 (d, J = 8.0, 2H, H-3' and H-5') 2.40 (s, 3H, CH_3-7'), 7.55 (s, 1H, H-6), 7.02 (s, 1H, H-3), 3.36 (s, 3H, -OCH_3-1''), 3.81 (m, 2H, H-1'''), 3.40 (t, J = 7.0, 2H, H-2'''). EIMS: *m/z* 542 $[M]^+$, 527 $[M\text{-}CH_3]^+$, 511 $[M\text{-}OCH_3]^+$, 478 $[M\text{-}SO_2]^+$, 155$[CH_3\text{-}C_6H_4SO_2]^+$

N-Benzyl-N-(4,5-dibromo-2-methoxyphenyl)-4-methylbenzenesulfonamide **(7h)**

White amorphous solid, yield 76%. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.59 (d, J = 8.0 Hz, 2H, H-2' and H-6'), 7.26(d, J = 8.0, 2H, H-3' and H-5') 2.42 (s, 3H, CH_3-7'), 7.24 (s, 1H, H-6), 6.94 (s, 1H, H-3), 3.36 (s, 3H, -OCH_3-1''), 4.68 (br s, 2H, H-7'''), 7.16 (brd, J = 8.0 Hz, 2H , H-2''' and H-6'''), 7.19 (brd, J = 7.5, 2H, H-3''' and H-5'''), 7.21 (brd, J =8.0 Hz ,1H, H-4'''). EIMS: *m/z* 525$[M]^+$, 510 $[M\text{-}CH_3]^+$, 494 $[M\text{-}OCH_3]^+$, 461 $[M\text{-}SO_2]^+$, 155 $[CH_3\text{-}C_6H_4SO_2]^+$.

N-(4,5-Dibromo-2-methoxyphenyl)-N-[(4-methylphenyl)sulfonyl]benzamide **(7i)**

White amorphous crystal solid, yield 96%. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.64 (d, J = 8.5 Hz, 2H, H-2' and H-6'), 7.21(d, J = 8.0, 2H, H-3' and H-5'), 2.49 (s, 3H, CH3-7'), 7.24 (s, 1H, H-6), 7.08 (s, 1H, H-3), 3.85 (s, 3H, -OCH_3-1''), 7.64(dd, J = 1.5, 7.0 Hz, 2H, H-2''' and H-6'''), 7.49(d, J = 8.0, 2H, H-3''',and H-5''') 7.55 (brt , J = 7.5, 1H, H-4'''). EIMS: *m/z* 539 $[M]^+$, 524 $[M\text{-}CH_3]^+$, 508 $[M\text{-}OCH_3]^+$, 475 $[M\text{-}SO_2]^+$, 155 $[CH_3\text{-}C_6H_4SO_2]^+$.

Table-1: Biological Screening of sulfa drugs (sulfonamides) **5a-e** and dibromosulfonamides **7a-i** (n=3, Mean ± SE).

Sample No.	AChE		BChE		Glucuronidase		α-Chymotrypsin	
	(%) at 0.5mM	(IC_{50}) µmoles	(%) at 0.5mM	(IC_{50}) µmoles	(%) at 0.5mM	(IC_{50}) µmoles	(%) at 0.5mM	(IC_{50}) µmoles
3	23.15±0.89	Nil	50.11±0.92	>500	2.15±1.12	Nil	38.80±0.44	Nil
5a	39.67±0.79	Nil	3.85±0.95	Nil	2.35±0.98	Nil	48.25±0.36	Nil
5b	58.04±0.87	>400	12.23±0.91	Nil	9.59±1.22	Nil	45.97±0.25	Nil
5c	99.02±0.84	137.41±0.99	97.62±0.98	127.14±0.98	95.14±1.09	238.32±0.88	43.36±0.65	Nil
5d	42.93±0.92	Nil	26.77±0.89	Nil	2.23±0.95	Nil	80.99±0.45	133.25±0.69
5e	10.65±0.95	Nil	37.85±0.79	Nil	10.23±1.21	Nil	36.90±0.89	Nil
6	93.26±0.77	198.32±0.95	89.23±0.98	17.25±0.99	89.77±0.66	97.87±0.99	56.33±0.23	Nil
7a	52.72±1.01	>500	11.72±1.02	Nil	31.82±0.92	Nil	42.32±0.58	Nil
7b	43.80±0.89	Nil	5.02±0.98	Nil	7.95±0.77	Nil	77.25±0.97	147.21±0.57
7c	47.41±0.77	Nil	17.45±0.75	Nil	53.18±0.84	>500	47.98±0.25	Nil
7d	74.02±0.85	281.51±0.99	10.54±0.87	Nil	40.23±0.45	Nil	81.87±0.57	152.41±0.11
7e	58.59±0.87	>400	44.92±0.45	Nil	37.14±1.07	Nil	93.45±0.47	123.81±0.74
7f	53.59±0.91	>500	22.85±0.48	Nil	17.71±0.93	Nil	57.05±0.31	Nil
7g	73.15±1.07	289.57±1.13	10.21±0.98	Nil	8.78±0.85	Nil	53.27±0.11	Nil
7h	73.80±0.93	299.11±1.09	7.72±1.11	Nil	4.09±0.72	Nil	35.39±0.78	Nil
7i	35.77±0.84	Nil	58.38±0.59	>400	28.78±0.79	Nil	54.28±0.72	Nil
Control	Eserine	0.04±0.001	Eserine	0.85±0.001	SAL	48.4±1.2	Chymostatin	8.24±0.11

