Synthesis of Some 1,4-Benzodioxane Containing Methanesulfonamides and Their Hemolytic Study on Human Blood

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Summary: The aim of this work was to synthesize some methanesulfonamides bearing 1,4-benzodioxane moiety and to evaluate their cytotoxicity profile through hemolytic study. The synthetic methodology involved the reaction of 1,4-benzodioxane-6-amine (**1**) with methanesulfonyl chloride (**2**) in aqueous alkaline medium under stirring for 2-3 hours and dynamic pH control at 9 to form *N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)methanesulfonamide (**3**). The synthesized sulfonamide, **3**, was dissolved in DMF and LiH was added as an activator. Then, it was stirred for 2-3 hours with a series of aralkyl/alkyl halides, **4a-g**, to yield *N*-alkyl/ aralkyl-*N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)methanesulfonamides (**5a-g**). The structural analysis of the derivatives was performed by FT-IR, ^{1}H-NMR and EI-MS. When these molecules were evaluated for their hemolytic study, the molecule **5g** was found to be least toxic amongst the series. Hence, this molecule might be utilized as safe therapeutic agent.

Keywords: Methanesulfonamides, 1,4-benzodioxane, Cytotoxicity, Spectral analysis, NMR, EI-MS.

Introduction

Sulfonamides are considered as one of the best anti-bacterial agents and act as anti-metabolites. Sulfa drugs were discovered in 1935 and amongst the first known is Red dye (Prontosil; (*E*)-4-[(2,4-diaminophenyl)azo]benzenesulfonamide; Fig. 1). It was found to be non-toxic and active in treating *streptococcal* infections in mice [1]. In the long run, it was investigated and declared that drug is metabolized by a bacterium present in small intestine and broken down to give sulfanilamide which was the true anti-bacterial agent [1-3]. Thus Protonsil was identified as first prodrug, which was synthesized largely in laboratory and became initial synthetic anti-bacterial which was found to be active against large number of infections [1]. Despite their undoubted benefits, sulfa drugs have proved ineffective against *Salmonella* species [1,2].

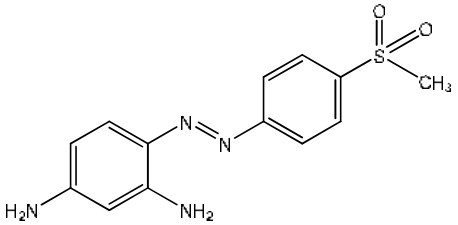

Fig. 1: (*E*)-4-[(2,4-Diaminophenyl)azo] benzenesulfonamide.

Sulfonamides are very famous anti-bacterial drugs because of low toxicity, low cost and excellent activity against the most bacterial strains. They are considered as potent translation initiation inhibitor [4], non-peptidic vasopressin receptor antagonistic [5], anti-inflammatory [6], anti-fungal [7], anti-protozoal [8] and anti-bacterial [9]. Sulfonamides are also very active in treatment of ophthalmic infections ulcerate colitis, scalds [10], rhemmatiod arthrist [11], male erectile dysfunction as the phosphodiesterase-5-inhibitor sildenafil [12] and obesity [13]. More recently, they are used in treatment of Alzheimer's disease [14], Amprenavir belongs to this class and is utilized as anti-HIV protease inhibitor [15] and also as anti-cancer agent [16].

Benzodioxane, another bioactive nucleus, is considered active as anti-inflammatory [17], anti-helmintic, anti-convulsant, anti-arthritic, antifungal, antioxidant, antitumor [16] and anti-hepatotoxic [18]. This nucleus also aids in reducing blood pressure for long term [19]. Silymarin isolated from *Silybum marianum* has been found to be a potent anti-hepatotoxic agent against a number of toxicants. It is considered to be a mixture of three flavonlignans in which silybin is the major component which make up about 20-30 % of total flavonolignans and contains 1,4-benodioxane ring system and possesses remarkable anti-hepatotoxic activity.

So based on aforementioned biological activities of compounds containing sulfamoyl and benzodioxane moieties, the present study was aimed to evaluate the cytotoxicity profile of new methane sulfunomides bearing 1,4-benzodioxane heterocycle.

