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Scientific paper

Design, Synthesis, Biological Evaluation and Molecular Docking Studies of Some New Sulfonamides Possessing 1,4-Benzodioxane Nucleus

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Abstract

In the current research work we have reported a series of *N*-aryl-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamides **3** and their *N*-substituted derivatives **6** and **7**, obtained from **3** with benzyl chloride and ethyl iodide, respectively. The synthesis was accomplished as a multistep sequence. The structural confirmations were established by ^{1}H NMR, IR and EIMS spectral techniques. Butyrylcholinesterase (BChE), acetylcholinesterase (AChE) and lipoxygenase (LOX) enzymes were used in this study. It was observed that most of the compounds prepared exhibit a moderate activity against BChE and AChE but promisingly good activity against lipoxygenase. Among the parent sulfonamides **3a**, **3b**, **3c** and **3e** showed the proficient antimicrobial activities, while from the derivatives **6a**, **6c**, **7a**, **7b** and **7c** were found active against the selected panel of bacterial and fungal species. Hemolytic activity was also conducted to check their therapeutic utility. All the compounds were computationally docked against LOX, BChE and AChE enzymes.

Keywords: 2,3-Dihydrobenzo[1,4]dioxine-6-sulfonyl chloride; lipoxygenase enzyme; antimicrobial and hemolytic activities; molecular docking.

1. Introduction

The very first prepared antimicrobial agents were sulfonamides, which are bacteriostatic agents and active against Gram negative and Gram positive bacteria. Basically, sulfonamides contain a benzene ring with sulfonyl and amino group at *para* position and have general formula Ar SO_2NHR. The nature of Arl and R varies from simple hydrogen to aliphatic carboaromatic, heterocyclic, OH, NH_2 or sugar scaffolds. SAR studies revealed that the liberated NH_2 group of sulfonamides is responsible for their activity. Low activity was observed in the case of attached substituents at *ortho* and *meta* positions.[1–5] Sulfonamides restrain folic acid in bacteria which facilitates the production of bacterial DNA and RNA. Dihydropteroic acid is precursor of folic acid. Dihydropteroate synthase is responsible for the transformation of dihydropteroate diphosphate and *P*-aminobenzoic acid into dihydropteroic

acid; on the other hand, sulfonamides hinder this enzyme.[6,7] These sulfonamide drugs can be classified as oral (absorbed and unabsorbed) and topical. Depending on the action absorbable sulfonamides can be short-, intermediate- and long-acting. Their absorption takes place through stomach and small intestine.[8] Commonly these sulfa drugs are synthesized by treating substituted sulfonyl chloride with suitable amine compounds. Sulfonamides are used to cure membrane infections, enteritis, ulcerative colitis and for skin treatments. In agriculture sulfonamides have antiherbicidal and antifungal uses. They have wide applications as antiobesity,[9] anticancer, antiviral,[3] protease inhibitors,[10] diuretic,[11,12] hypoglycemic,[13] antithyroid,[14] antitumor,[15–17] anti-neuropathic pain,[18] antibacterial,[19,20] cyclooxygenase[21] and carbonic anhydrase inhibitory agents.[22]

Compounds containing a 1,4-benzodioxane skeleton display wider range of biological activities,[23] such as anticancer,[24,25] immunosuppressive,[26] antiinflammatory,[27] antibacterial[28] and some others.[29,30]

Additionally, interaction modes of the synthesized compounds were examined by molecular docking approach. The purpose of docking methodologies was to forecast the ligand and target complex and to align the molecular database (designed inhibitors) on the basis of binding affinity to that of target. The MOE-Dock was used for docking of all the synthesized inhibitors with the binding site of target enzymes. The eventual objective of molecular docking was to get ligands with better characteristics displaying good inhibition potential.[31] This research work is a productive effort to bring in close proximity the pharmacologically significant moieties. Keeping continuity in connection with the research work on sulfonamide molecules,[32] the different *N*-substituted sulfonamides derived from 1,4-benzodioxine-6-sulfonyl chloride were synthesized. It was aimed that the new drugs bearing sulfamoyl functionality would have striking activity and be able to cope with the challenges.

2. Experimental

2. 1. General

2,3-Dihydrobenzo[1,4]dioxine-6-sulfonyl chloride, different aryl amines and the other electrophilic reagents were purchased from local suppliers of chemicals. All the solvents were used after distillation and purification. Griffin and George melting point instrument was employed to record melting points of the target compounds. The open capillary tube was used and melting points are reported uncorrected. The TLC was utilized to check the purity and reaction progress. TLC plates were finished from pre-coated silica gel G-25-UV_{254}. The gradient solvent systems of ethyl acetate ($C_4H_8O_2$) and *n*-hexane (C_6H_{14}) were applied. The detection was carried out at 254 nm and TLC plates were developed by ceric sulphate reagent. On a Jasco-320-A spectrophotometer the IR spectra were recorded. The pellet of a sample was made with KBr and functional group stretchings are reported in wave numbers (cm^{-1}). NMR spectra were recorded on Bruker spectrometers. The deutrated methanol and chloroform were used as the solvents. The operating frequencies thus utilized were 300 and 400 MHz and chemical shifts are given in ppm. On a JMS-HX-110 spectrometer the mass spectra (EIMS) were measured with a data system.

2. 2. Synthesis

2. 2. 1. General Procedure for the Synthesis of *N*-Aryl-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamides (3a–e) in Aqueous Medium

0.85 mmol of various substituted aryl amines **2a–e** were dispensed in 50 mL of distilled water contained in a 250 mL round-bottom flask. The pH of the reacting medium was maintained at 9.0–10.0. The aqueous solution of Na_2CO_3 was added drop wise at 25 °C. Subsequently 0.85 mmol (0.20 g) of 2,3-dihydrobenzo[1,4]dioxine-6-sulfonyl chloride (**1**) was added slowly to the reaction mass over 10–15 min. The reaction mixture was kept on stirring at RT till the TLC confirmation indicated the completion of the reaction. The workup was done by slowly adding conc. aq. HCl (2 mL) and dropping pH to 2.0. The title compounds **3a–e** were collected as solid precipitates on filtration and washed with immense volume of distilled water.

***N*-(2,3-Dimethylphenyl)-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide (3a)**

White solid; yield: 80% (217.7 mg); m.p. 102–104 °C; molecular formula: $C_{16}H_{17}NO_4S$; molecular weight: 319 g/mol; HR-MS: $[M]^+$ 319.3772 (Calcd. for $C_{16}H_{17}NO_4S$; 319.3854); IR (KBr, cm^{-1}): ν_{max} stretching: 3419 (N-H), 3026 (C-H Ar ring), 2914 (CH_2), 1613 (C=C Ar ring), 1325 (SO_2),1125 (C-O-C of ether); ^{1}H NMR (400 MHz,C-DCl_3): δ (ppm) 1.99 (s, 3H, CH_3-2"), 2.27 (s, 3H, CH_3-1"), 4.24–4.27 (m, 4H, CH_2-2, CH_2-3), 6.73 (brd, J = 7.6 Hz, 1H, H-4'), 6.78 (t, J = 7.6 Hz, 1H, H-5'), 6.80 (d, J = 8.0 Hz, 1H, H-6'), 6.95 (d, J = 8.4 Hz, 1H, H-8), 7.18 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H, H-7), 7.25 (d, J = 2.0 Hz, 1H, H-5); EIMS: *m/z* 319 $[M]^+$, 255 $[M\text{-}SO_2]^+$, 214 $[C_6H_3C_2H_4O\text{-}_2SO_2NH]^+$, 199 $[C_6H_3C_2H_4O_2SO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 105 $[C_6H_3(CH_3)_2]^+$, 90 $[C_6H_3CH_3]^+$, 79 $[C_4H(CH_3)_2]^+$, 75 $[C_6H_3]^+$, 64 $[C_4HCH_3]^+$.

***N*-(2,4-Dimethylphenyl)-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide (3b)**

White solid; yield: 89% (242.2 mg); m.p. 108–110 °C; molecular formula: $C_{16}H_{17}NO_4S$; molecular weight: 319 g/mol; HR-MS: $[M]^+$ 319.3772 (Calcd. for $C_{16}H_{17}NO_4S$; 319.3854); IR (KBr, cm^{-1}): ν_{max} stretching: 3435 (N-H), 3021 (C-H Ar ring), 2917 (-CH_2-), 1615 (C=C Ar ring), 1324 (-SO_2-), 1140 (C-O-C of ether); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.99 (s, 3H, CH_3-2"), 2.24 (s, 3H, CH_3-1"), 4.24–4.26 (m, 4H, CH_2-2, CH_2-3), 6.80 (s, 1H, H-3'),

6.84 (d, J = 8.0 Hz, 1H, H-5'), 6.92 (d, J = 8.4 Hz, 1H, H-6'), 7.12 (d, J = 8.0 Hz, 1H, H-8), 7.17 (dd, J_1= 8.4 Hz, J_2 = 2.0 Hz, 1H, H-7), 7.26 (d, J = 2.0 Hz, 1H, H-5); EIMS: *m/z* 319 $[M]^+$, 255 $[M\text{-}SO_2]^+$, 214 $[C_6H_3C_2H_4O_2SO_2NH]^+$, 199 $[C_6H_3C_2H_4O_2SO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 105 $[C_6H_3(CH_3)_2]^+$, 90 $[C_6H_3CH_3]^+$, 79 $[C_4H(CH_3)_2]^+$, 75 $[C_6H_3]^+$, 64 $[C_4HCH_3]^+$.

