Synthesis and Enzyme Inhibitory Studies of Some New *N*-Alkylated/Aralkylated *N*-(4-Ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxin-6-sulfonamides

[1]Muhammad Athar Abbasi*, [1]Mudassar Islam, [1]Aziz-Ur-Rehman, [1]Sabahat Zahra Siddiqui,
[2]Khalid Mohammed Khan, [3]Muhammad Ashraf and [3]Humna Asghar
[1]*Department of Chemistry, Government College University, Lahore-54000, Pakistan.*
[2]*HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.*
[3]*Department of Chemistry, The Islamia University of Bahawalpur, Bahawalpur-63100, Pakistan.*
atrabbasi@yahoo.com; abbasi@gcu.edu.pk*

(Received on 17th June 2015, accepted in revised form 16th May 2016)

Summary: The research endeavor was aimed to synthesize *N*-alkyl/aralkylated-*N*-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamides and to evaluate their enzyme inhibitory potential. The target molecules were synthesized in two steps. The first step involved the reaction of 4-ethoxyaniline (1) with *N*-2,3-dihydrobenzo[1,4]-dioxin-6-sulfonyl chloride (2) under dynamic pH control maintained by 10% aqueous Na_2CO_3 to yield *N*-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide (3). In second step parent compound 3 was reacted with various alkyl/aralkyl halides (4a-l) in *N,N'*-dimethylformamide and catalytic amount of lithium hydride to accomplish some new *N*-alkyl/aralkylated-*N*-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamides (5a-l). Probable structures of the synthesized compounds were characterized by contemporary spectral techniques i.e. IR, ^{1}H-NMR and EIMS and were finally evaluated for enzyme inhibitory potential against α-glucosidase and urease. The synthesized compounds exhibited moderate to weak therapeutic potential throughout the series.

Keywords: *N*-(4-Ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide, Alkyl/Aralkyl halides, Spectral analysis, Enzyme Inhibition Activity.

Introduction

Sulfonamides; commonly known as sulfa drugs are widely utilized against eradicating various infections in animal and humans. [1]. Sulfonamides possess broad range of various bioactivities and are considered as important pharmaceutical candidates. They are extensively consumed as anti-bacterial agents [2]. Different analogues of sulfonamides are known to possess anti-microbial, anti-convulsant, hypoglycemic and diuretic properties. Furthermore, they act as cysteine protease and carbonic anhydrase inhibitors [3, 4]. The sulfa drug moiety is a part of potent anti-tumor drugs and osteogenic agents. The amides of sulfonic acids have also been involved in the synthesis of heterocyclic compounds [5] and dendrimers [6]. During division of microtubules aryl sulfonamides are act as antitumor agent in G1 phase of cell cycle [7].

Sulfonamides containing benzodioxane ring systems possess diverse biological activities e.g. anti-inflammatory [10] and α-adrenergic blocking agent [12]. *Silybum marianum* isolate Silymarin has been found to exhibit potent anti-hepatotoxic activity The 1,4-benzodioxane moiety has valuable importance in biological active compounds like Americana A6 and haedoxan A7 having anti-hepatotoxic and insecticidal activity. [11]. It has been known to contain three flavonolignan isomers i.e. silybin, silydianin and silychristin amongst which Silybin, is the main component, containing benzodioxane ring system and constitute about 20-30 % of total flavonolignans [13, 14]. Amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors are potentiated by amides of benzodioxin-6-piperonylic and carboxylic acids. Such compounds are of great interest for treatment of various neurodegenerative diseases e.g. Alzheimer's disease [15, 16]. Pyruvate kinase PKM2 activators contain benzodioxane moiety e.g. 2-[2,3-dihydrobenzo(1,4)-dioxin-6-ylthio]-1-(2-methyl-1-(methylsulfonyl)-indolin-5-yl)ethanone (Fig. 1) which perturb cellular proliferation and influence cancer metabolism [17, 18].