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Experimental

General

All chemicals were purchased from Sigma Aldrich and Fluka through local suppliers and were used without purification. Purity of compounds was checked by thin layer chromatography with solvent system comprising of various percentages of ethyl acetate and *n*-hexane. The reactions were also monitored by pre-coated silica gel G-25UV254 plates and visualized by UV lamp at 254 nm. The melting points were checked using open capillary tube on Gallenkamp melting point apparatus and were uncorrected. FT-IR spectra were recorded on midac m 2000 spectrometer. ^{1}H-NMR spectra were recorded in $CDCl_3$ on Burker spectrometer, operating at 400 MHz at 25 °C. The chemical shifts δ are given in ppm and coupling constant (*J*) in hertz (Hz). The abbreviations used in ^{1}H-NMR spectral interpretation were; s = singlet, d = doublet, dd = doublet of doublet, t = triplet, br.t = broad triplet, q = quartet, quint = quintet, sex = sextet, sep = septet and m = multiplet. Finnigan MAT-312 instrument was used to measure mass spectra (EI-MS).

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

1,4-Benzodioxane-6-amine (3 mL, 0.02 mol; **1**) was suspended in 50 mL distilled water in a 250 mL round bottomed flask and 10 mL aqueous Na_2CO_3 (10%) was added to the mixture which was stirred for 0.5 hour. After half an hour methanesulfonyl chloride (1.91 mL, 0.02 mol; **2**) was added to the reaction mixture slowly and reaction mixture was stirred further for 2-3 hours at room temperature and monitored with TLC till single spot for completion of reaction. The product was collected by filtration, washed with distilled water and dried to achieve pure *N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)methanesulfonamide (**3**) as brownish amorphous powder. Yield: 95 %; m.p.: 107-109 °C; Molecular formula: $C_9H_{11}NO_4S$; Molecular weight: 229 gmol^{-1}. HR-MS: $[M]^{\bullet+}$ 229.0416 (Calcd. for $C_9H_{11}NO_4S$; 229.0409; Mol. formula: $C_9H_{11}NO_4S$; Mol. weight: 229 gmol^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 6.96 (d, *J* = 2.4 Hz, 1H, H-5), 6.82 (d, *J* = 8.0 Hz, 1H, H-8), 6.78 (dd, *J* = 2.4, 8.0 Hz, 1H, H-7), 4.24 (br.s, 4H, CH_2-2 & CH_2-3), 2.88 (s, 3H, CH_3-1′).

Synthesis of N-alkyl/aralkyl-N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)methanesulfonamides **(5a-g)**

N-(2,3-Dihydrobenzo[1,4]dioxin-6-yl)methanesulfonamide (0.1 g, 0.30 mmol; **3**) was solubilized in *N,N*-dimethyl formamide (DMF, 10.0 mL) in 50 mL round bottomed flask, followed by the addition of lithium hydride (0.1 mmol) to the reaction mixture which was stirred for 30 minutes at 25 °C. Different aralkyl/alkyl halides (**4a-g**; 0.30 mmol) were added in reaction mixture and further stirred for 2-3 hours. The reaction was monitored by TLC till single spot. After completion, the reaction mixture was poured on crushed ice and precipitates were filtered, washed and air-dried to obtain pure products (**5a-g**).