_N_-(2,5-Dimethylphenyl)-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide (3c)

White solid; yield: 82% (223.1 mg); m.p. 124–126 °C; molecular formula: $C_{16}H_{17}NO_4S$; molecular weight: 319 g/mol; HR-MS: $[M]^+$ 319.3772 (Calcd. for $C_{16}H_{17}NO_4S$; 319.3854); IR (KBr, cm^{-1}): ν_{max} stretching: 3415 (N-H), 3024 (C-H Ar ring), 2912 (-CH_2-), 1619 (C=C Ar ring), 1325 (-SO_2-), 1122 (C-O-C of ether); ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 1.96 (s, 3H, CH_3-2"), 2.20 (s, 3H, CH_3-1"), 4.22–4.30 (m, 4H, CH_2-2, CH_2-3), 6.85 (d, J = 7.6 Hz, 1H, H-4'), 6.89 (d, J = 7.5 Hz, 1H, H-3'), 6.91 (s, 1H, H-6'), 6.99 (d, J = 8.1 Hz, 1H, H-8), 7.09 (d, J = 2.1 Hz, 1H, H-5), 7.13 (dd, J_1 = 7.8 Hz, J_2 = 2.1 Hz, 1H, H-7); EIMS: *m/z* 319 $[M]^+$, 255 $[M\text{-}SO_2]^+$, 214 $[C_6H_3C_2H_4O_2SO_2NH]^+$, 199 $[C_6H_3C_2H_4O_2SO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 105 $[C_6H_3(CH_3)_2]^+$, 90 $[C_6H_3CH_3]^+$, 79 $[C_4H(CH_3)_2]^+$, 75 $[C_6H_3]^+$, 64 $[C_4HCH_3]^+$.

_N_-(2,6-Dimethylphenyl)-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide (3d)

White solid; yield: 71% (193.2 mg); m.p. 151–153 °C; molecular formula: $C_{16}H_{17}NO_4S$; molecular weight: 319 g/mol; HR-MS: $[M]^+$ 319.3772 (Calcd. for $C_{16}H_{17}NO_4S$; 319.3854); IR (KBr, cm^{-1}): ν_{max} stretching: 3410 (N-H), 3045 (C-H Ar ring), 2909 (-CH_2-), 1614 (C=C), 1326 (-SO_2-), 1125 (C-O-C of ether); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.02 (s, 6H, CH_3-1", CH_3-2"), 4.25–4.30 (m, 4H, CH_2-2, CH_2-3), 6.93 (brd, J = 7.8 Hz, 1H, H-4'), 7.01 (d, J = 8.4 Hz, 2H, H-3', H-5'), 7.03 (d, J = 8.4 Hz, 1H, H-8), 7.38 (d, J = 2.4 Hz, 1H, H-5), 7.48 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H, H-7); EIMS: *m/z* 319 $[M]^+$, 255 $[M\text{-}SO_2]^+$, 214 $[C_6H_3C_2H_4O_2SO_2NH]^+$, 199 $[C_6H_3C_2H_4O_2SO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 105 $[C_6H_3(CH_3)_2]^+$, 90 $[C_6H_3CH_3]^+$, 79 $[C_4H(CH_3)_2]^+$, 75 $[C_6H_3]^+$, 64 $[C_4HCH_3]^+$.

_N_-(3,4-Dimethylphenyl)-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide (3e)

White solid; yield: 86% (234.0 mg); m.p. 124–126 °C; molecular formula: $C_{16}H_{17}NO_4S$; molecular weight: 319 g/mol; HR-MS: $[M]^+$ 319.3772 (Calcd. for $C_{16}H_{17}NO_4S$; 319.3854); IR (KBr, cm^{-1}): ν_{max} stretching: 3422 (N-H), 3018 (C-H Ar ring), 2919 (-CH_2-), 1615 (C=C Ar ring), 1323 (-SO_2), 1118 (C-O-C of ether); ^{1}H NMR (400 MHz,$CDCl_3$): δ (ppm) 2.15 (s, 6H, CH_3-1", CH_3-2"), 4.23–4.25 (m, 4H, CH_2-2, CH_2-3), 6.76 (d, J = 7.6 Hz, 1H, H-5'), 6.81 (s, 1H, H-2'), 6.84 (d, J = 8.0 Hz, 1H, H-6'), 6.96 (d, J = 8.0 Hz, 1H, H-8), 7.22 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H, H-7), 7.28 (d, J = 2.0 Hz, 1H, H-5); EIMS: *m/z* 319 $[M]^+$, 255 $[M\text{-}SO_2]^+$, 214 $[C_6H_3C_2H_4O_2SO_2NH]^+$, 199 $[C_6H_3C_2H_4O_2SO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 105 $[C_6H_3(CH_3)_2]^+$, 90 $[C_6H_3CH_3]^+$, 79 $[C_4H(CH_3)_2]^+$, 75 $[C_6H_3]^+$, 64 $[C_4HCH_3]^+$.

2. 2. 2. General Procedure for the Synthesis of Compounds 6a–e and 7a–e

0.40 mmol (0.01 g) of lithum hydride was added to a solution containing of 0.1 g of compounds **3a–e** in 25 mL of aprotic solvent (DMF) and kept in round-bottom flask at 25 °C. On complete addition, the reaction mixture was stirred for half an hour. The benzyl chloride (**4**) and ethyl iodide (**5**) were added into the reaction mixture to establish **6a–e** and **7a–e** series, respectively. The stirring lasted for 1–2 h. The monitoring of the reaction completion was done by TLC. The reaction contents were quenched with cold distilled water after ensuring the complete conversion of the reactants. The corresponding *N*-benzyl/ethyl derivatives of *N*-aryl-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamides **6a–e** and **7a–e** were obtained as solid precipitates on filtration. The subsequent washing with distilled water and drying yielded our target molecules. The greasy and sticky compounds were isolated through solvent extraction by using chloroform.

***N*-Benzyl-*N*-(2,3-dimethylphenyl)-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide (6a)**

Yellow sticky solid; yield: 86% (110.3 mg); molecular formula: $C_{23}H_{23}NO_4S$; molecular weight: 409 g/mol; HR-MS: $[M]^+$ 409.4991 (Calcd. for $C_{23}H_{23}NO_4S$; 409.5187); IR (KBr, cm^{-1}): ν_{max} stretching: 3416 (N-H), 3015 (C-H Ar ring), 2918 (-CH_2-), 1617 (C=C Ar ring), 1329 (-SO_2-), 1135 (C-O-C of ether); ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 2.00 (s, 3H, CH_3-2'"), 2.06 (s, 3H, CH_3-1'"), 4.25–4.29 (m, 4H, CH_2-2, CH_2-3), 4.63 (s, 2H, CH_2-7"), 6.81 (d, J = 7.6 Hz, 1H, H-4'), 6.87–6.95 (m, 5H, H-2" to H-6"), 7.02 (d, J = 7.2 Hz, 1H, H-6'), 7.10 (t, J = 7.6 Hz, 1H, H-5'), 7.31 (d, J = 8.4 Hz, 1H, H-8), 7.34 (d, J = 2.0 Hz, 1H, H-5), 7.38 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H, H-7); EIMS: *m/z* 409 $[M]^+$, 345 $[M\text{-}SO_2]^+$, 304 $[C_6H_3C_2H_4O_2SO_2NCH_2C_6H_5]^+$, 199 $[C_6H_3C_2H_4O_2SO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$,105 $[C_6H_3(CH_3)_2]^+$, 91 $[C_7H_7]^+$, 90 $[C_6H_3CH_3]^+$, 79 $[C_4H(CH_3)_2]^+$, 75 $[C_6H_3]^+$, 65 $[C_5H_5]^+$, 64 $[C_4HCH_3]^+$.

***N*-Benzyl-*N*-(2,4-dimethylphenyl)-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide (6b)**

Light grey sticky solid; yield: 78% (100.0 mg); molecular formula: $C_{23}H_{23}NO_4S$; molecular weight: 409 g/mol; HR-MS: $[M]^+$ 409.4991 (Calcd. for $C_{23}H_{23}NO_4S$; 409.5187); IR (KBr, cm^{-1}): ν_{max} stretching: 3423 (N-H), 3067 (C-H Ar ring), 2932 (-CH_2-), 1617 (C=C Ar ring), 1326 (-SO_2), 1145 (C-O-C of ether); ^{1}H NMR (300 MHz,CD_3OD): δ (ppm) 1.90 (s, 3H, CH_3-2'"), 2.22 (s, 3H,

CH_3-1'''), 4.19 (s, 2H, CH_2-7''), 4.32–4.34 (m, 4H, CH_2-2, CH_2-3), 6.55 (d, *J* = 8.1 Hz, 1H, H-5'), 6.86 (d, *J* = 7.8 Hz, 1H, H-6'), 6.90 (s, 1H, H-3'), 6.92–7.01 (m, 5H, H-2'' to H-6''), 7.10 (d, *J* = 8.1 Hz, 1H, H-8), 7.17 (d, *J* = 2.1 Hz, 1H, H-5), 7.19 (dd, J_1 = 8.1 Hz, J_2 = 2.1Hz, 1H, H-7); EIMS: *m/z* 409 $[M]^+$, 345 $[M\text{-}SO_2]^+$, 304 $[C_6H_3C_2H_4O_2SO_2N\text{-}CH_2C_6H_5]^+$, 199 $[C_6H_3C_2H_4O_2SO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$,105 $[C_6H_3(CH_3)_2]^+$, 91 $[C_7H_7]^+$, 90 $[C_6H_3CH_3]^+$, 79 $[C_4H(CH_3)_2]^+$, 75 $[C_6H_3]^+$, 65 $[C_5H_5]^+$, 64 $[C_4HCH_3]^+$.