Note: IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ–Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA).
AChE = Acetyl cholinesterase.
BChE = Butyryl cholinesterase. SAL = D-Saccharic acid 1,4-lactone

Acetylcholinesterase Assay

The AChE inhibition activity was performed according to the reported method [16] with slight modifications. Total volume of the reaction mixture was 100 µL. It contained 60 µL Na_2HPO_4 buffer with concentration of 50 mM and pH 7.7. Ten µL test compound (0.5 mM well-1) was added, followed by the addition of 10 µL (0.005 unit well-1) enzyme. The contents were mixed and pre-read at 405 nm. Then contents were pre-incubated for 10 min at 37ºC. The reaction was initiated by the addition of 10 µL of 0.5 mM well-1 substrate (acetylthiocholine iodide), followed by the addition of 10 µL DTNB (0.5 mM well-1). After 15 min of incubation at 37ºC, absorbance was measured at 405 nm. Synergy HT (BioTek, USA) 96-well plate reader was used in all experiments. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM well-1) was used as a positive control. The percent inhibition was calculated by the help of following equation.

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

Butyrylcholinesterase Assay

The BchE inhibition activity was performed according to the reported method [16] with slight modifications. Total volume of the reaction mixture was 100 µL containing 60 µL, Na_2HPO_4 buffer, 50 mM and pH 7.7. Ten µL test compound 0.5 mM well-1, followed by the addition of 10 µL (0.5 unit well-1) BchE. The contents were mixed and pre-read at 405 nm and then pre-incubated for 10 mins at 37ºC. The reaction was initiated by the addition of 10 µL of 0.5 mM well-1 substrate (butyrylthiocholine bromide) followed by the addition of 10 µL DTNB, 0.5 mM well-1. After 15 min of incubation at 37 ºC, absorbance was measured at 405 nm. Synergy HT (BioTek, USA) 96-well plate reader was used in all experiments. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM well-1) was used as positive control. The percent inhibition was calculated by the help of following equation.

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

IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

α-*Chymotrypsin Assay*

α-Chymotrypsin inhibition assay was carried out according to the reported method [17, 18]. A total

volume of 100 µl reaction mixture contained 60 µl of 50 mM Tris-HCl buffer (pH 7.6), 10 µl of 0.5 mM test compound and 15 µl (0.9 units) of enzyme (Sigma, USA) prepared in the above buffer. The contents were mixed, preincubated for 15 min at 37 ^{o}C and preread at 410 nm. The reaction was initiated by the addition of 15 µl of 1.3 mM substrate, N-succinyl phenylalanine-*p*-nitroanilide (Sigma, USA). Absorbance was measured at 410 nm using Synergy HT microplate reader after 30-60 min when absorbance values of uninhibited enzyme assay reached 0.7-0.9. The positive and negative controls were included. All experiments were carried out in triplicate. The percent inhibition was calculated by following equation. Inhibition (%) = (Control –Test / Control) x 100. IC_{50} values (concentration at which enzyme inhibition is 50%) were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

β-Glucuronidase Assay

β-Glucuronidase (EC 3.2.1.31, Type B1 from bovine liver, Sigma) activity was determined by measuring the absorbance of *p*-nitophenol formed from the substrate at 405 nm by the method of Collins[19] with minor modifications. Total reaction volume of 100 µl contained 70 µl of 0.1M acetate buffer pH 5, 10 µl of enzyme (12 U), and 0.5 mM test compound. Contents were preincubated for 5 min. Reaction was started by the addition of 10 µl of 0.5 mM *p*-nitrophenyl-*β-D*-glucuronide and incubated at 37^{o}C for 30 min. The reaction was stopped by the addition of 50 µl of 0.2M Na_2CO_3 solution. An appropriate control without test compound and with standard inhibitor was also run in parallel. The plate was read at 405 nm using 96 well plate reader (Synergy HT, BioTek, USA). The percent inhibition was calculated by the help of following equation.

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

IC_{50} values of selected compounds were calculated using EZ–Fit Enzyme Kinetics software (Perella Scientific Inc. Amherst, USA).

Conclusion

It was concluded from our study that the inhibitory activity of the synthesized sulfa drugs is affected by the substituent on the nitrogen atom. The compound *N*-propyl-*N*-(2-methoxyphenyl)-4-methylbenzenesulfonamide (**5c**) and *N*-(4,5-dibromo-2-methoxyphenyl)-4-methylbenzenesulfonamide (**6**) depicted over all better inhibitory potential against the acetylcholinesterase, butyrylcholinesterase and glucuronidase enzymes as compared to other synthesized derivatives perhaps due to suitable chain substitution (*n*-propyl) and un-substitution respectively in these molecules. However, dibromination along with substitution of *N*-methyl group was found to be more suitable entity as in **7e** for the inhibition of α-chymotrysin enzyme.

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