Spectral Characterization

N-(2,3-Dihydrobenzo[1,4]dioxin-6-yl)-N-(ethyl)methanesulfonamide **(5a)**

Brown solid; yield: 80%; m.p. 118 °C; m.f.: $C_{11}H_{15}NO_4S$; molecular weight: 257 g mol^{-1}; IR (KBr, cm^{-1}): υ_{max}: 3071 (C-H stretching of aromatic ring), 1514 (C=C stretching of aromatic ring), 1338 (-SO_2 stretching), 1151 (C-O-C stretching of ether); ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm): δ 6.94 (br.s, 1H, H-5), 6.84 (d, *J* = 8.0 Hz, 1H, H-8), 6.79 (dd, *J* = 2.0, 8.0 Hz, 1H, H-7), 4.24 (br.s, 4H, CH_2-2 & CH_2-3), 3.64 (q, *J* = 7.2 Hz, 1H, H-1″), 2.86 (s, 3H, CH_3-1′), 1.11 (t, *J* = 7.2 Hz, 1H, H-2″). HR-MS: $[M]^{\bullet+}$ 257.0731 (Calcd. for $C_{11}H_{15}NO_4S$; 257.0722); EI-MS: *m/z* 257 $[C_{11}H_{15}NO_4S]^{+}$ (51.2%), 178 $[C_{10}H_{12}NO_2]^{+}$ (62%), 135 $[C_8H_7O_2]^{+}$ (7.6%), 107 $[C_7H_3O_2]^{+}$ (3.9%), 79 $[Me\text{-}SO_2]^{+}$ (7.7%), 75 $[C_6H_4]^{+}$ (1.9%).

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

Grey amorphous solid; Yield: 80 %; m.p.: 119-120 °C; Molecular formula: $C_{16}H_{17}NO_4S$; Molecular weight: 319 gmol^{-1}; IR (KBr, cm^{-1}): υ_{max}: 3077 (C-H stretching of aromatic ring), 1505 (C=C stretching of aromatic ring), 1335 (-SO_2 stretching), 1156 (C-O-C stretching of ether); ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm): δ 7.25-7.24 (m, 5H, H-2" to H-6"), 6.75 (signal merged in doublet of H-8, 1H, H-5), 6.74 (d, *J* = 8.4 Hz, 1H, H-8), 6.69 (dd, *J* = 2.4, 8.8 Hz, 1H, H-7), 4.74 (s, 2H, CH_2-7"), 4.19 (br.s, 4H, CH_2-2 & CH_2-3), 2.91 (s, 3H, CH_3-1'); HR-MS: $[M]^{\bullet+}$ 319.0891 (Calcd. for $C_{16}H_{17}NO_4S$; 319.0878); EI-MS: *m/z* 319 $[C_{16}H_{17}NO_4S]^{+}$ (50.3%), 240 $[C_{15}H_{14}NO_2]^{+}$ (100%), 135 $[C_8H_7O_2]^{+}$ (3%), 107 $[C_6H_3O_2]^{+}$ (3%), 91 $[C_7H_7]^{+}$ (37.8%), 80 $[C_4HO_2]^{+}$ (1.4%), 79 $[Me\text{-}SO_2]^{+}$ (4.5%), 77 $[C_6H_5]^{+}$(3.2%), 65 $[C_5H_5]^{+}$ (48%).

N-(2,3-Dihydrobenzo[1,4]dioxin-6-yl)-N-(3-phenylpropyl)methanesulfonamide **(5c)**

Grey amorphous solid; Yield: 78%; m.p.: 104-105 °C; Molecular formula: $C_{18}H_{21}NO_4S$; Molecular weight: 347 g mol^{-1}; IR (KBr, cm^{-1}): υ_{max}: 3034 (C-H stretching of aromatic ring), 1504 (C=C stretching of aromatic ring), 1333 (-SO_2 stretching), 1143 (C-O-C stretching of ether); ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm): δ 7.16-7.09 (m, 5H, H-2" to H-6"), 6.85 (d, *J* = 7.6 Hz, 1H, H-8), 6.83 (br.s, H-5), 6.80

(dd, *J* = 1.8, 7.6 Hz, 1H, H-7), 4.24 (br.s, 4H, CH_2-2 & CH_2-3), 3.61 (t, *J* = 7.2 Hz, 2H, CH_2-9"), 2.84 (s, 3H, CH_3-1'), 2.63 (t, *J* = 7.2 Hz, 2H, CH_2-7"), 1.78 (quint, *J* = 7.2 Hz, 2H, CH_2-8"); HR-MS: $[M]^{\bullet+}$ 347.1199 (Calcd. for $C_{18}H_{21}NO_4S$; 347.1191; EI-MS: *m/z* 347 $[C_{18}H_{21}NO_4S]^{\bullet+}$ (50.1%), 268 $[C_{17}H_{18}NO_2]^+$ (62%), 135 $[C_8H_7O_2]^+$ (3%), 107 $[C_6H_3O_2]^+$ (3%), 91 $[C_7H_7]^+$ (37.8%), 80 $[C_4HO_2]^+$ (1.4%), 79 $[Me\text{-}SO_2]^+$ (4.5%), 77 $[C_6H_5]^+$ (3.2%), 65 $[C_5H_5]^+$ (48%).