N-Benzyl-N-(2,5-dimethylphenyl)-2,3-dihydrobenzo [1,4]dioxine-6-sulfonamide (6c)

Yellow sticky solid; yield: 76% (97.4 mg); molecular formula: $C_{23}H_{23}NO_4S$; molecular weight: 409 g/mol; HR-MS: $[M]^+$ 409.4991 (Calcd. for $C_{23}H_{23}NO_4S$; 409.5187); IR (KBr, cm^{-1}): ν_{max} stretching: 3431 (N-H), 3041 (C-H Ar ring), 2922 (-CH_2-), 1612 (C=C Ar ring), 1328 (-SO_2-), 1131 (C-O-C of ether); ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 1.89 (s, 3H, CH_3-2'''), 2.14 (s, 3H, CH_3-1'''), 4.30–4.36 (m, 4H, CH_2-2, CH_2-3), 4.56 (s, 2H, CH_2-7''), 6.95 (dd, J_1 = 8.4 Hz, J_2 = 2.1 Hz, 1H, H-4'), 7.00 (d, *J* = 8.4 Hz, 1H, H-3'), 7.03 (s, 1H, H-6'), 7.09–7.20 (m, 5H, H-2'' to H-6''), 7.56 (d, *J* = 8.4 Hz, 1H, H-8), 7.62 (d, *J* = 2.1 Hz, 1H, H-5), 7.71 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H, H-7); EIMS: *m/z* 409 $[M]^+$, 345 $[M\text{-}SO_2]^+$, 304 $[C_6H_3C_2H_4O_2SO_2N\text{-}CH_2C_6H_5]^+$, 199 $[C_6H_3C_2H_4O_2SO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$,105 $[C_6H_3(CH_3)_2]^+$, 91 $[C_7H_7]^+$, 90 $[C_6H_3CH_3]^+$, 79 $[C_4H(CH_3)_2]^+$, 75 $[C_6H_3]^+$, 65 $[C_5H_5]^+$, 64 $[C_4HCH_3]^+$.

N-Benzyl-N-(2,6-dimethylphenyl)-2,3-dihydrobenzo [1,4]dioxine-6-sulfonamide (6d)

Creamy white crystalline solid; yield: 72% (92.3 mg); m.p. 156–158 °C; molecular formula: $C_{23}H_{23}NO_4S$; molecular weight: 409 g/mol; HR-MS: $[M]^+$ 409.4991 (Calcd. for $C_{23}H_{23}NO_4S$; 409.5187); IR (KBr, cm^{-1}): ν_{max} stretching: 3422 (N-H), 3013 (C-H Ar ring), 2927 (-CH_2-), 1643 (C=C Ar ring), 1325 (-SO_2-), 1126 (C-O-C of ether); ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 2.68 (s, 6H, CH_3-1''', CH_3-2'''), 4.23–4.33 (m, 4H, CH_2-2, CH_2-3), 4.61 (s, 2H, CH_2-7''), 6.14–6.94 (m, 5H, H-2'' to H-6''), 6.96 (brd, *J* = 8.8 Hz, 2H, H-3', H-5'), 7.01 (brd, *J* = 8.8 Hz, 1H, H-4'), 7.27 (d, *J* = 8.0 Hz, 1H, H-8), 7.32 (d, *J* = 2.0 Hz, 1H, H-5), 7.58 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H, H-7), EIMS: *m/z* 409 $[M]^+$, 345 $[M\text{-}SO_2]^+$, 304 $[C_6H_3C_2H_4O_2SO_2NCH_2C_6H_5]^+$, 199 $[C_6H_3C_2H_4O_2SO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$,105 $[C_6H_3(CH_3)_2]^+$, 91 $[C_7H_7]^+$, 90 $[C_6H_3CH_3]^+$, 79 $[C_4H(CH_3)_2]^+$, 75 $[C_6H_3]^+$, 65 $[C_5H_5]^+$, 64 $[C_4HCH_3]^+$.

N-Benzyl-N-(3,4-dimethylphenyl)-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide (6e)

White solid; yield: 90% (115.4 mg); m.p. 118–120 °C; molecular formula: $C_{23}H_{23}NO_4S$; molecular weight: 409 g/mol; HR-MS: $[M]^+$ 409.4991 (Calcd. for $C_{23}H_{23}NO_4S$; 409.5187); IR (KBr, cm^{-1}): ν_{max} stretching: 3416 (N-H), 3055 (C-H Ar ring), 2943 (-CH_2-), 1622 (C=C Ar ring), 1322 (-SO_2), 1134 (C-O-C of ether); ^{1}H NMR (400 MHz,$CDCl_3$): δ (ppm) 2.10 (s, 3H, CH_3-2'''), 2.14 (s, 3H, CH_3-1'''), 4.28–4.31 (m, 4H, CH_2-2, CH_2-3), 4.65 (s, 2H, CH_2-7''), 6.66 (d, *J* = 7.2 Hz, 1H, H-5'), 6.78 (s, 1H, H-2'), 6.81 (d, *J* = 7.2 Hz, 1H, H-6'), 6.88–6.90 (m, 5H, H-2'' to H-6''), 6.92 (d, *J* = 8.4 Hz, 1H, H-8), 7.12 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H, H-7), 7.21 (d, *J* = 2.0 Hz, 1H, H-5); EIMS: *m/z* 409 $[M]^+$, 345 $[M\text{-}SO_2]^+$, 304 $[C_6H_3C_2H_4O_2SO_2N\text{-}CH_2C_6H_5]^+$, 199 $[C_6H_3C_2H_4O_2SO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$,105 $[C_6H_3(CH_3)_2]^+$, 91 $[C_7H_7]^+$, 90 $[C_6H_3CH_3]^+$, 79 $[C_4H(CH_3)_2]^+$, 75 $[C_6H_3]^+$, 65 $[C_5H_5]^+$, 64 $[C_4HCH_3]^+$.

N-(2,3-Dimethylphenyl)-N-ethyl-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide (7a)

Yellow sticky solid; yield: 82 % (89.2 mg); molecular formula: $C_{18}H_{21}NO_4S$; molecular weight: 347 g/mol; HR-MS: $[M]^+$ 347.4302 (Calcd. for $C_{18}H_{21}NO_4S$;347.4587); IR (KBr, cm^{-1}): ν_{max} stretching: 3435 (N-H), 3032 (C-H Ar ring), 2929 (-CH_2-), 1625 (C=C Ar ring), 1320 (-SO_2), 1123 (C-O-C of ether); ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 0.96 (t, *J* = 7.2 Hz, 3H, CH_3-2''), 2.00 (s, 3H, CH_3-2'''), 2.06 (s, 3H, CH_3-1'''), 3.63 (q, *J* = 7.2 Hz, 2H, CH_2-1''), 4.20–4.29 (m, 4H, CH_2-2, CH_2-3), 6.81 (d, *J* = 7.6 Hz, 1H, H-4'), 6.93 (t, *J* = 7.6 Hz, 1H, H-5'), 7.02 (d, *J* = 7.2 Hz, 1H, H-6'), 7.21 (d, *J* = 8.0 Hz, 1H, H-8), 7.63 (d, *J* = 2.4 Hz, 1H, H-5), 7.75 (dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 1H, H-7); EIMS: *m/z* 347 $[M]^+$, 283 $[M\text{-}SO_2]^+$, 268 $[C_6H_3C_2H_4O_2NCH\text{-}_2C_6H_3(CH_3)_2]^+$, 242 $[C_6H_3C_2H_4O_2SO_2NC_2H_5]^+$, 240 $[C_6H_3O_2NCH_2C_6H_3(CH_3)_2]^+$, 227 $[C_6H_3C_2H_4O_2SO_2NCH_2]^+$, 199 $[C_6H_3C_2H_4O_2SO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 105 $[C_6H_3(CH_3)_2]^+$, 90 $[C_6H_3CH_3]^+$, 79 $[C_4H(CH_3)_2]^+$, 75 $[C_6H_3]^+$, 64 $[C_4HCH_3]^+$.

N-(2,4-Dimethylphenyl)-N-ethyl-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide (7b)

Greenish brown sticky solid; yield: 73% (79.4 mg); molecular formula: $C_{18}H_{21}NO_4S$; molecular weight: 347 g/mol; HR-MS: $[M]^+$ 347.4302 (Calcd. for $C_{18}H_{21}NO_4S$; 347.4587); IR (KBr, cm^{-1}): ν_{max} stretching: 3430 (N-H), 3044 (C-H Ar ring), 2918 (-CH_2-), 1629 (C=C Ar ring), 1328 (-SO_2-), 1120 (C-O-C of ether); ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 1.00 (t, *J* = 7.2 Hz, 2H, CH_3-2''), 2.20 (s, 3H, CH_3-2'''), 2.27 (s, 3H, CH_3-1'''), 3.77 (q, *J* = 7.6 Hz, 2H, CH_2-1''), 4.28–4.33 (m, 4H, CH_2-2, CH_2-3), 6.48 (s, 1H, H-3'), 6.50 (d, *J* = 7.6Hz, 1H, H-5'), 6.90 (d, *J* =7.6 Hz, 1H, H-6'), 6.97 (d, *J* = 8.0 Hz, 1H, H-8), 7.10 (d, *J* = 2.4 Hz, 1H, H-5), 7.12 (dd, J_1 = 8.2 Hz, J_2 = 2.0 Hz, 1H, H-7); EIMS: *m/z* 347 $[M]^+$, 283 $[M\text{-}SO_2]^+$, 268 $[C_6H_3C_2H_4O_2NCH\text{-}_2C_6H_3(CH_3)_2]^+$, 242 $[C_6H_3C_2H_4O_2SO_2NC_2H_5]^+$, 240 $[C_6H_3O_2NCH_2C_6H_3(CH_3)_2]^+$, 227 $[C_6H_3C_2H_4O_2SO_2NCH_2]^+$, 199 $[C_6H_3C_2H_4O_2SO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 105 $[C_6H_3(CH_3)_2]^+$, 90 $[C_6H_3CH_3]^+$, 79 $[C_4H(CH_3)_2]^+$, 75 $[C_6H_3]^+$, 64 $[C_4HCH_3]^+$.

