Synthesis, Spectral Characterization and Biological Screening of *N*-Substituted Derivatives of *N*-(1-Hydroxy-2-methylpropan-2-yl)benzenesulfonamide

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In the present study, a series of *N*-substituted derivative of *N*-(1-hydroxy-2-methylpropan-2-yl)benzenesulfonamide was synthesized. Firstly, the reaction of 2-amino-2-methyl propan-1-ol (**1**) with benzenesulfonyl chloride (**2**) yielded *N*-(1-hydroxy-2-methylpropan-2-yl)benzene sulfonamide (**3**) and secondly on treatment with different electrophiles (**4a-i**) in the presence of *N,N*-dimethyl formamide and sodium hydride which act as a base furnished into *N*-substituted derivatives of *N*-(1-hydroxy-2-methylpropan-2-yl)benzenesulfonamide (**5a-i**). All these derivatives along with their parent compounds were characterized by IR, EI-MS and ¹H NMR spectra. These compounds were assayed for their antioxidant activities by using 2,2-diphenyl-1-picrylhydrazil (DPPH) scavenging and other biological activities *via* screening them against acetylcholinesterase, butyrylcholinesterase and lipoxygenase enzymes, however, these showed prominent activity against butyrylcholinesterase enzyme. It is clearly evident from the results that compounds, *N*-(butan-2-yl)-*N*-(1-hydroxy-2-methylpropan-2-yl)benzene sulfonamide (**5e**) and *N*-(allyl)-*N*-(1-hydroxy-2-methylpropan-2-yl)benzenesulfonamide (**5f**) were found to be potent inhibitor having IC₅₀ value of 65.47 ± 0.69 and 79.36 ± 0.92 μmol, respectively, relative to eserine, a reference standard with IC₅₀ value of 0.85 ± 0.001 μmol. These two compounds **5e** and **5f** were also showed good scavenging activity against DPPH.

Key Words: 2-Amino-2-methyl propan-1-ol, Benzenesulfonyl chloride, Sulfonamides, Butyrylcholinesterase enzyme, DPPH.

INTRODUCTION

Sulfonamides are a very important class of compounds in the pharmaceutical industry, being widely used as anticancer, antiinflammatory and antiviral agents¹. They are well known for their antimicrobial activity because of their being less-expensive, less toxic and having superb inhibitory effect against bacterial infections². Nucleophilic substitution reactions occur during sulfonamide synthesis. Amine acts as nucleophile, attacks on sulfonyl chloride³. Sulfonamides are effective inhibitors of carbonic anhydrase⁴ and are wide spectrum antibiotics. More than 30 drugs bearing this functionality have been reported to apparent clinical applications⁵. These compounds are most effective inhibitor against HIV protease. These also take account of anticonvulsants, diuretics, hypoglycemic functionality. Sulfonamides also find their wide use in case of animal husbandry and food additives⁶.

Acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8) consist of a family of

enzymes which comprise serine hydrolases. The different substrates specificities 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 main function of AChE and BChE is to catalyze the hydrolysis of the neurotransmitter acetylcholine and termination of the nerve impulse in cholinergic synapses⁷. It has been found that BChE is present in extensively higher quantities in Alzheimer's plaques than in the normal age related non-dementia of brains. H₁ and H₂ receptor antagonists possess AChE inhibitory activities. Cholinesterase inhibitors raise the amount of acetylcholine accessible for neuronal and neuromuscular transmission through their capacity to reversibly or irreversibly⁸. Hence, the search for new cholinesterase inhibitors is considered a significant and ongoing strategy to introduce new drug candidates for the treatment of Alzheimer's disease and other associated diseases⁹.

Literature review showed that minor modification in the structure of sulfonamides can lead to quantitative as well as qualitative changes in the biological activity. It encouraged us to synthesize various *N*-substituted derivatives of sulfonamides with an objective to search new contenders of drug having significant enhanced activity and can be helpful in controlling many degenerative diseases.

In this work, we report various *N*-substituted derivatives of *N*-(1-hydroxy-2-methylpropan-2-yl)benzenesulfonamide. First, a parent sulfonamide was prepared by reacting benzene-sulfonyl chloride with 2-amino-2-methyl propan-1-ol at room temperature. Simple stirring in basic aqueous media gave the desired compound in excellent yield that was further reacted with different electrophiles to acquire *N*-alkyl substituted sulfonamides.