N-(2,3-Dihydrobenzo[1,4]dioxin-6)-yl)-N-(2-methylbenzyl)methanesulfonamide **(5d)**

Dark brown amorphous solid; Yield: 85%; m.p.: 133-134 °C; Molecular formula: $C_{17}H_{19}NO_4S$; Molecular weight: 333 $gmol^{-1}$; IR (KBr, cm^{-1}): υ_{max}: 3016 (C-H stretching of aromatic ring), 1505 (C=C stretching of aromatic ring), 1337 (-SO_2 stretching), 1152 (C-O-C stretching of ether); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 7.17 (br.d, *J* = 7.2 Hz, 1H, H-4"), 7.11-7.05 (m, 3H, H-3", H-5", H-6"), 6.76 (d, *J* = 2.4 Hz, 1H, H-5), 6.72 (d, *J* = 8.4 Hz, 1H, H-8), 6.69 (dd, *J* = 2.4, 8.4 Hz, 1H, H-7), 4.77 (s, 2H, CH_2-7"), 4.18 (br.s, 4H, CH_2-2 & CH_2-3), 2.92 (s, 3H, CH_3-1'), 2.29 (s, 3H, CH_3-8"); HR-MS: $[M]^{\bullet+}$ 333.1042 (Calcd. for $C_{17}H_{19}NO_4S$; 333.1035); EI-MS: *m/z* 333 $[C_{17}H_{19}NO_4S]^{\bullet+}$ (29%), 253 $[C_{16}H_{15}NO_2]^+$ (1.7%), 135 $[C_8H_7O_2]^+$ (4.1%), 107 $[C_6H_3O_2]^+$ (4.5%), 105 $[C_8H_9]^+$ (100%), 91 $[C_7H_7]^+$ (3.9%), 80 $[C_4H_2O_2]^+$ (2%), 79$[Me\text{-}SO_2]^+$ (10%), 77 $[C_6H_5]^+$ (7.6%).

N-(2-Chlorobenzyl)-N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)methanesulfonamide **(5e)**

Light brown amorphous solid; Yield: 80%; m.p.: 127-128 °C; Molecular formula: $C_{16}H_{16}ClNO_4S$; Molecular weight: 353 $gmol^{-1}$; IR (KBr, cm^{-1}): υ_{max}: 3096 (C-H stretching of aromatic ring), 1505 (C=C stretching of aromatic ring), 1327 (-SO_2 stretching), 1167 (C-O-C stretching of ether); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 7.49 (dd, *J* = 0.8, 7.2 Hz, 1H, H-3"), 7.25 (m, 1H, H-5"), 7.20-7.14 (m, 2H, H-4" & 'H-5'), 6.83 (br.s, 1H, H-5), 6.77-6.76 (m, 2H, H-7 & H-8), 4.92 (br.s, 2H, CH_2-7"), 4.19 (br.s, 4H, CH_2-2 & CH_2-3), 2.96 (s, 3H, CH_3-1'); HR-MS: $[M]^{\bullet+}$ 353.0496 (Calcd. for $C_{16}H_{16}ClNO_4S$; 353.0489); EI-MS: *m/z* 353 $[C_{16}H_{16}ClNO_4S]^{\bullet+}$ (52.6%), 274 $[C_{15}H_{13}ClNO_2]^+$ (100%), 135 $[C_8H_8O_2]^+$ (4.4%), 125 $[C_7H_6Cl]^+$ (26.9%), 99 $[C_5H_4Cl]^+$ (1.9%), 80 $[C_4HO_2]^+$ (2.4%), 79 $[Me\text{-}SO_4]^+$ (7.2%), 75 $[C_6H_3]^+$ (1.3%), 65 $[C_5H_5]^+$ (1.9%), 51 $[C_4H_3]^+$ (3.9%).