***N*-(2,5-Dimethylphenyl)-*N*-ethyl-2,3-dihydrobenzo [1,4]dioxine-6-sulfonamide (7c)**

Light yellow sticky solid; yield: 69% (75.1 mg); molecular formula: $C_{18}H_{21}NO_4S$; molecular weight: 347 g/mol; HR-MS: $[M]^+$ 347.4302 (Calcd. for $C_{18}H_{21}NO_4S$; 347.4587); IR (KBr, cm^{-1}): ν_{max} stretching: 3438 (N-H), 3021 (C-H Ar ring), 2927 (-CH_2-), 1618 (C=C Ar ring), 1326 (-SO_2-), 1129 (C-O-C of ether); ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) 0.99 (t, *J* =7.0 Hz, 3H, CH_3-2"); 2.16 (s, 3H, CH_3-2'"), 2.27 (s, 3H, CH_3-1'"), 3.60 (q, *J* = 7.5 Hz, 2H, CH_2-1"), 4.28–4.34 (m, 4H, CH_2-2, CH_2-3), 6.39 (s, 1H, H-6'), 6.99 (d, *J* = 8.5 Hz, 1H, H-3'), 7.04 (dd, *J* = 2.0, 8.0 Hz, 1H, H-4'), 7.09 (d, *J* = 2.5 Hz, 1H, H-5), 7.13 (dd, J_1 = 8.5 Hz, J_2 = 2.0 Hz, 1H, H-7), 7.16 (d, *J* = 7.5 Hz, 1H, H-8); EIMS: *m/z* 347 $[M]^+$, 283 $[M\text{-}SO_2]^+$, 268 $[C_6H_3C_2H_4O_2NCH_2C_6H_3(CH_3)_2]^+$, 242 $[C_6H_3C_2H_4O_2SO_2NC_2H_5]^+$, 240 $[C_6H_3O_2NCH_2C_6H_3(CH_3)_2]^+$, 227 $[C_6H_3C_2H_4O_2SO_2NCH_2]^+$, 199 $[C_6H_3C_2H_4O_2SO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 105 $[C_6H_3(CH_3)_2]^+$, 90 $[C_6H_3CH_3]^+$, 79 $[C_4H(CH_3)_2]^+$, 75 $[C_6H_3]^+$, 64 $[C_4HCH_3]^+$.

***N*-(2,6-Dimethylphenyl)-*N*-ethyl-2,3-dihydrobenzo [1,4]dioxine-6-sulfonamide (7d)**

White solid; yield: 78% (84.9 mg); m.p; 109–111 °C; molecular formula: $C_{18}H_{21}NO_4S$; molecular weight: 347 g/mol; HR-MS: $[M]^+$ 347.4302 (Calcd. for $C_{18}H_{21}NO_4S$; 347.4587); IR (KBr, cm^{-1}): ν_{max} stretching: 3445 (N-H), 3017 (C-H Ar ring), 2921 (-CH_2-), 1612 (C=C Ar ring), 1325 (-SO_2), 1134 (C-O-C of ether); ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 1.07 (t, *J* = 7.2 Hz, 3H, CH_3-2"), 2.06 (s, 6H, CH_3-1'", CH_3-2'"), 3.56 (q, *J* = 7.2 Hz, 2H, CH_2-1"), 4.28–4.32 (m, 4H, CH_2-2, CH_2-3), 6.99 (d, *J* = 8.4 Hz, 2H, H-3', H-5'), 7.02 (d, *J* = 8.4 Hz, 1H, H-4'), 7.06 (d, *J* = 8.4 Hz, 1H, H-8), 7.12 (d, *J* = 2.1 Hz, 1H, H-5), 7.24 (dd, J_1 = 8.4 Hz, J_2 = 2.1 Hz, 1H, H-7); EIMS: *m/z* 347 $[M]^+$, 283 $[M\text{-}SO_2]^+$, 268 $[C_6H_3C_2H_4O_2NCH_2C_6H_3(CH_3)_2]^+$, 242 $[C_6H_3C_2H_4O_2SO_2NC_2H_5]^+$, 240 $[C_6H_3O_2NCH_2C_6H_3(CH_3)_2]^+$, 227 $[C_6H_3C_2H_4O_2SO_2NCH_2]^+$, 199 $[C_6H_3C_2H_4O_2SO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 105 $[C_6H_3(CH_3)_2]^+$, 90 $[C_6H_3CH_3]^+$, 79 $[C_4H(CH_3)_2]^+$, 75 $[C_6H_3]^+$, 64 $[C_4HCH_3]^+$.

***N*-(3,4-Dimethylphenyl)-*N*-ethyl-2,3-dihydrobenzo [1,4]dioxine-6-sulfonamide (7e)**

Yellowish brown sticky solid; yield: 86% (93.6 mg); molecular formula: $C_{18}H_{21}NO_4S$; molecular weight: 347 g/mol; HR-MS: $[M]^+$ 347.4302 (Calcd. for $C_{18}H_{21}NO_4S$; 347.4587); IR (KBr, cm^{-1}): ν_{max} stretching: 3432 (N-H), 3017 (C-H Ar ring), 2923 (-CH_2-), 1612 (C=C Ar ring), 1325 (-SO_2-), 1134 (C-O-C of ether); ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 1.02 (t, *J* = 7.2 Hz, 3H, CH_3-2"), 2.19 (s, 3H, CH_3-2'"), 2.24 (s, 3H, CH_3-1'"), 3.55 (q, *J* = 7.2 Hz, 2H, CH_2-1"), 4.26–4.36 (m, 4H, CH_2-2, CH_2-3), 6.62 (d, *J* = 8.4 Hz, 1H, H-5'), 6.71 (dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 1H, H-6'), 6.81 (d, *J* = 2.0 Hz, 1H, H-2'), 6.94 (d, *J* = 8.4 Hz, 1H, H-8), 7.01 (d, *J* = 2.0 Hz, 1H, H-5), 7.06 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H, H-7); EIMS: *m/z* 347 $[M]^+$, 283 $[M\text{-}SO_2]^+$, 268 $[C_6H_3C_2H_4O_2NCH_2C_6H_3(CH_3)_2]^+$, 242 $[C_6H_3C_2H_4O_2SO_2NC_2H_5]^+$, 240 $[C_6H_3O_2NCH_2C_6H_3(CH_3)_2]^+$, 227 $[C_6H_3C_2H_4O_2SO_2NCH_2]^+$, 199 $[C_6H_3C_2H_4O_2SO_2]^+$, 135 $[C_6H_3C_2H_4O_2]^+$, 107 $[C_6H_3O_2]^+$, 105 $[C_6H_3(CH_3)_2]^+$, 90 $[C_6H_3CH_3]^+$, 79 $[C_4H(CH_3)_2]^+$, 75 $[C_6H_3]^+$, 64 $[C_4HCH_3]^+$.

2. 3. Enzyme Inhibition Studies

2. 3. 1. Cholinesterase Assay

The BChE and AChE inhibition activities were assayed in concurrence with the reported method after making minor changes.[33] Overall total volume of 100 µL was pertained to reaction mixture; made by mixing 60 µL of Na_2HPO_4 buffer (pH 7.7), 10 µL of each test sample and the enzyme. The sample and buffer strength was kept at 0.5 mM per well. BChE was added 0.5 unit per well and a quantity of 0.005 units was made constant for AChE in each well. After mixing the contents, the reading was recorded at 405 nm. Before this, incubation of 10 min at 37 °C was ensured. On adding 10 µL of both substrate (0.5 mM per well) and DTNB (0.5 mM per well) the reaction was allowed to start. The substrates for BChE and AChE were butyrylthiocholine chloride and acetylthiocholine iodide, respectively. Again the incubation was done for 15 min at 37 °C. Later on, the absorbance was measured at 405 nm. The instrument used was 96-well plate reader Synergy HT, Biotek, USA. 0.5 mM per well of Eserine was exploited as the positive control. The experiments were carried out in triplicate. The % inhibition was calculated as:

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

Here, total enzyme activity without inhibitor is described as control, whereas test corresponds to its activity in the presence of our synthesized molecules. EZ–Fit Enzyme Kinetics software was employed to determine IC_{50} values. The software was provided by Perrella Scientific Inc. Amherst, USA.

2. 3. 2. Lipoxygenase Assay

For lipoxygenase assay the total volume of the mixture was maintained at 200 µL. It contained 150 µL of 100 mM Na_3PO_4 buffer having pH 8.0, 10 µL of 0.5 mM per well of the test compound and 15 µL of the enzyme. The lipoxygenase was added in 600 units in each well. After mixing and pre-incubation (10 min; 25 °C) the contents were pre-read at 234 nm. On adding 25 µL of substrate solution the reaction was initiated. The absorbance was measured at 234 nm using 96-well plate reader Synergy HT, Biotek, USA. Baicalein was used as the positive control having 0.5 mM per well in the assay. The % inhibition and rest of calculation was executed by the same method as described above.[34–36]

2. 3. 3. Statistical Analysis

All experimental measurements were recorded three folds. The statistical analysis was achieved by Microsoft Excel 2010.

2. 4. Antimicrobial Activity

2. 4. 1. Microbial Strains

A set of microorganisms were used to conduct antimicrobial activity. All the synthesized compounds were tested against fungal, Gram negative and Gram positive bacterial strains. In this study *Bacillus subtilis* (*B. subtilis*) JS 2004 and *Staphylococcus aureus* (*S. aureus*) API Staph TAC 6736152 were used as Gram positive bacteria, whereas *Pasteurella multocida* (*P. multocida*) and *Escherichia coli* (*E. coli*) ATCC 25922 were among Gram negative bacteria. The four pathogenic fungi: *Microsporum canis (M. canis)*, *Candida albicans (C. albican)*, *Aspergillus flavus* (*A. flavus*) and *Fusarium solani* (*F. solani*) were also the constituents of the assay. All the pure strains were provided by CMS department of UAF, Faisalabad. The Department of microbiology checked their purity and confirmed their identification. In nutrient agar (NA, Oxoid) bacterial strains were cultured overnight at 37 °C. For fungal strains Potato Dextrose Agar (PDA, Oxoid) was chosen and subjected to culture at 28 °C overnight.[37]

2. 4. 2. Disc Diffusion Method

Disc diffusion method was used to determine antimicrobial activity of synthesized compounds. Suspension of tested microorganisms (100 μL) comprised 10^7 colony-forming units (CFU)/mL of bacterial cells. They were expanded on NA medium. The suspension with 10^6 spores/mL of fungi were expended on PDA medium. The solution of compound was applied to saturate filter discs. Sample free discs were used for negative control. For the comparison of sensitivity of strain/isolate in the analyzed microbial species, positive reference used for fungi and bacteria were Flumequine (30 μg/disk) (Oxoid, UK) and Amoxycillin (30 μg/dish) (Oxoid, UK), respectively. After keeping it at 4 °C for 2 hours, plates were incubated for 24 hours at 28 °C for fungal strains and for 18 hours at 37 °C for bacteria. Antimicrobial activity was assessed by calculating the diameter (mm of growth) of inhibition zones using zone reader. Later on it was compared with the controls.[37]