EXPERIMENTAL

TLC was performed on pre-coated silica gel G-25-UV₂₅₄ 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. The IR spectra were recorded in KBr on a Jasco-320-A spectrophotometer. ¹H NMR spectra were recorded in CDCl₃ on a Bruker spectrometers operating at 400 MHz. Chemical shifts are given in ppm. Mass spectra (EIMS) were measured on Finnigan MAT-112 instrument. EI-MS were recorded in were recorded on a JMS-HX-110 spectrometer, with a data system. The melting points were recorded on a Griffin & George melting point apparatus by open capillary tube and were uncorrected.

Procedure for the synthesis of sulfonamide in aqueous media: The nucleophilic substitution reaction of amine with benzene sulfonyl chloride was carried out as follows: a mixture of benzene sulfonyl chloride (10.0 mmol; 1.27 mL) and 2-amino-2-methyl propan-1-ol (10.0 mmol; 0.95 mL) was suspended in 50 mL water. The pH of the suspension was adjusted and maintained at 9-10 by adding aqueous solution of a base (Na₂CO₃) at room temperature. The reaction mixture was stirred and progress of completion was monitored by thin layer chromatography. After the completion of reaction, concentrated HCl was added gradually to adjust the pH to 2. The precipitates were quenched by filtration, washed with distilled water and dried to afford the title compound **3**. The product was dissolved in methanol and re-crystallized by slow evaporation of the solvent, to generate colourless bead like crystals of *N*-(1-hydroxy-2-methylpropan-2-yl)benzenesulfonamide. Yield 91 %; m.p. 124 °C.

General procedure for the synthesis of *N*-alkyl substituted sulfonamides in DMF: The calculated amount of *N*-(1-hydroxy-2-methylpropan-2-yl)benzenesulfonamide (0.1 mmol) was taken in a round bottomed flask (50 mL), then *N,N*-dimethyl formamide (DMF) (10 mL) was added to dissolve it followed by the addition of sodium hydride (0.1 mmol) to the mixture. The reaction mixture was stirred for 0.5 h at room temperature and then slowly added the alkyl halide to the mixture and the solution was further stirred for 2 h. The progress of reaction was monitored *via* TLC till single spot. Distilled water was added in the flask and the product was received by solvent extraction.

Acetylcholinesterase assay: The AChE inhibition activity was performed according to the reported method¹⁰ with slight modifications. Total volume of the reaction mixture was 100 μL. It contained 60 μL Na₂HPO₄ buffer with concentration of 50 mM and pH 7.7. 10 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 37 °C. 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 37 °C the absorbance was measured at 405 nm using 96-well plate reader Synergy HT, Biotek, USA. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM well⁻¹) was used as a positive control. The per cent inhibition was calculated by the help of following equation

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

IC₅₀ values were calculated using EZ-Fit enzyme kinetics software (Perrella Scientific Inc. Amherst, USA).

Butyrylcholinesterase assay: The BChE inhibition activity was performed according to the reported method¹⁰ with slight modifications. Total volume of the reaction mixture was 100 μL containing 60 μL, Na₂HPO₄ buffer, 50 mM and pH 7.7. 10 μL test compound 0.5 mM well⁻¹ was added followed by the addition of 10 μL (0.5 unit well⁻¹) BChE (Sigma Inc.). The contents were mixed and pre-read at 405 nm and then pre-incubated for 10 min at 37 °C. The reaction was initiated by the addition of 10 μL of 0.5 mM well⁻¹ substrate (butyrylthiocholine chloride). Followed by the addition of 10 μL DTNB, 0.5 mM well⁻¹. After 15 min of incubation at 37 °C, absorbance was measured at 405 nm using 96-well plate reader Synergy HT, Biotek, USA. 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.

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

IC₅₀ values were calculated using EZ-Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA).

Lipoxygenase assay: Lipoxygenase (LOX) activity was assayed according to the method^{11,12} with slight modifications. A total volume of 200 μL lipoxygenase assay mixture contained 150 μL sodium phosphate buffer (100 mM, pH 8.0), 10 μL test compound and 15 μL purified lipoxygenase enzyme (600 units well⁻¹, Sigma Inc.). The contents were mixed and pre-read at 234 nm and preincubated for 10 min at 25 °C. The reaction was initiated by addition of 25 μL substrate solution. The change in absorbance was observed after 6 min at 234 nm using 96-well plate reader Synergy HT, Biotek, USA. All reactions were performed in triplicates. The positive and negative controls were included in the assay. Baicalin (0.5 mM well⁻¹) was used as a positive control. The percentage inhibition (%) was calculated by formula given below.