N-(4-Chlorobenzyl)-N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)methanesulfonamide **(5f)**

Light brown amorphous solid; Yield: 76%; m.p.: 113-114 °C ; Molecular formula: $C_{16}H_{16}ClNO_4S$; Molecular weight: 353 $gmol^{-1}$; IR (KBr, cm^{-1}): υ_{max}: 3047 (C-H stretching of aromatic ring), 1505 (C=C stretching of aromatic ring), 1327 (-SO_2 stretching), 1168 (C-O-C stretching of ether), 685 (C-Cl stretching); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 7.19 (br.d merged in signal of $CDCl_3$, *J* = 8.1 Hz, 2H, H-3" & H-5"), 7.19 (br.d, *J* = 8.1 Hz, 2H, H-2" & H-6"), 6.77 (d, *J* = 8.8 Hz, 1H, H-8), 6.73 (d, *J* = 2.4 Hz, 1H, H-5), 6.66 (dd, *J* = 2.0, 8.4 Hz, 1H, H-7), 4.70 (s, 2H, CH_2-7"), 4.20 (br.s, 4H, CH_2-2 & CH_2-3), 2.91 (s, 3H, CH_3-1'); HR-MS: $[M]^{\bullet+}$ 353.0491 (Calcd. for $C_{16}H_{16}ClNO_4S$; 353.0489); EI-MS: *m/z* 353 $[C_{16}H_{16}ClNO_4S]^{\bullet+}$ (51.5%), 274 $[C_{15}H_{13}ClNO_2]^+$ (100%), 135 $[C_8H_8O_2]^+$ (4.5%), 125 $[C_7H_6Cl]^+$ (60.5%), 111 $[C_6H_4Cl]^+$ (1.5%), 99 $[C_5H_4Cl]^+$ (2.0%), 80 $[C_4HO_2]^+$ (1.9%), 79 $[Me\text{-}SO_4]^+$ (5.9%), 75 $[C_6H_3]^+$ (1.2%), 65 $[C_5H_5]^+$ (1.8%), 51 $[C_4H_3]^+$ (2.5%).

N-(4-Bromobenzyl)-N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)methanesulfonamide **(5g)**

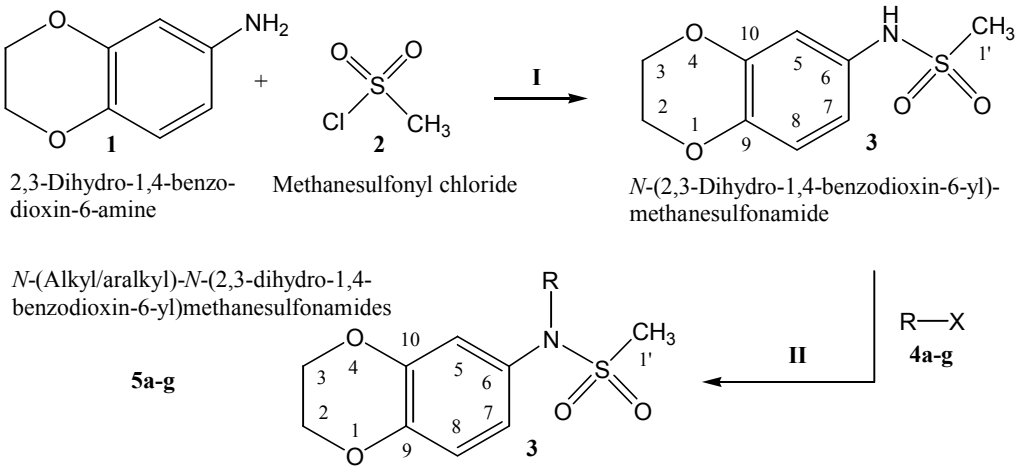
Light brown solid; Yield: 95%; m.p.: 133-134 °C ; Molecular formula: $C_{16}H_{16}BrNO_4S$; Molecular weight: 397 $gmol^{-1}$**;** IR (KBr, cm^{-1}): υ_{max}: 2872 (C-H stretching of aromatic ring), 1506 (C=C stretching of aromatic ring), 1326 (-SO_2 stretching), 1150 (C-O-C stretching of ether), 614 (C-Br stretching); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 7.38 (br.d, *J* = 8.0 Hz, 2H, H-3" & H-5"), 7.12 (br.d, *J* = 8.4 Hz, 2H, H-2" & H-6"), 6.77 (d, *J* = 8.8 Hz, 1H, H-8), 6.73 (d, 1H, *J* = 2.4 Hz, H-5), 6.66 (dd, *J* = 2.4, 8.8 Hz, 1H, H-7), 4.69 (s, 2H, CH_2-7"), 4.20 (br.s, 4H, CH_2-2 & CH_2-3), 2.91 (s, 3H, CH_3-1'); HR-MS: $[M]^{\bullet+}$ 396.9974 (Calcd. for $C_{16}H_{16}BrNO_4S$; 396.9983); EI-MS: *m/z* 399 $[C_{16}H_{16}BrNO_4S+2]^{\bullet+}$ (41.6 %), 397 $[M]^{\bullet+}$ (40.8 %), 318 $[C_{15}H_{13}BrNO_4S]^+$ (100%), 169 $[C_7H_6Br]^+$ (52.3%), 154 $[C_6H_4Br]^+$ (2.6%), 135 $[C_8H_7O_2]^+$ (5.6%), 129 $[C_4H_2Br]^+$ (1.5%), 107 $[C_7H_3O_2]^+$ (4.9%), 81 $[C_4H_1O_2]^+$ (1.6%), 79 $[Me\text{-}SO_4]^+$ (7.7%), 75 $[C_6H_4]^+$ (1.9%), 65 $[C_5H_5]^+$ (2.2%), 51 $[C_4H_3]^+$ (4.7%).