2. 4. 3. Hemolytic Activity

To study the hemolytic activity of the compounds 3 mL of fresh blood of heparinized human was collected. After consent the bovine from volunteers of the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan was bleeded. Centrifugation of blood was done at 1000 × *g* for 5 min. Plasma was discarded. 5 mL of chilled (4 °C) sterile isotonic Phosphate Buffered Saline (PBS) with pH 7.4 was used to wash the cells. This task was performed for three times. For each assay erythrocytes were maintained 10^8 cells per mL. 100 μL of each compound was taken and mixed with 10^8 cells/mL of human separately. The incubation of samples was done at 37 °C for 35 min. After incubation these samples were settled for 10 min and later on kept on agitation. The samples were placed on ice for 5 min immediately after incubation then centrifuged at 1000 × *g* for 5 min. From each tube 100 μL of supernatant was taken and then diluted with chilled (4 °C) PBS 10 times. As the positive control, Triton X-100 (0.1% v/v) was used; while phosphate buffer saline (PBS) acted as the negative control. The μQuant's life science instrument licensed by Biotek, USA was used to record the absorbance. The absorbance was taken at 576 nm. The % RBCs lysis for each sample was measured.[38,39]

2. 5. Molecular Docking

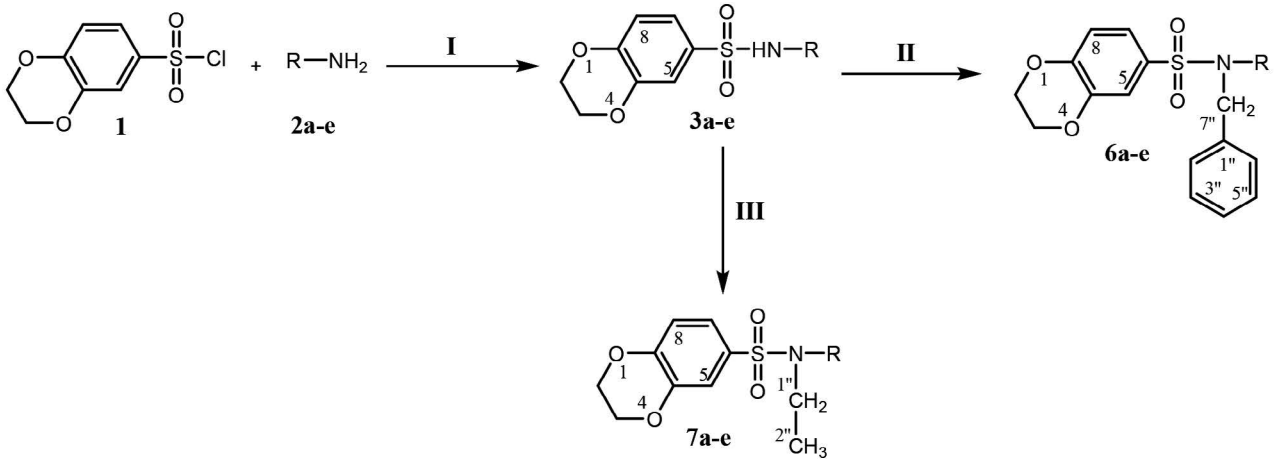
The structures of all the synthesized inhibitors were constructed using MOE-Builder tool. The default parameters of MOE-Dock program were used for the molecular docking of the ligands. Ligands were allowed to be flexible in order to find the accurate conformations of the ligands and to obtain minimum energy structures. At the end of docking, the best conformations of the ligands were analyzed for their binding interactions.[40]

3. Results and Discussion

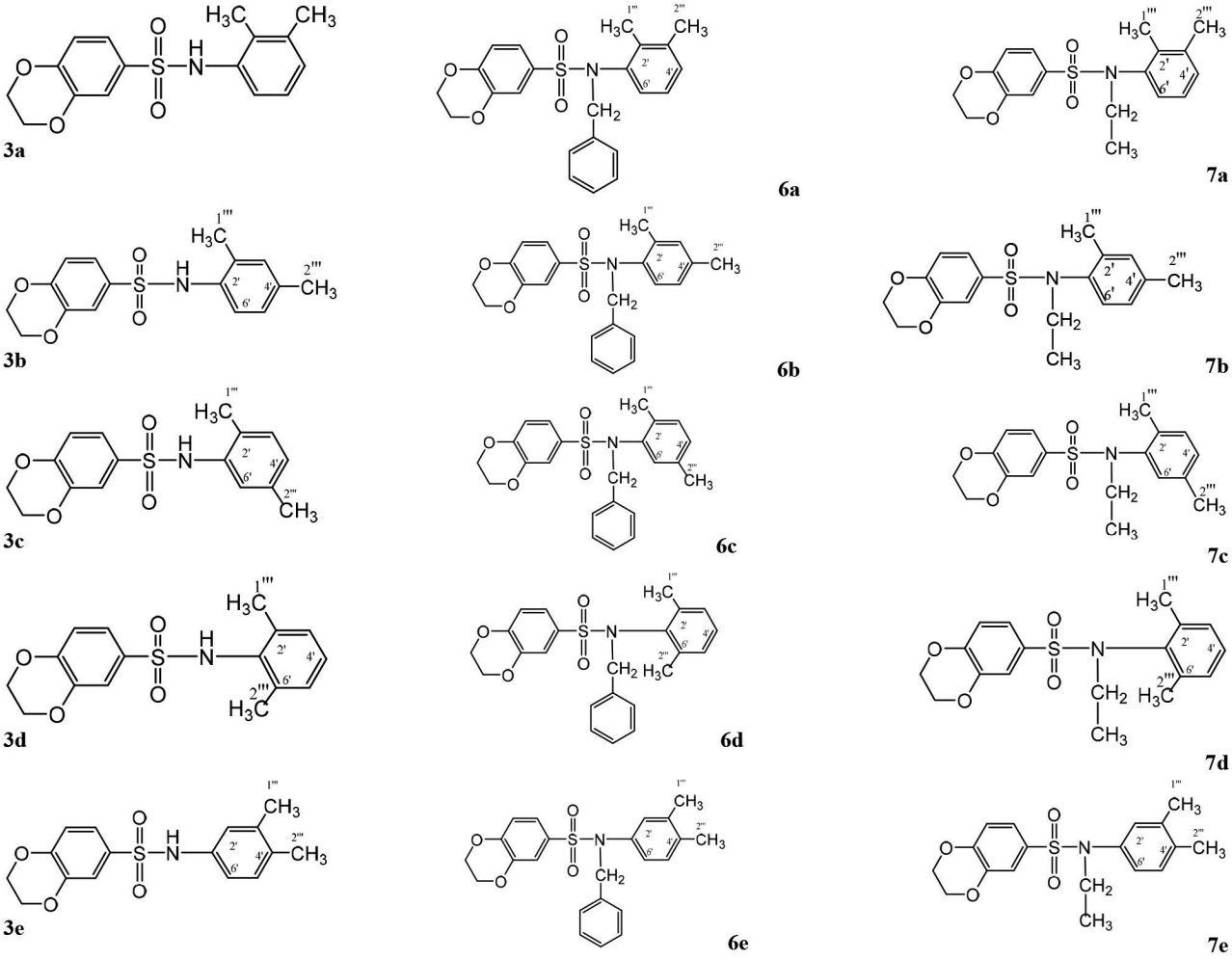
3. 1. Chemistry

We report herein the synthesis of a series of heterocyclic compounds containing 1,4-benzodioxane nucleus. The precursor molecules *N*-aryl-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide **3a**–**e**, were prepared by condensing 1,4-benzodioxane-6-sulfonyl chloride (**1**) and aryl amines **2a**–**e** in basic aqueous medium. Two series of *N*-substituted derivatives (**6a**–**e** and **7a**–**e**) were formed by the reactions of **3a**–**e** with two different electrophiles. The substitution reactions yielded *N*-benzyl (**6a**–**e**) or *N*-ethyl (**7a**–**e**) derivatives of *N*-aryl-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamides as represented in Scheme 1. The derivatives **6a**–**e** and **7a**–**e** were obtained in DMF as the solvent and with LiH as the base and activator. The reaction products were obtained within 30 to 70 min of stirring at room temperature. By adding cold distilled water along with vigorous shaking of the reaction mixtures, the products were precipitated; isolation through filtration yielded pure solid targets. On the other hand, some sticky and greasy compounds were recovered through solvent extraction method using chloroform as the extracting solvent. Here the description of the parent compound **3a** is given as an example for elaborating the spectral data. **3a** was synthesized as a white powder with good yield (80%) and m.p. was recorded as 102–104 °C. High Resolution Mass Spectrome-

try (HR-MS) showed $M^{\cdot+}$ (molecular ion peak) at *m/z* 319.3772. Therefore, molecular formula of the compound was established as $C_{16}H_{17}NO_4S$. The total number of protons was confirmed from ^1H NMR spectrum. The IR spectrum of **3a** confirmed the presence of different functionalities. Like the absorption bands were observed at 3419



Reagents and conditions: (I) Na_2CO_3 (aq), pH 9–10, stir, 3–4h, rt, (II) $C_6H_5CH_2Cl$ (**4**), DMF, LiH, stir, 4–5 h, rt (III) CH_3CH_2I (**5**), DMF, LiH, stir, 4–5 h, rt



Scheme 1. Synthetic scheme and sulfonamide compounds bearing benzodioxane nucleus.

cm^{-1} for N-H stretching of sulfamoyl group and at 3026 cm^{-1} for aromatic C-H stretching. A peak at 1613 cm^{-1} depicted the presence of C=C stretching of Arl ring. A stretching band at 1325 cm^{-1} confirmed the SO_2 stretching of sulfonyl group in the molecule. A characteristic band at 1125 cm^{-1} was assigned to C-O-C stretching of ether, respectively. The EI-MS gave characteristic peaks at *m/z* 199 and 90. These were attributed to the formation of