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

where, Control = total enzyme activity without inhibitor. Test = Activity in the presence of test compound. IC₅₀ values was calculated using EZ-Fit Enzyme Kinetics software (Perrella Scientific Inc. Amherst, USA).

DPPH assay: The stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for the determination of antioxidant activity. Different concentrations of compounds in respective solvents were added at an equal volume (10 µL) to 90 µL of 100 µM methanolic DPPH in a total volume of 100 µL in 96-well plates. The contents were mixed and incubated at 37 °C for 0.5 h. The absorbance was measured at 517 nm using Synergy HT BioTek® USA microplate reader. Quercetin and L-ascorbic acid were used as standard antioxidants. The experiments were carried out in triplicates. IC₅₀ values were calculated using EZ-Fit5 Perrella Scientific Inc. Amherst USA software. The decrease in absorbance indicates increased radical scavenging activity which was determined by the following formula¹³.

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

Statistical analysis: All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2003. Results are presented as mean ± sem.

Spectral characterization of the synthesized compounds

***N*-(1-Hydroxy-2-methylpropan-2-yl)benzenesulfonamide (3):** White amorphous powder, Yield 89 %, m.p. 124 °C. IR (KBr, ν_{max} , cm⁻¹): 3432 (*N*-H, stretching), 3058 (C-H, stretching of aromatic ring), 1623, 1517 (C=C, aromatic stretching), 1341 (-SO₂-NH-, stretching); ¹H NMR (500 MHz, CDCl₃): δ 7.90 (dd, *J* = 8.5, 2.1 Hz, 2H, H-2', H-6'), 7.56 (m, 1H, H-4'), 7.49 (m, 2H, H-3', H-5'), 2.27 (s, 2H, CH₂-1) and 1.14 (s, 6H, CH₃-3, CH₃-4); EIMS: *m/z* 229 [M]⁺, 165 [M-SO₂]⁺, 141 [C₆H₅SO₂]⁺, 73 [M-C₆H₅CH₂NH]⁺.

***N*-(1-Hydroxy-2-methylpropan-2-yl)-*N*-methylbenzenesulfonamide (5a):** Brown greasy liquid, Yield 75 %. IR (KBr, ν_{max} , cm⁻¹): 3052 (C-H, stretching of aromatic ring), 1628, 1523 (C=C, aromatic stretching), 1344 (-SO₂-NH-, stretching); ¹H NMR (500 MHz, CDCl₃): δ 7.85 (dd, *J* = 8.5, 2.1 Hz, 2H, H-2', H-6'), 7.56 (m, 1H, H-4'), 7.49 (m, 2H, H-3', H-5'), 3.72 (s, 2H, CH₂-1), 2.98 (s, 3H, CH₃-1'') and 1.21 (s, 6H, CH₃-3, CH₃-4); EIMS: *m/z* 243 [M]⁺, 179 [M-SO₂]⁺, 102 [M-C₆H₅SO₂]⁺, 141 [C₆H₅SO₂]⁺.

***N*-Ethyl-*N*-(1-hydroxy-2-methylpropan-2-yl)benzenesulfonamide (5b):** Transparent gammy liquid, Yield 70 %. IR (KBr, ν_{max} , cm⁻¹): 3054 (C-H, stretching of aromatic ring), 1621, 1522 (C=C, aromatic stretching), 1342 (-SO₂-NH-, stretching); ¹H NMR (500 MHz, CDCl₃): δ 7.87 (dd, *J* = 8.5, 2.1 Hz, 2H, H-2', H-6'), 7.54 (m, 1H, H-4'), 7.48 (m, 2H, H-3', H-5'), 3.66 (s, 2H, CH₂-1), 3.44 (q, 2H, CH₂-1''), 1.31 (s, 6H, CH₃-3, CH₃-4) and 1.18 (t, *J* = 7.0, 3H, CH₃-2''); EIMS: *m/z* 257 [M]⁺, 193 [M-SO₂]⁺, 116 [M-C₆H₅SO₂]⁺, 141 [C₆H₅SO₂]⁺.