Hemolytic Study

Hemolytic activity was studied by the reported method of Powell *et. al.* [20,21]. 3.0 mL fresh heparin added human blood obtained from volunteers after guidance from the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. The study protocol was approved from the director graduate studies (Institutional Ethical Committee) vide notification No. DGS/8786-89 dated 09-03-2015, University of Agriculture, Faisalabad, Pakistan [22] and was conducted in accordance with the 1964 declaration of Helsinki and its later amendments [23].

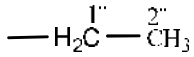
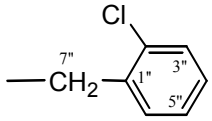
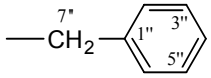
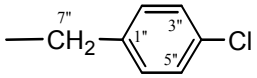
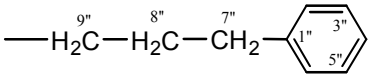
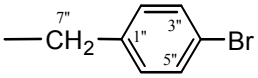
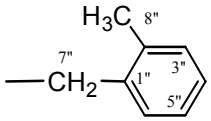
Results and Discussion:

A series of compounds bearing sulfamoyl functionality along with 1,4-benzodioxane moiety have been synthesized by the protocol sketched in Scheme-1. Different alkyl/aralkyl groups utilized in the synthesis are given in Table-1. The synthesized compounds were evaluated for their hemolytic activity to ascertain their cytotoxicity profile.



Scheme-1: Outline for the synthesis of *N*-(alkyl/aralkyl)-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)methanesulfonamides (**5a-g**). Reagents & Conditions: (I) Aq. 10% Na_2CO_3 soln./pH 9-10/stirring at RT for 3-4 hrs. (II) DMF/LiH/stirring at RT for 2-3 hrs.

Table-1: Different alkyl/aralkyl halides (**4a-g**) utilized in the synthesis of N-(alkyl/aralkyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)methanesulfonamides (**5a-g**).