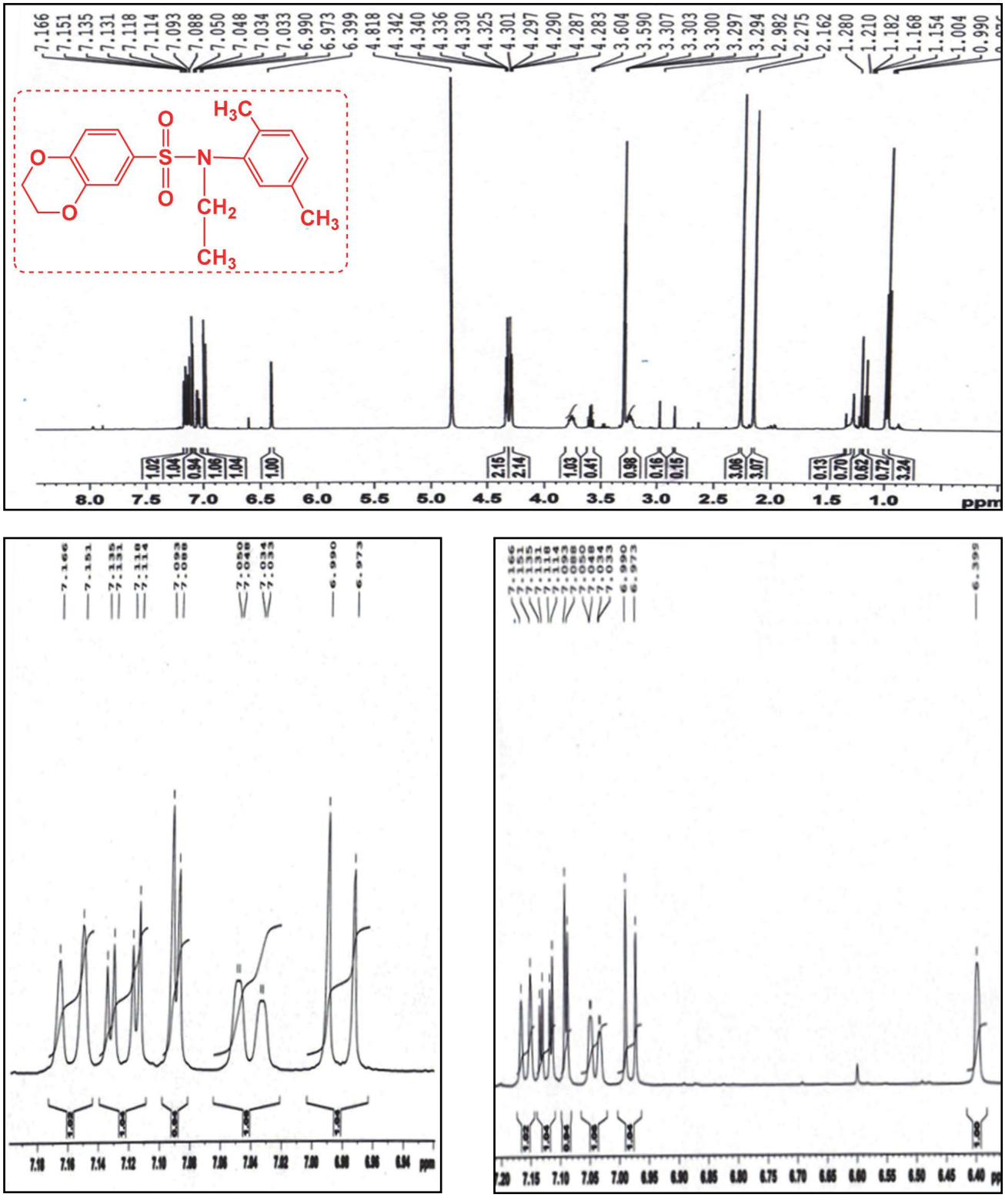


Figure 1a. ^{1}H NMR spectrum of *N*-(2,5-dimethylphenyl)-*N*-ethyl-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide **7c** (aromatic region)

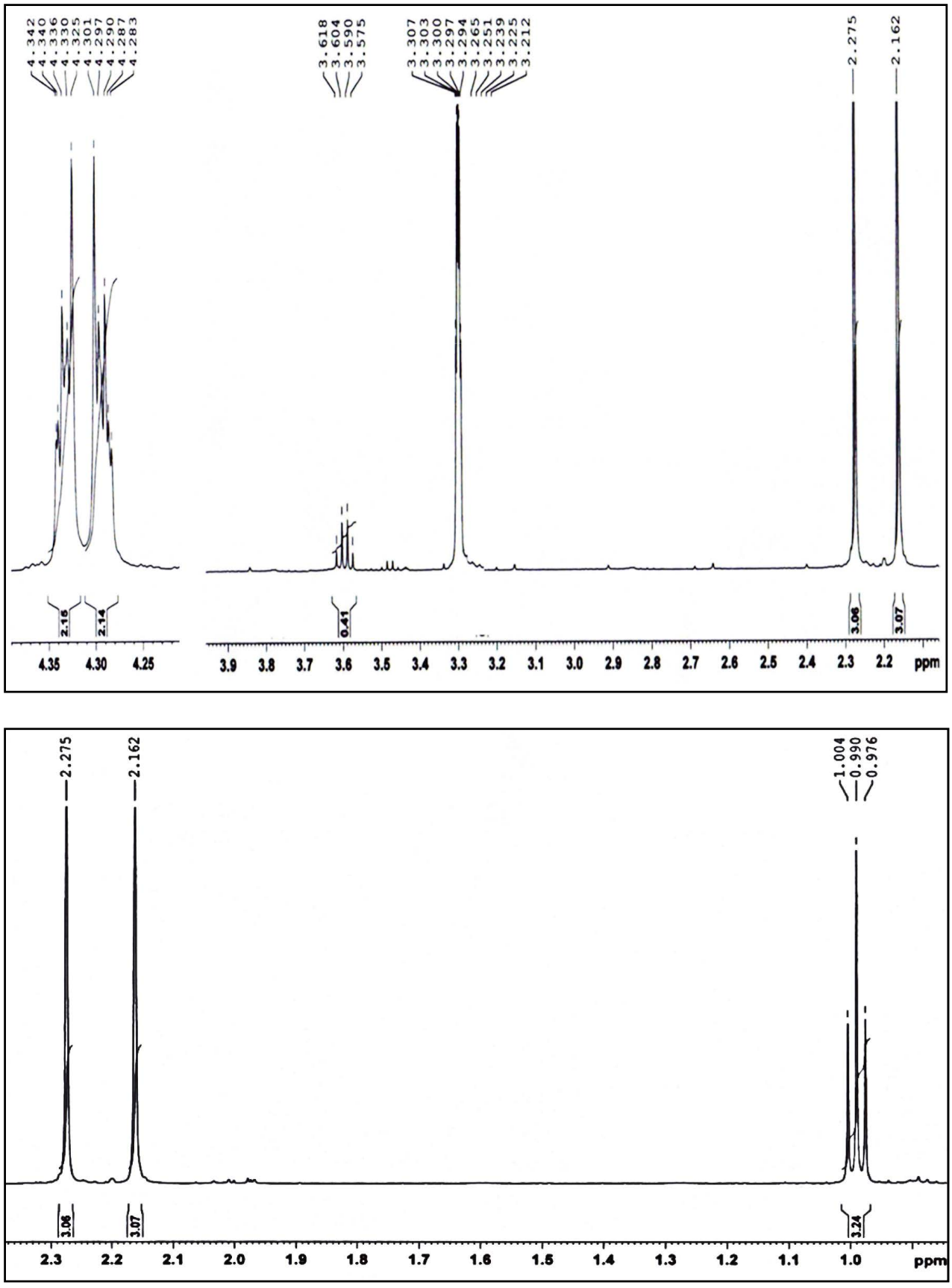


Figure 1b. ^{1}H NMR spectrum of *N*-(2,5-dimethylphenyl)-*N*-ethyl-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide **7c** (aliphatic region)

$C_6H_3C_2H_4O_2SO_2^+$ and $C_6H_3CH_3^+$ cations, respectively. In the aromatic region of the ^{1}H NMR spectrum signals appeared at δ 7.25 as doublet (small coupling constant) confirming H-5, doublet of doublet at chemical shift of 7.18 showed presence of H-7 and another doublet at δ 6.95 (large coupling constant) indicated H-8 of phenyl ring attached to the sulfonyl group. Whereas three aromatic signals at δ 6.80 (brd), 6.78 (t) and 6.73 (brd) were assigned to the benzene ring of 2,3-dimethylphenyl group. In the shielded, upfield and aliphatic region of the spectrum three distinct signals appeared. The multiplet ranging from 4.24–4.27 (CH_2-2, CH_2-3), singlet at 2.27 (CH_3-1") and relatively higher upfield singlet at 1.99 (CH_3-2") indicated the presence of 1,4-dioxane nucleus and two methyl groups attached to the second and third position of aniline in the molecule. The structure of **3a** was established as (2,3-di-