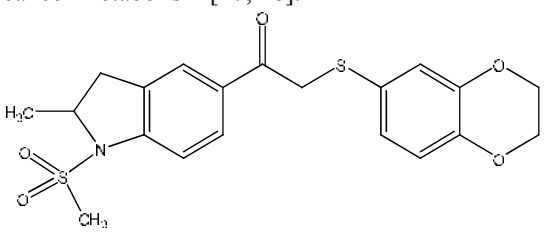


Fig. 1: Structure of 2-[2,3-dihydrobenzo(1,4)-dioxin-6-ylthio]-1-(2-methyl-1-(methylsulfonyl)-indolin-5-yl)ethanone

*To whom all correspondence should be addressed.

Aryl sulfonamides bearing benzodioxane moiety have been identified as ExoU inhibitors with no cytotoxicity [19]. The sulfonamides have gained interest of researchers because of anti-inflammatory, anti-thyroid, anti-cancer, anti-viral, etc. activities and as inhibitors of cyclohydrogenase, lipoxygenase, HIV protease, etc [20-24]. Due to these remarkable pharmacological properties and their development in pharmaceutical sector encouraged us to synthesize new sulfonamides derivatives encompassing 1,4-benzodioxane core.

In present work, a series of *N*-alkyl/aralkylated-*N*-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamides (**5a-m**) were synthesized and were screened for anti-enzymatic potential and the results revealed that they displayed moderate to weak inhibitory potential against *α*-glucosidase and urease.

Experimental

General

The chemicals consumed in the research were acquired from Sigma Aldrich/Fluka. The solvents used were of analytical grade. Progress of reactions was monitored on pre-coated silica gel G-25-UV_{254} in various proportions of *n*-hexane and ethyl acetate. Melting points of compounds were recorded on Gallen Kamp melting point apparatus by open capillary tube. FTIR spectra were recorded on MIDAC M 2000 photon spectrometer. Burker spectrometer, operating at 25 °C at 400 MHz, was used to record the ^{1}H-NMR spectra in $CDCl_3$. The coupling constant (*J*) is given in hertz (Hz) and chemical shift *δ* in ppm. Finnigan MAT-312 spectrometer was used to measure mass spectra.

Synthesis

N-(4-Ethoxyphenyl)-2,3-dihydrobenzo-[1,4]dioxin-6-sulfonamide (**3**)

4-Ethoxyaniline (0.25 mL; 0.002 mol; **1**) was suspended in 25 mL distilled water and 10 % aqueous Na_2CO_3 was added to maintain the pH at 9-10 and reaction mixture were stirred for half an hour after which 2,3-dihydrobenzo[1,4]-dioxin-6-sulfonyl chloride (0.46 g; 0.002 mol; **2**) was added in the mixture along with gradual stirring and was further stirred for 2 hours. The completion of reaction was monitored by TLC till single spot. The product was precipitated at pH 2 using conc. HCl and was filtered, washed with distilled water and air-dried to achieve *N*-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]dioxin-6-sulfonamide **3** as off-white powder; Yield: 87 %; m.p: 189 °C; Molecular formula: $C_{16}H_{17}NO_5S$; Molecular weight: 335 gmol^{-1}; IR (KBr, cm^{-1}): ν_{max}: 3045 (C-H stretching of aromatic ring), 2988 (-CH_2 stretching), 1639 (C=C stretching of aromatic ring) and 1380 (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 500 MHz, *δ* in ppm): 7.14 (d, *J* = 7.6 Hz, 2H, H-2' & H-6'), 6.91 (d, *J* = 7.6 Hz, 2H, H-3' & H-5'), 6.67 (d, *J* = 1.9 Hz, 1H, H-5), 6.54 (dd, *J* = 1.4, 8.0 Hz, 1H, H-7), 6.21 (d, *J* = 6.4 Hz, 1H, H-8), 4.19-4.04 (m, 4H, CH_2-2 & CH_2-3), 3.21 (q, *J* = 7.2 Hz, 2H, -$\underline{OCH_2}$), 1.29 (t, *J* = 7.0 Hz, 3H, $OCH_2\underline{CH_3}$).