Compd.	-R	Compd.	-R
4a, 5a		4e, 5e	
4b, 5b		4f, 5f	
4c, 5c		4g, 5g	
4d, 5d			

Chemistry

The structures of all the synthesized compounds were confirmed through spectral data of IR, ^{1}H-NMR, HR-MS and EI-MS. The structural analysis of one of the compounds is discussed hereby in detail for the expediency of the readers. The molecule **5g** was obtained as light brown solid in 95% yield, having a melting point of 133-134 °C. The IR spectrum of compound **5g** displayed C-H stretching of aromatic ring at 2872 cm^{-1}, C=C stretching of aromatic ring 1506 cm^{-1}, SO_2 stretching at 1326 cm^{-1} and C-O-C stretching of ether at 1150 cm^{-1}. Its molecular formula was confirmed through its EI-MS showing molecular ion peak at *m/z* 397 and by counting the number of protons in its ^{1}H-NMR spectrum (Fig. 1). An isotopic peak at *m/z* 399 was also observed in the EI-MS spectrum due to bromine atom and hence assigned as $[M+2]^+$ peak. The suggested mass fragmentation pattern of this molecule has been outlined in Fig.2. The 4-bromobenzyl moiety in the molecule was corroborated by an A_2B_2 spin system represented by two *ortho*-coupled doublets at δ 7.38 (br.d, J = 8.0 Hz, 2H, H-3" & H-5") and 7.12 (br.d, J = 8.4 Hz, 2H, H-2" & H-6") in the aromatic region of its ^{1}H-NMR spectrum along with a benzylic methylene singlet at δ 4.69 (s, 2H, CH_2-7"). Similarly, the 1,4-benzodioxane moiety in the molecule was depicted by an ABX spin system represented by an *ortho*-coupled doublet at δ 6.77 (d, J = 8.8 Hz, 1H, H-8), a *meta*-coupled doublet at δ 6.73 (d, 1H, J = 2.4 Hz, H-5), and corresponding doublet of doublets at δ 6.66 (dd, J = 2.4, 8.8 Hz, 1H, H-7). The two symmetrical methylenes appeard as a broad singlet at δ 4.20 (4H, CH_2-2 & CH_2-3). The most upfield singlet δ 2.91 (3H, CH_3-1') was peculiar for the methyl group attached with a sulfonyl moiety. Hence, on the basis of aforementioned cumulative evidences, the structure of **5g** was confirmed as *N*-(4-bromobenzyl)-*N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)methanesulfonamide. In a similar way, the structures of all other synthesized molecules were deduced through such spectral investigations.

Table-2: Cytotoxicity (hemolytic) data of synthesized *N*-aralkyl/alkyl-*N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)methanesulfonamides (**5a-g**).

Compound	5a	5b	5c	5d	5e	5f	5g	Control	
Cytotoxicity%	4.40	6.25	2.46	4.27	7.21	3.36	1.42	PBS 0.5	Triton X 100

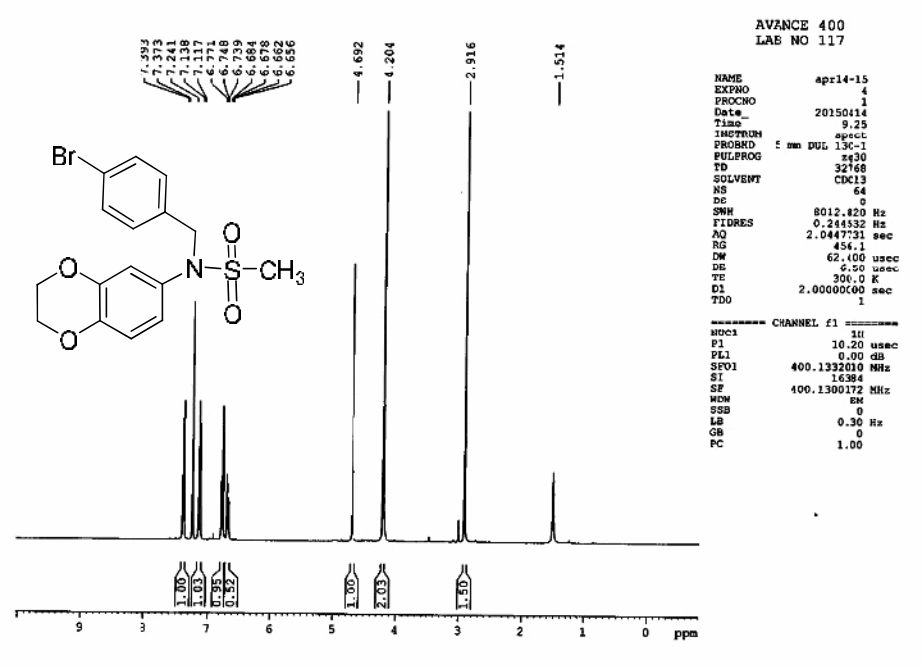


Fig. 1: ^{1}H-NMR Spectrum of *N*-(4-bromobenzyl)-*N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)methanesulfonamide (**5g**).