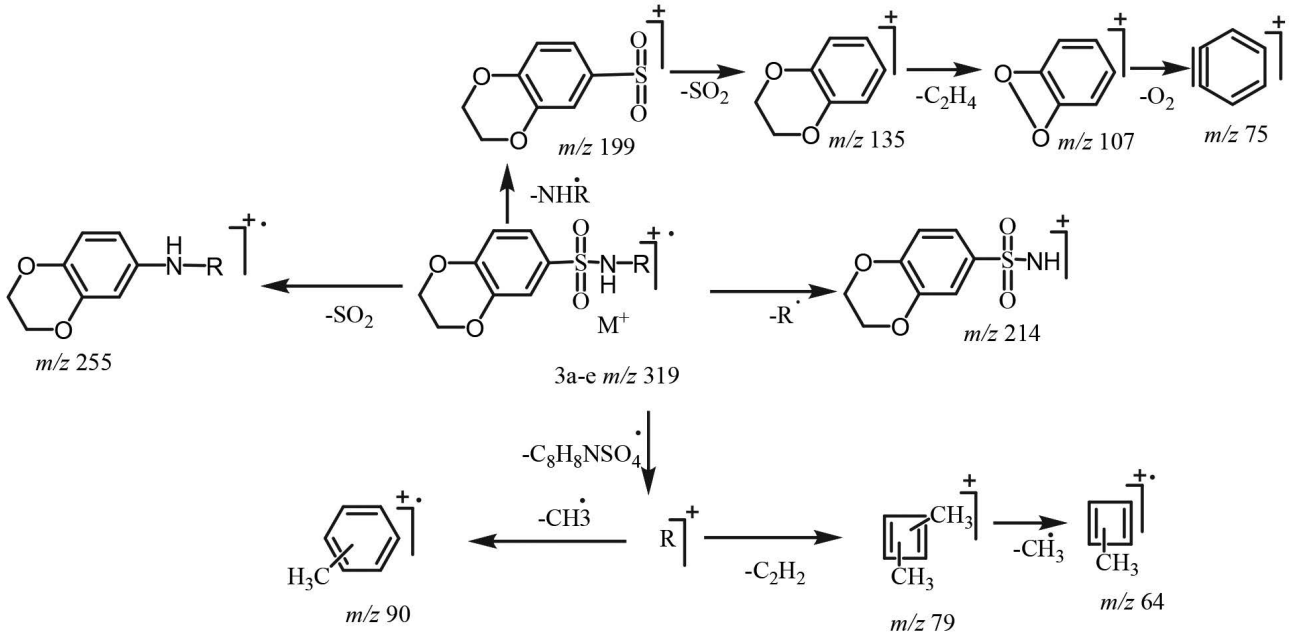


Figure 2. Mass fragmentation pattern of *N*-aryl-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamides **3a**–**e**

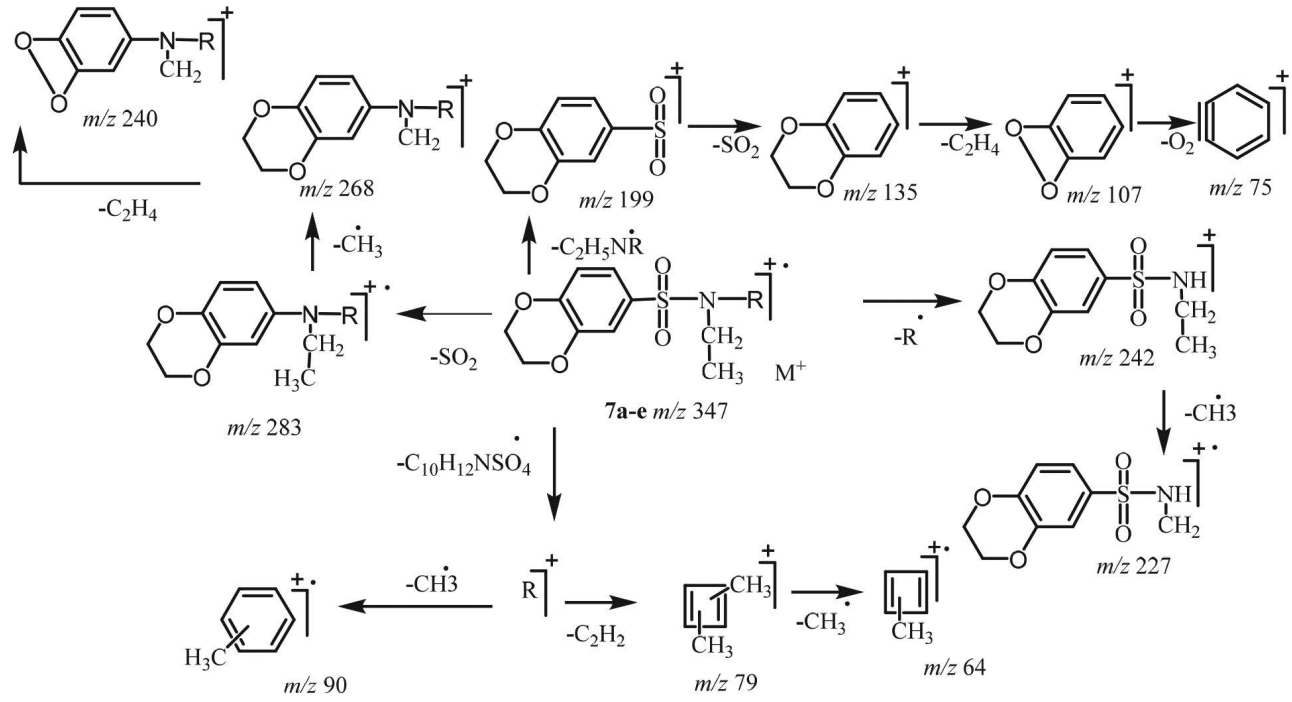


Figure 3. Mass fragmentation pattern of *N*-ethyl-*N*-(dimethylphenyl)-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide **7a**–**e**

methylphenyl)-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide on these collective confirmations. The structures of other derivatives were also ascertained on the similar pattern. The ^{1}H NMR spectrum of molecule **7c** is shown in Figure 1. The mass fragmentation pattern of parent sulfonamides **3a–e** and their *N*-ethyl derivatives **7a–e** are provided in Figures 2 and 3.

3. 2. Enzyme Inhibition

The screening of all the synthesized compounds against butyrylcholinesterase enzyme revealed that only three compounds showed better activity; *N*-(2,3-dimethylphenyl)-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide (**3a**), *N*-benzyl-(2,5-dimethylphenyl)-2,3-dihydro- benzo[1,4]dioxine-6-sulfonamide (**6c**) and *N*-ethyl-(2,4-dimethylphenyl)-2,3 -dihydro- benzo[1,4]dioxine-6-sulfonamide (**7b**) having IC_{50} values of 374.11 ± 0.01, 387.51 ± 0.48 and 353.13 ± 0.86 μmol/L respectively, relative to eserine, a reference standard with IC_{50} value of 0.85 ± 0.0001 μmol/L (Table 1). The activity of these compounds was most probably due to the presence of methyl groups at different positions of phenyl ring in **3a**, benzyl group in **6c** along with alkyl groups and an additional ethyl group for **7b** attached to the nitrogen of sulfonamide. The screening against acetylcholinesterase enzyme of all the synthesized compounds showed that only four compounds were moderately active against it; *i.e.* *N*-(3,4dimethylphenyl)-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide (**3e**), *N*-benzyl-*N*-(2,4-dimethylphenyl)-2,3-dihydrobenzo[1,4] dioxine-6-sulfonamide (**6b**), *N*-ethyl-*N*-(2,4-dimethylphenyl)-2,3-dihy-drobenzo[1,4]dioxine-6-sulfonamide (**7b**) and *N*-ethyl-*N*-(2,6-dimethylphenyl) -2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide (**7d**) having IC_{50} values ranging from 178.51 ± 0.14 to 364.27 ± 0.54 μmol/L. The relative reference standard was eserine, with IC_{50} value of 0.04 ± 0.0001 μmol/L. The % inhibition associated with these compounds arrayed from 59.91 ± 0.96 to 76.56 ± 0.19 μmol/L respectively. The proficient activity was observed for **7b** and **7d**; this was most likely due to the occurrence of *N*-ethyl group in these compounds in comparison to the other series members. Against lipoxygenase enzyme, all the synthesized compounds showed beneficially good activity but the most active were *N*-(2,6-dimethylphenyl)-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide (**3d**), *N*-(2,4-dimethylphenyl)-2,3-dihydrobenzo [1,4]dioxine-6-sulfonamide (**3b**), *N*-benzyl-*N*-(2,6-dimethylphenyl)-2,3-dihydrobenzo [1,4] dioxine-6-sulfonamide (**6d**) and *N*-(3,4-dimethylphenyl)-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide (**3e**) having IC_{50} values of 34.21 ± 0.12, 64.21 ± 0.05, 84.61 ± 0.11 and 87.61 ± 0.14 μmol/L respectively, relative to baicalein, a reference standard with IC_{50}

Table 1. Enzyme inhibition studies of the synthesized compounds.

Compound	BChE			AChE			LOX		
	Conc./well (mM)	Inhibition (%)	IC_{50} μM	Conc. (mM)	Inhibition (%)	IC_{50} (μmol.)	Conc./well (mM)	Inhibition (%)	IC_{50} μM
3a	0.5	59.98 ± 0.45	374.11 ± 0.07	0.5	34.68 ± 0.68	–	0.5	81.34 ± 0.33	89.25 ± 0.17
3b	0.5	36.56 ± 0.66	–	0.5	18.34 ± 0.25	–	0.5	89.11 ± 0.22	64.21 ± 0.05
3c	0.5	36.44 ± 0.22	–	0.5	16.91 ± 0.11	–	0.5	88.45 ± 0.82	92.25 ± 0.11
3d	0.5	29.12 ± 0.55	–	0.5	30.39 ± 0.85	–	0.5	96.86 ± 0.36	34.21 ± 0.12
3e	0.5	56.85 ± 0.91	>400	0.5	59.91 ± 0.96	302.11 ± 0.14	0.5	82.22 ± 0.67	87.61 ± 0.14
6a	0.5	32.32 ± 0.19	>500	0.5	4.37 ± 0.15	>500	0.5	1.04 ± 0.22	–
6b	0.5	54.10 ± 0.17	>500	0.5	76.56 ± 0.19	267.17 ± 0.21	0.25	35.55 ± 0.35	–
6c	0.5	58.72 ± 0.13	387.51 ± 0.48	0.5	64.77 ± 0.36	364.27 ± 0.54	0.25	43.01 ± 0.64	–
6d	0.5	43.41 ± 0.82	–	0.5	15.85 ± 0.55	–	0.5	82.11 ± 0.14	84.61 ± 0.11
6e	0.5	46.26 ± 0.62	–	0.5	46.99 ± 0.38	–	0.5	63.26 ± 0.55	238.71 ± 0.14
7a	0.5	28.85 ± 0.14	>500	0.5	1.97 ± 0.25	>500	0.5	13.79 ± 0.15	–
7b	0.5	67.69 ± 0.18	353.13 ± 0.86	0.5	73.08 ± 0.76	223.1 ± 0.18	0.25	53.44 ± 0.66	>400
7c	0.5	54.09 ± 0.15	>500	0.5	46.67 ± 0.23	>500	0.5	5.21 ± 0.11	–
7d	0.5	42.65 ± 0.61	–	0.5	70.52 ± 0.61	178.51 ± 0.14	0.5	48.46 ± 0.19	–
7e	0.5	42.03 ± 0.58	–	0.5	32.36 ± 0.14	–	0.5	43.84 ± 0.19	–
Control	**Eserine**	82.82 ± 1.09	0.85 ± 0.0001	**Eserine**	91.29 ± 1.17	0.04 ± 0.0001	**Baicalein**	93.79 ± 1.27	**22.4 ± 1.3**