N-Alkyl/aralkylated-N-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamides (**5a-l)**

N-(4-Ethoxyphenyl)-2,3-dihydrobenzo-[1,4]dioxin-6-sulfonamide (0.2 g; 0.57 mmol; **3**) in *N,N'*-dimethylformamide (DMF, 10 mL) was taken in 50 mL round-bottomed flask along with lithium hydride (LiH, 0.004 g). The reaction mixture was stirred for 30 min at 25 °C then alkyl/aralkyl halides (0.57 mmol; **4a-l**) were added in reaction mixture which was further stirred for 3 h. The reaction was monitored by TLC till single spot. After completion the reaction mixture was quenched with ice and precipitates were filtered, washed and air-dried to obtain pure *N*-alkyl/aralkylated-*N*-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]dioxin-6-sulfonamides (**5a-l**).

N-Ethyl-N-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide (**5a**)

Grayish semi-solid; Yield: 82 %; Molecular formula: $C_{18}H_{21}NO_5S$; Molecular weight: 363 gmol^{-1}; HR-MS: $[M]^+$ 363.4295 (calculated. For $C_{18}H_{21}NO_5S$; 363.4296); IR (KBr, cm^{-1}): ν_{max}: 3047 (C-H stretching of aromatic ring), 2981 (-CH_2 stretching), 1633 (C=C stretching of aromatic ring), 1379 (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 500 MHz, *δ* in ppm): 7.20 (d, *J* = 2.2 Hz, 1H, H-5), 7.08 (dd, *J* = 2.2, 9.5 Hz, 1H, H-7), 6.98 (d, *J*= 9.5 Hz, 2H, H-2' & H-6'), 6.89 (d, *J* = 8.5 Hz, 1H, H-8), 6.82 (d, *J*= 9.5 Hz, 2H, H-3' & H-5'), 4.34-4.29 (m, 4H, CH_2-2 & CH_2-3), 4.04 (q, *J* = 7.0 Hz, 2H, -$\underline{OCH_2}$), 3.56 (q, *J* = 7.1 Hz, 2H, CH_2-1"), 1.43 (t, *J* = 9.0 Hz, 3H, $OCH_2\underline{CH_3}$), 1.07 (t, *J* = 7.1 Hz, 3H, CH_3-2"); EIMS: (*m/z*) $[M]^+$ 363 $(C_{18}H_{21}NO_5S)^{\cdot+}$, 335 $(C_{16}H_{17}NO_5S)^{\cdot+}$, 299 $(C_{18}H_{21}NO_3)^{\cdot+}$, 242 $(C_{10}H_{12}NO_4S)^+$, 178 $(C_{10}H_{12}NO_2)^+$, 164 $(C_{10}H_{14}NO)^+$, 29 $(C_2H_5)^+$.

N-Iso-propyl-N-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide (**5b**)

Grey powder; Yield: 85 %; m.p: 143 °C; Molecular formula: $C_{19}H_{23}NO_5S$; Molecular weight:

377 $gmol^{-1}$; HR-MS: $[M]^+$ 377.4565 (calculated. For $C_{19}H_{23}NO_5S$; 377.4566); IR (KBr, cm^{-1}): v_{max}: 3040 (C-H stretching of aromatic ring), 2980 (-CH_2 stretching), 1639 (C=C stretching of aromatic ring), 1383 (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 7.31 (d, *J* = 1.7 Hz, 1H, H-5), 7.23 (dd, *J* = 1.8, 6.4 Hz, 1H, H-7), 7.00 (d, *J* = 7.1 Hz, 2H, H-2' & H-6'), 6.90 (d, *J* = 7.1 Hz, 1H, H-8), 6.84 (d, *J* = 7.1 Hz, 2H, H-3' & H-5'), 4.39-4.29 (m, 4H, CH_2-2 & CH_2-3), 4.56 (sept., *J* = 5.6 Hz, CH-1"), 4.05 (q, *J* = 5.8 Hz, 2H, -$\underline{OCH_2}$), 1.44 (t, *J* = 5.0 Hz, 3H, $OCH_2\underline{CH_3}$), 1.07 (d, *J* = 5.6 Hz, 6H, CH_3-2" & CH_3-3"); EIMS: (*m/z*) $[M]^+$ 377 $(C_{19}H_{23}NO_5S)^{\cdot+}$, 349 $(C_{17}H_{19}NO_5S)^{\cdot+}$, 313 $(C_{19}H_{23}NO_3)^{\cdot+}$, 256 $(C_{11}H_{14}NO_4S)^+$, 178 $(C_{10}H_{13}NO_2)^+$, 43 $(C_3H_7)^+$.

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

White amorphous solid; Yield: 89 %; m.p: 145 °C; Molecular formula: $C_{23}H_{23}NO_5S$; Molecular weight: 425 $gmol^{-1}$; HR-MS: $[M]^+$ 425.4984 (calculated. For $C_{23}H_{23}NO_5S$; 425.4982); IR (KBr, cm^{-1}): v_{max}: 3055 cm^{-1} (C-H stretching of aromatic ring), 2928 cm^{-1} (-CH_2 stretching), 1620 cm^{-1} (C=C stretching of aromatic ring) and 1305 cm^{-1} (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 7.28 (d, *J* = 2.0 Hz, 1H, H-5), 7.25-7.22 (m-merged in $CDCl_3$ signal, 5H, H-2" to H-6"), 7.15 (dd, *J* = 2.0, 8.4 Hz, 1H, H-7), 6.94 (d, *J* = 8.4 Hz, 1H, H-8), 6.90 (d, *J* = 8.8 Hz, 2H, H-3' & H-5'), 6.72 (d, *J* = 8.8 Hz, 2H, H-2' & H-6'), 4.69 (s, 2H, CH_2-7"), 4.38-4.32 (m, 4H, CH_2-2 & CH_2-3), 3.97 (q, *J* = 6.8 Hz, 2H, -$O\underline{CH_2}$), 1.39 (t, *J* = 6.8 Hz, 3H, $OCH_2\underline{CH_3}$); EIMS: (*m/z*) $[M]^+$ 425 $(C_{23}H_{23}NO_5S)^{\cdot+}$, 397 $(C_{21}H_{19}NO_5S)^{\cdot+}$, 361 $(C_{23}H_{23}NO_3)^{\cdot+}$, 304 $(C_{15}H_{14}NO_4S)^+$, 240 $(C_{15}H_{14}NO_4)^+$, 226 $(C_{15}H_{16}NO)^+$, 199 $(C_8H_7O_4S)^+$, 135 $(C_8H_7O_2)^+$, 121 $(C_8H_9O)^+$, 91 $(C_7H_7)^+$, 95 $(C_6H_7O)^+$, 65 $(C_5H_5)^+$.

N-2-Ethylphenyl-N-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide (**5d**)