***N*-Butyl-*N*-(1-hydroxy-2-methylpropan-2-yl)benzenesulfonamide (5c):** Transparent greasy liquid, Yield 78 %. IR (KBr, ν_{max} , cm⁻¹): 3046 (C-H, stretching of aromatic ring), 1623, 1525 (C=C, aromatic stretching), 1346 (-SO₂-NH-, stretching); ¹H NMR (500 MHz, CDCl₃): δ 7.91 (dd, *J* = 8.5, 2.1 Hz, 2H, H-2', H-6'), 7.63 (m, 1H, H-4'), 7.55 (m, 2H, H-3', H-5'), 3.65

(s, 2H, CH₂-1), 3.46 (t, *J* = 7.0, 2H, CH₂-1''), 1.75 (m, 2H, CH₂-2''), 1.31 (m, 2H, CH₂-3'') 1.30 (s, 6H, CH₃-3, CH₃-4) and 0.91 (t, *J* = 7.5, 3H, CH₃-4''); EIMS: *m/z* 285 [M]⁺, 221 [M-SO₂]⁺, 144 [M-C₆H₅SO₂]⁺, 141 [C₆H₅SO₂]⁺.

***N*-(1-Hydroxy-2-methylpropan-2-yl)-*N*-pentylbenzenesulfonamide (5d):** Light brown greasy liquid, Yield 63 %. IR (KBr, ν_{max} , cm⁻¹): 3053 (C-H, stretching of aromatic ring), 1625, 1523 (C=C, aromatic stretching), 1343 (-SO₂-NH-, stretching); ¹H NMR (500 MHz, CDCl₃): δ 7.90 (dd, *J* = 8.5, 2.1 Hz, 2H, H-2', H-6'), 7.57 (m, 1H, H-4'), 7.49 (m, 2H, H-3', H-5'), 3.65 (s, 2H, CH₂-1), 3.36 (t, 2H, *J* = 7.0 Hz, CH₂-1''), 1.73 (m, 2H, CH₂-2''), 1.34 (m, 4H, CH₂-3'' CH₂-4'') 1.23 (s, 6H, CH₃-3, CH₃-4) and 0.90 (t, *J* = 7.5, 3H, CH₃-5''); EIMS: *m/z* 299 [M]⁺, 235 [M-SO₂]⁺, 158 [M-C₆H₅SO₂]⁺, 141 [C₆H₅SO₂]⁺.

***N*-(Butan-2-yl)-*N*-(1-hydroxy-2-methylpropan-2-yl)benzenesulfonamide (5e):** Light brown greasy liquid, yield 68 %. IR (KBr, ν_{max} , cm⁻¹): 3053 (C-H, stretching of aromatic ring), 1625, 1523 (C=C, aromatic stretching), 1343 (-SO₂-NH-, stretching); ¹H NMR (500 MHz, CDCl₃): δ 7.92 (dd, *J* = 8.5, 2.1 Hz, 2H, H-2', H-6'), 7.55 (m, 1H, H-4'), 7.48 (m, 2H, H-3', H-5'), 3.66 (s, 2H, CH₂-1), 2.98 (d, *J* = 7.5 Hz, 2H, CH₂-1''), 1.74 (m, 1H, H-2''), 1.13 (s, 6H, CH₃-3, CH₃-4) and 0.91 (d, 6H, *J* = 7.5 Hz, CH₃-3'', CH₃-4''); EIMS: *m/z* 285 [M]⁺, 221 [M-SO₂]⁺, 144 [M-C₆H₅SO₂]⁺, 141 [C₆H₅SO₂]⁺.

***N*-Allyl-*N*-(1-hydroxy-2-methylpropan-2-yl)benzenesulfonamide (5f):** Brown greasy liquid, yield 75 %. IR (KBr, ν_{max} , cm⁻¹): 3052 (C-H, stretching of aromatic ring), 1628, 1523 (C=C, aromatic stretching), 1344 (-SO₂-NH-, stretching); ¹H NMR (500 MHz, CDCl₃): δ 7.85 (dd, *J* = 8.5, 2.1 Hz, 2H, H-2', H-6'), 7.56 (m, 1H, H-4'), 7.49 (m, 2H, H-3', H-5'), 5.17 (dd, *J* = 1.6, 17.3 Hz, H_b-3''), 5.13 (dd, *J* = 1.2, 10 Hz, H_a-3''), 5.61 (m, 1H, H-2''), 3.79 (m, 2H, CH₂-1''), 3.72 (s, 2H, CH₂-1) and 1.21 (s, 6H, CH₃-3, CH₃-4); EIMS: *m/z* 269 [M]⁺, 205 [M-SO₂]⁺, 128 [M-C₆H₅SO₂]⁺, 141 [C₆H₅SO₂]⁺.