Note: 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). LOX = Lipoxygenase. AChE = Acetyl cholinesterase. BChE = Butyryl cholinesterase.

value of 22.4 ± 1.3 μmol/L. The proficient activity of first and second compounds was most probably due to the occurrence of two alkyl groups, one at the second position and the other one at the sixth and fourth positions of aniline ring, respectively. In **3e** these two alkyl groups were situated at the third and fourth positions and resulted in less inhibition in comparison to **3d** and **3b**. For **6d** the credibly was due to the presence of two alkyl groups at the second and sixth position of aniline ring along with benzyl group attached to the nitrogen of sulfonamide. From the activity of molecules against LOX it might be concluded that free nitrogen of sulfamoyl group could be more effective in blocking the active site of enzyme; instead when we substitute it with ethyl or benzyl groups. All the parent compounds **3a**–**e** can be further utilized for the synthesis of new derivatives with other different electrophiles to enhance their biological, antimicrobial and other activities.

3. 3. Antimicrobial Activity

The *in vitro* antimicrobial properties of the parent compounds **3** and their derivatives were tested. Against the selected panel of both bacterial and fungal species parent compounds **3a**, **3b**, **3c** and **3e** exhibited moderate antimicrobial activity; and **3c** showed only antibacterial activity as is evident from Table 2. Regarding these parent sulfonamides **3**, the compound **3e** showed relatively higher activity but lower than that of the standard compound. Among the derivatives of **6a**–**e** series **6a** and **6c** have shown both the antibacterial and antifungal activities; and **6a** demonstrated higher activities in contrast to the other series members. **7a**, **7b** and**7c** are the members of **7a**–**e** series which were active against both bacterial and fungal strains; among these **7b** exhibited relatively better results against both microbes. The remaining compounds possess very low or no activity against the assessed microorganisms. The highest hemolytic activity was shown by **3b** (92%) but lower than the positive control (Triton-X-100). The lowest hemolytic activity was shown by **7b** and **7c** (2.1% and 0.7%, respectively) but higher than the negative controls (PBS). Overall it can be concluded here that **7b** and **7c** were the better members overall from all these compounds and ligands; because they have displayed better antimicrobial potential and less hemolytic activity. On the basis of the presented results we may assume that the synthesized sulfonamides may be suitable leads for further improvement to address different targets.

Table 2. Antibacterial and antifungal studies on synthesized compounds.

Compound	Antibacterial activity				Hemolytic activity	Antifungal activity			
	Staphylococcsa ureus	*Bacillus subtilis*	*Pasturella multocida*	*Escherichia coli*	(Mean) % ± S.D.	*Candida albicans*	*Microsporum canis*	*Aspergillus flavus*	*Fusarium solani*
	Zone of inhibition (mm)						Zone of inhibition (mm)		
3a	14	14	16	16	71.847 ± 0.093	14	14	16	16
3b	16	16	12	14	91.721 ± 1.121	16	16	12	14
3c	14	14	12	14	88.162 ± 0.278	–	–	–	–
3d	–	–	–	–	88.328 ± 3.122	–	–	–	–
3e	18	16	14	14	86.918 ± 0.226	19	16	14	14
6a	16	12	14	16	91.684 ± 3.221	16	18	14	14
6b	-	-	-	-	88.372 ± 0.192	–	–	–	–
6c	14	16	12	14	73.814 ± 0.464	13	18	11	12
6d	–	–	–	–	81.770 ± 3.060	–	–	–	–
6e	–	–	–	–	88.481 ± 0.309	–	–	–	–
7a	14	14	16	12	71.472 ± 0.087	16	14	18	16
7b	16	18	14	14	2.186 ± 0.124	19	17	18	16
7c	16	14	14	16	0.743 ± 0.062	17	12	15	18
7d	–	–	–	–	72.372 ± 0.526	–	–	–	–
7e	–	–	–	–	91.668 ± 5.508	–	–	–	–
Streptomycin	30	8	28	30	Flumequine	29	27	26	31
PBS					0.00 ± 0.0				
Triton(toxicity)					100 ± 0.0				

4. Molecular Docking

The results obtained from *in silico* approach were also favoring the fact that the synthesized sulfonamides have shown good interactions with the target site. The interaction analysis has shown that in every compound the sulfonamide group is contributing to the interactions. The interactions of compounds **3b** and **7d** with the active site of lipoxygenase (LOX) and acetylcholinesterase (AChE) are shown in Figures 4 and 5, respectively.

The interaction analysis of compound **7d** against acetylcholinesterase depicted that the residues of the binding pocket interacted with two functionalities of the compound. Tyr121 developed links with the one oxygen of SO_2 group in sulfonamide moiety and Ser122 interacted with the one oxygen atom (at fourth position) of 1,4-dioxane ring (Figure 5). Whereas in compound **3b** His518 has developed interactions with oxygen of sulfamoyl group and His513 has interacted with oxygen of the dioxane functionality (Figure 4).

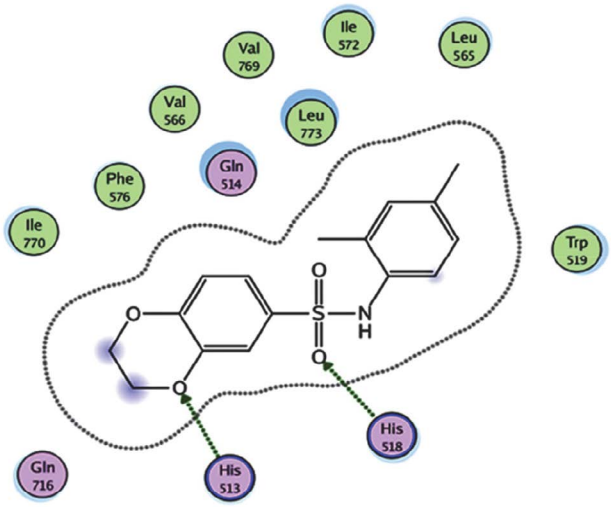


Figure 4: 2D interaction of *N*-(2,4-dimethylphenyl)-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide (**3b**) against lipoxygenase.

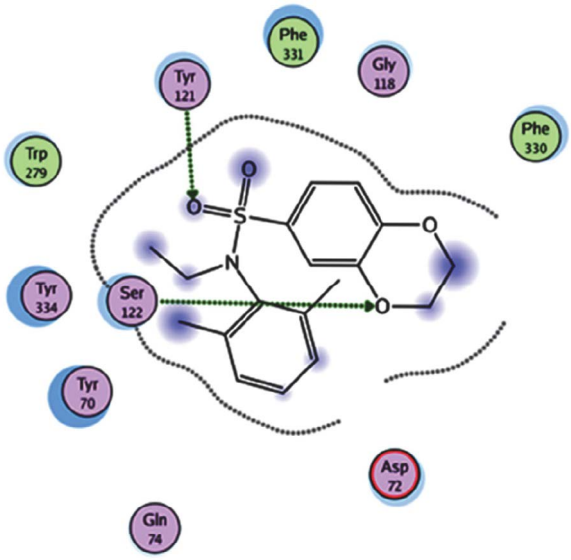


Figure 5: 2D image of compound *N*-(2,6-dimethylphenyl)-*N*-ethyl-2,3-dihydro- benzo[1,4]dioxine-6-sulfonamide (**7d**) against acetylcholinesterase.

5. Conclusion

A new series of sulfonamides bearing 1,4-benzodioxane ring systems were synthesized. These were characterized by IR, ^{1}H NMR and EIMS. All the compounds were screened for their antibacterial and antifungal activity by disc diffusion method. Compounds **3e**, **7b** and **7c** exhibited good antimicrobial activity among all the synthesized compounds but lower than that of the standard drug streptomycin. Compound **7b** was better inhibitor against BchE and **7d** for AchE, while **3d** exhibited good inhibition potential against LOX. Most of the synthesized compounds exhibited an overall bearable toxicity level and could be utilized as possible therapeutic entrants after making structural modifications.

6. Acknowledgements

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Conflict of Interest:

Authors declare no conflict of interest.

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Povzetek

V predstavljenem raziskovalnem delu poročamo o seriji *N*-aril-2,3-dihidrobenzo[1,4]dioksin-6-sulfonamidov **3** in njihovih novih *N*-substituiranih derivatih **6** in **7**, ki smo jih iz **3** pripravili z benzil kloridom oz. etil jodidom. Sintezo smo izvedli v več stopnjah. Strukture produktov smo določili z ^{1}H NMR, IR in EIMS spektroskopskimi tehnikami. Kot encime smo v študiji uporabili butirilholinesterazo (BChE), acetilholinesterazo (AChE) in lipoksigenazo (LOX). Ugotovili smo, da večina spojin izkazuje zmerno aktivnost proti BChE in AChE in obetavno dobro aktivnost proti lipoksigenazi. Med matičnimi sulfonamidi so **3a**, **3b**, **3c** in **3e** izkazali najbolj učinkovite antimikrobne aktivnosti, po drugi strani pa so derivati **6a**, **6c**, **7a**, **7b** in **7c** izkazali dobro aktivnosti proti izbranim bakterijam in glivam. Hemolitsko aktivnost smo določili, da bi ugotovili morebitno terapevtsko uporabnost pripravljenih spojin. Vse spojine smo tudi računsko sidrali v encime LOX, BChE in AChE.