Brownish pallets; Yield: 94 %; m.p: 112 °C; Molecular formula: $C_{24}H_{25}NO_5S$; Molecular weight: 439 $gmol^{-1}$; HR-MS: $[M]^+$ 439.5255 (calculated. For $C_{24}H_{25}NO_5S$; 439.5254); IR (KBr, cm^{-1}): v_{max}: 3049 (C-H stretching of aromatic ring), 2930 (-CH_2 stretching), 1629 (C=C stretching of aromatic ring), 1359 (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 7.25-7.21 (m-merged in $CDCl_3$ signal, 2H, H-3" to H-5"), 7.18 (d, *J* = 2.5 Hz, 1H, H-5), 7.12 (d, *J* = 7.1 Hz, 1H, H-8), 7.08 (d, *J* = 9.0 Hz, 2H, H-2" & H-6"), 6.99 (dd, *J* = 2.5, 9.0 Hz, 1H, H-7), 6.94 (d, *J* = 10.0 Hz, 2H, H-2' & H-6'), 6.81 (d, *J* = 10.0 Hz, 2H, H-3' & H-5'), 4.28-4.26 (m, 4H, CH_2-2 & CH_2-3), 4.02 (q, *J* = 9.0 Hz, 2H, -$O\underline{CH_2}$), 3.68 (t, *J* = 9.5 Hz, 2H, CH_2-8"), 2.73 (t, *J* = 9.5 Hz, 2H, CH_2-7"), 1.40 (t, *J* = 9.0 Hz, 3H, $OCH_2\underline{CH_3}$); EIMS: (*m/z*) $[M]^+$ 439 $(C_{24}H_{25}NO_5S)^{\cdot+}$, 411 $(C_{22}H_{21}NO_5S)^{\cdot+}$, 375 $(C_{24}H_{25}NO_3)^{\cdot+}$, 318 $(C_{16}H_{16}NO_4S)^{\cdot+}$, 254 $(C_{16}H_{16}NO_2)^+$, 240 $(C_{16}H_{18}NO)^+$, 105 $(C_8H_9)^+$, 199 $(C_8H_7O_4S)^+$, 135 $(C_8H_7O_2)^+$, 121 $(C_8H_9O)^+$, 91 $(C_7H_7)^+$, 95 $(C_6H_7O)^+$, 65 $(C_5H_5)^+$.

N-2-Propylphenyl-N-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide (**5e**)

Brown pallets; Yield: 92 %; m.p: 138 °C; Molecular formula: $C_{25}H_{27}NO_5S$; Molecular weight: 453 $gmol^{-1}$; HR-MS: $[M]^+$ 453.5523 (calculated. For $C_{25}H_{27}NO_5S$; 453.5524); IR (KBr, cm^{-1}): v_{max}: 3245 (N-H stretching), 3055 (C-H stretching of aromatic ring), 2933 (-CH_2 stretching), 1638 (C=C stretching of aromatic ring), 1379 (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 7.28 (br.s, 1H, H-5), 7.19-7.16 (m, 3H, H-3" to H-5"), 7.11 (d, *J* = 7.1 Hz, 2H, H-2" & H-6"), 7.08 (dd, *J* = 1.3, 7.0 Hz, 1H, H-7), 7.00 (d, *J* = 7.2 Hz, 2H, H-2' & H-6'), 6.99 (d, *J* = 7.2 Hz, 1H, H-8), 6.83 (d, *J* = 7.2 Hz, 2H, H-3' & H-5'), 4.33-4.29 (m, 4H, CH_2-2 & CH_2-3), 4.04 (q, *J* = 5.7 Hz, 2H, -$O\underline{CH_2}$), 3.56 (t, *J* = 5.7 Hz, 2H, CH_2-9"), 2.67 (t, *J* = 6.5 Hz, 2H, -CH_2-7"), 1.76 (quint., *J* = 5.8 Hz, 2H, -CH_2-8"), 1.43 (t, *J* = 5.7 sHz, 3H, $OCH_2\underline{CH_3}$); EIMS: (*m/z*) $[M]^+$ 453 $(C_{25}H_{27}NO_5S)^{\cdot+}$, 425 $(C_{23}H_{23}NO_5S)^{\cdot+}$, 389 $(C_{25}H_{27}NO_3)^{\cdot+}$, 334 $(C_{16}H_{16}NO_5S)^{\cdot+}$, 270 $(C_{16}H_{16}NO_3)^+$, 254 $(C_{17}H_{20}NO)^+$, 119 $(C_9H_{11})^+$, 91 $(C_7H_7)^+$, 65 $(C_5H_5)^+$.