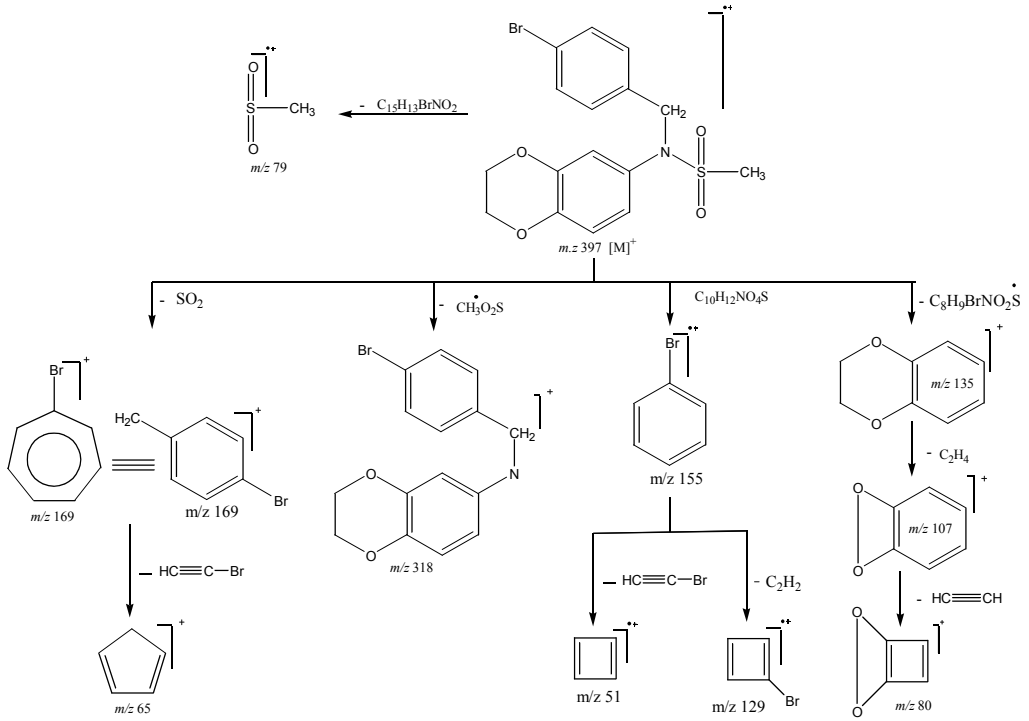


Fig. 2: Suggested mass fragmentation pattern of *N*-(4-bromobenzyl)-*N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)methanesulfonamide (**5g**).

Hemolytic Activity

The synthesized *N*-(alkyl/aralkyl)-*N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)methanesulfonamides (**5a-g**) were evaluated for their cytotoxicity profile through hemolytic study on fresh heparin added human blood. The results are tabulated in (Table-2). Overall, the synthesized molecules, **5a-g**, exhibited very weak cytotoxicity levels. Amongst them, **5g**, possessed the least cytotoxicity (1.42%), where, PBS (0.5%) and Triton X (100%) were used as controls. The least cytotoxity of this molecule mignt be attributed the presence of 4-bromobenzyl group in it. Similarly, the molecule **5c** also exhibited a low cytotoxicity with percentage hemolysis of 2.46%. Here it was concluded that the insertion of methylene groups (as in 3-phenylpropyl group) can render low toxicity to molecule. It was also ascribed that the substitution of any substituent at *ortho*-position in a benzyl unit was outcoming in an increase in the cytotoxicy of the molecules. This trend was apparent by the hemolysis values of **5d** (4.27%) and **5e** (7.21%) in the series.

Conclusion

The synthesized molecules exhibited very modest cytotoxicity and hence these might be utilized as possible therapeutic agents.

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