***N*-Benzyl-*N*-(1-hydroxy-2-methylpropan-2-yl)benzenesulfonamide (5g):** Gammy liquid, yield 71 %. IR (KBr, ν_{max} , cm⁻¹): 3042 (C-H, stretching of aromatic ring), 1620, 1524 (C=C, aromatic stretching), 1340 (-SO₂-NH-, stretching); ¹H NMR (500 MHz, CDCl₃): δ 7.90 (dd, *J* = 8.5, 2.1 Hz, 2H, H-2', H-6'), 7.61 (m, 1H, H-4'), 7.50 (m, 2H, H-3', H-5'), 7.36-7.42 (m, 5H, H-2'' to H-6''), 3.62 (s, 2H, CH₂-1), 3.56 (s, 2H, CH₂-7'') and 1.32 (s, 6H, CH₃-3, CH₃-4); EIMS: *m/z* 319 [M]⁺, 255 [M-SO₂]⁺, 178 [M-C₆H₅SO₂]⁺, 141 [C₆H₅SO₂]⁺, 91 [C₇H₇]⁺.

***N*-(2-Phenylethyl)-*N*-(1-hydroxy-2-methylpropan-2-yl)benzenesulfonamide (5h):** Gammy liquid, Yield 71 %. IR (KBr, ν_{max} , cm⁻¹): 3038 (C-H, stretching of aromatic ring), 1624, 1521 (C=C, aromatic stretching), 1349 (-SO₂-NH-, stretching); ¹H NMR (500 MHz, CDCl₃): δ 7.98 (dd, *J* = 8.5, 2.1 Hz, 2H, H-2', H-6'), 7.67 (m, 1H, H-4'), 7.55 (m, 2H, H-3', H-5'), 7.27-7.14 (m, 5H, H-2'' to H-6''), 3.58 (t, *J* = 8.0 Hz, 2H, H-8''), 3.15 (t, *J* = 7.0 Hz, 2H, H-7''), 3.70 (s, 2H, CH₂-1) and 1.32 (s, 6H, CH₃-3, CH₃-4); EIMS: *m/z* 333 [M]⁺, 269 [M-SO₂]⁺, 192 [M-C₆H₅SO₂]⁺, 141 [C₆H₅SO₂]⁺, 91 [C₇H₇]⁺.

***N*-(3-Phenylpropyl)-*N*-(1-hydroxy-2-methylpropan-2-yl)benzenesulfonamide (5i):** Yellow liquid, Yield 71 %. IR (KBr, ν_{max} , cm⁻¹): 3028 (C-H, stretching of aromatic ring), 1619, 1528 (C=C, aromatic stretching), 1351 (-SO₂-NH-, stretching);

^1H NMR (500 MHz, CDCl_3): δ 7.97 (dd, $J = 8.5, 2.1$ Hz, 2H, H-2', H-6'), 7.65 (m, 1H, H-4'), 7.50 (m, 2H, H-3', H-5'), 7.28–7.15 (m, 5H, H-2'' to H-6''), 3.33 (t, $J = 7.0$ Hz, 2H, H-9''), 2.80 (t, $J = 7.5$ Hz, 2H, H-7''), 2.15 (t, $J = 7.5$ Hz, 2H, H-8''), 3.78 (s, 2H, CH_2 -1) and 1.35 (s, 6H, CH_3 -3, CH_3 -4); EIMS: m/z 347 $[\text{M}]^+$, 283 $[\text{M}-\text{SO}_2]^+$, 206 $[\text{M}-\text{C}_6\text{H}_5\text{SO}_2]^+$, 141 $[\text{C}_6\text{H}_5\text{SO}_2]^+$, 91 $[\text{C}_7\text{H}_7]^+$.

RESULTS AND DISCUSSION

The objective of our research work was to synthesize *N*-substituted derivatives of *N*-(1-hydroxy-2-methyl propan-2-yl)benzenesulfonamide and to screen out their enzymatic activities. Parent compound **3** was synthesized by the reaction of 2-amino-2-methyl propan-1-ol (**1**) with benzene sulfonyl chloride (**2**) in aqueous media¹⁴. Further, a series of *N*-substituted derivatives (**Scheme-I**) were brought about by treating compound **3** with different electrophiles, **4a-i**, in the presence of *N,N*-dimethyl formamide and NaH which acts as a base. Reactants completely converted into products by simple stirring after 1–3 h. Precipitates were quenched by treating

with cold water and recrystallized by using methanol. The parent compound **3** was synthesized as white amorphous powder. The molecular formula $\text{C}_{10}\text{H}_{15}\text{NO}_3\text{S}$ was established by HR-MS showing molecular ion peak at m/z 229.343 (calcd. (%) for $\text{C}_{14}\text{H}_{15}\text{NO}_2\text{S}$, 229.340). The IR spectrum revealed the presence of a sulfonyl group ($1338, 1447\text{ cm}^{-1}$) and $-\text{NH}-$ (3100 cm^{-1}) group in the molecule. The EI-MS gave a distinct peak at m/z 165 after the removal $-\text{SO}_2$ group and further a peak was observed at m/z 141 which showed the presence of benzenesulfonyl group in the molecule. In the aromatic region of the ^1H NMR spectrum signals appeared at δ 7.90 (dd, $J = 8.5, 2.1$ Hz, 2H, H-2', H-6'), 7.56 (m, 1H, H-4') and 7.49 (m, 2H, H-3', H-5'), due to downfield shift, protons were assigned to the mono substituted sulfonyl ring. In the aliphatic region of the ^1H NMR spectrum, signals appeared at δ 2.27 (s, 2H, CH_2 -1) and 1.14 (s, 6H, CH_3 -3, CH_3 -4); indicated the presence of one methylene and two methyl groups in the molecule. On the basis of above evidences the structure of compound **3** was assigned as *N*-(1-hydroxy-2-methylpropan-2-yl)benzene sulfonamide. The structure was also confirmed from its crystal

Compound	R	Compound	R
5a	$-\text{CH}_3$ 1''	5f	$\begin{array}{c} \text{H} \quad \quad \text{H}_a \\ \diagdown \quad \diagup \\ \text{C}=\text{C} \\ \diagup \quad \diagdown \\ -\text{CH}_2 \quad 2'' \quad 3'' \quad \text{H}_b \\ 1'' \end{array}$
5b	$-\text{CH}_2-\text{CH}_3$ 1'' 2''	5g	$\begin{array}{c} 7'' \\ \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_4 \\ \\ 4'' \end{array}$
5c	$-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$ 1'' 2'' 3'' 4''	5h	$\begin{array}{c} 7'' \\ \\ \text{CH}_2-8'' \\ \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_4 \\ \\ 4'' \end{array}$
5d	$-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$ 1'' 2'' 3'' 4'' 5''	5i	$\begin{array}{c} 7'' \quad 9'' \\ \quad \\ \text{CH}_2-\text{CH}_2 \\ \quad \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_4 \\ \\ 4'' \end{array}$
5e	$\begin{array}{c} 3'' \\ \\ -\text{CH}_2-\text{CH} \\ \quad \\ 1'' \quad 2'' \quad \text{CH}_3 \end{array}$	—	—

Scheme-I: Outline for the synthesis of *N*-substituted derivatives of *N*-(1-hydroxy-2-methyl propan-2-yl)benzenesulfonamide

TABLE-1
EVALUATION OF BIOLOGICAL ACTIVITIES OF *N*-SUBSTITUTED DERIVATIVES OF
N-(1-HYDROXY-2-METHYL PROPAN-2-YL)BENZENESULFONAMIDE

Sample No.	DPPH		AChE		BChE		LOX	
	Inhibition (%) at 0.5 mM	IC ₅₀ (μmol)	Inhibition (%) at 0.5 mM	IC ₅₀ (μmol)	Inhibition (%) at 0.5 mM	IC ₅₀ (μmol)	Inhibition (%) at 0.5 mM	IC ₅₀ (μmol)
3	92.67±0.59	89.21±0.89	35.29±0.47	Nil	60.23±0.58	165.36±0.85	13.41±0.66	Nil
5a	91.63±0.78	97.43±0.98	20.41±0.95	Nil	69.23±0.87	159.36±0.91	38.20±0.45	Nil
5b	74.42±0.75	103.12±0.91	50.02±0.83	<500	71.60±0.45	139.71±0.85	44.91±1.06	Nil
5c	84.58±0.91	75.57±0.79	23.13±1.01	Nil	71.03±1.02	149.99±0.89	36.41±0.92	Nil
5d	83.83±0.84	81.78±0.87	23.45±0.93	Nil	79.87±1.11	111.54±0.92	41.44±0.72	<500
5e	63.23±0.73	59.19±0.95	37.02±0.89	Nil	87.92±0.98	65.47±0.69	38.08±0.76	Nil
5f	71.57±1.02	61.88±0.97	20.85±0.77	Nil	65.23±0.82	79.36±0.92	26.59±0.84	Nil
5g	83.61±0.78	111.41±0.92	32.41±0.95	Nil	62.22±0.83	179.33±0.97	48.21±0.44	<500
5h	61.60±0.72	154.43±0.91	29.34±0.91	Nil	71.23±0.87	185.36±0.98	48.20±0.45	<500
5i	73.80±0.74	101.73±0.77	36.41±0.85	Nil	69.85±1.13	131.51±0.91	31.41±0.70	Nil
Control	Quercetin	16.96±0.14	Eserine	0.04±0.001	Eserine	0.85±0.001	Baicalein	22.4±1.3

Note: IC₅₀ values (concentration at which there is 50 % enzyme inhibition) of compounds were calculated using EZ-Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA). AChE = Acetyl cholinesterase. BChE = Butyrylcholinesterase. LOX = Lipoxigenase. DPPH = 1,1-diphenyl-2-picrylhydrazyl radical.

data¹⁵. During the course of this study we obtained some *N*-substituted derivatives of sulfonamides in good yield and others moderate to low. On the basis of data from IR, EIMS and ¹H NMR, the structures of *N*-substituted derivatives of *N*-(1-hydroxy-2-methyl propan-2-yl)benzenesulfonamides were elucidated as described in experimental section.

Enzyme inhibition activity: The screening of these synthesized compounds against acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and lipoxigenase enzymes (LOX) revealed that they were exhibited good inhibitory potential against butyrylcholinesterase as it was evident from their IC₅₀ values but remained inactive against acetylcholinesterase and lipoxigenase enzymes. The results are depicted in Table-1. It is clearly evident from results that compounds *N*-(butan-2-yl)-*N*-(1-hydroxy-2-methylpropan-2-yl)benzene sulfonamide (**5e**) and *N*-(allyl)-*N*-(1-hydroxy-2-methylpropan-2-yl)benzenesulfonamide (**5f**) was found to be potent inhibitor having IC₅₀ value of 65.47 ± 0.69 and 79.36 ± 0.92 μmol, relative to eserine, a reference standard with IC₅₀ value of 0.85 ± 0.001 μmol, probably due to the *N*-substitution of isobutyl and allyl groups in the molecules, respectively. DPPH is a stable free radical at room temperature. DPPH radical is scavenged by antioxidants through the donation of a proton and form reduced DPPH. The colour changes from violet to yellow after reduction of DPPH and it can be quantified by decrease of absorbance at wavelength 517 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition. The colour change from violet to yellow and fall in absorbance of the stable radical DPPH was measured for three different concentrations of samples and the results are shown in Table-1. These results showed that the compounds **5e** and **5f** also showed good scavenging activity against DPPH. The IC₅₀ value for these compounds 59.19 ± 0.95 and 61.88 ± 0.97 μmol, relative to quercetin, a reference standard with IC₅₀ value of 16.96 ± 0.14 μmol, was calculated from the curves plotted. IC₅₀ is the concentration of fraction causing 50 % inhibition of absorbance and lower its value means greater antioxidant activity of the fraction.

Conclusion

The proposed structure of the synthesized compound is well supported by spectroscopic data. From the enzyme inhibition data (Table-1), it may be concluded that the two compounds **5e** and **5f** have talented activity against butyrylcholinesterase enzyme with IC₅₀ value 65.47 ± 0.69 and 79.36 ± 0.92 μmol, respectively but all other compounds showed moderate activity. All the synthesized compounds also showed good scavenging activity against DPPH but all were remained inactive against acetylcholinesterase and lipoxigenase enzymes. Hence on the basis of aforesaid results, these synthesized derivatives provide an overall indispensable basis to introduce new drug candidates for the treatment of Alzheimer's disease and other associated diseases. Due to having antioxidant properties, these can be helpful in controlling many degenerative diseases.

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