N-2-Chlorobenzyl-N-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide (**5f**)

Brown semi-solid; Yield: 91 %; Molecular formula: $C_{23}H_{22}ClNO_5S$; Molecular weight: 459 $gmol^{-1}$; HR-MS: $[M]^+$ 459.9436 (calculated. For $C_{23}H_{22}ClNO_5S$; 459.9437); IR (KBr, cm^{-1}): v_{max}: 3251(N-H stretching), 3049 (C-H stretching of aromatic ring), 2966 (-CH_2 stretching), 1635 (C=C stretching of aromatic ring), 1369 (-SO_2 stretching), 704 (C-Cl stretching); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 7.65 (br.d, *J* = 8.4 Hz, 1H, H-3"), 7.62 (ddd, *J* = 1.2, 6.2, 8.6 Hz, 1H, H-4"), 6.81 (br.t, *J* = 8.0 Hz, 1H, H-5"), 6.79 (br.d, *J* = 6.8 Hz, 1H, H-6"), 7.28 (d, *J* = 8.2 Hz, 2H, H-2' & H-6'), 6.77 (d, *J* = 8.2 Hz, 2H, H-3' & H-5'), 6.72 (d, *J* = 1.8 Hz, 1H, H-5), 6.29 (dd, *J* = 1.2, 8.0 Hz, 1H, H-7), 6.04 (d, *J* = 8.2 Hz, 1H, H-8), 4.47 (s, 2H, CH_2-7"), 4.37-4.33 (m, 4H, CH_2-2 & CH_2-3), 4.30 (s, 2H, -CH_2-7"), 3.39 (q, *J* = 8.4 Hz, 2H, -$O\underline{CH_2}$), 1.32 (t, *J* = 8.0 Hz, 3H, $OCH_2\underline{CH_3}$); EIMS (*m/z*): 459 $(C_{23}H_{22}ClNO_5S)^{\cdot+}$, 395 $(C_{23}H_{22}ClNO_3)^{\cdot+}$, 274 $(C_{15}H_{13}ClNO_2)^{\cdot+}$, 260

$(C_{15}H_{15}ClNO)^+$, 199 $(C_8H_7O_4S)^+$, 135 $(C_8H_7O_2)^+$, 125 $(C_7H_6Cl)^+$, 95 $(C_6H_7O)^+$, 86 $(C_4H_3Cl)^+$.

N-3-Chlorobenzyl-N-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide (**5g**)

Brown semi-solid; Yield: 90 %; Molecular formula: $C_{23}H_{22}ClNO_5S$; Molecular weight: 459 $gmol^{-1}$; HR-MS: $[M]^+$ 459.9436 (calculated. For $C_{23}H_{22}ClNO_5S$; 459.9437); IR (KBr, cm^{-1}): ν_{max}: 3251(N-H stretching), 3049 (C-H stretching of aromatic ring), 2966 (-CH_2 stretching), 1635 (C=C stretching of aromatic ring), 1369 (-SO_2 stretching), 704 (C-Cl stretching); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 7.59 (br.s, 1H, H-2"), 7.23 (br.s, 1H, H-5), 7.16-7.14 (m, 3H, H-4" to H-6"), 6.93 (d, J = 7.1 Hz, 1H, H-8), 6.91 (d, J = 7.5 Hz, 2H, H-2' & H-6'), 6.74 (d, J = 7.5 Hz, 2H, H-3' & H-5'), 6.41 (br.d, J = 5.0 Hz, 1H, H-7), 4.66 (s, 2H, CH_2-7"), 4.35-4.32 (m, 4H, CH_2-2 & CH_2-3), 3.97 (q, J = 5.7 Hz, 2H, -O$\underline{CH_2}$), 1.40 (t, J = 5.7 Hz, 3H, $OCH_2\underline{CH_3}$); EIMS (*m/z*): 459 $(C_{23}H_{22}ClNO_5S)^{\cdot+}$, 395 $(C_{23}H_{22}ClNO_3)^{\cdot+}$, 274 $(C_{15}H_{13}ClNO_2)^{\cdot+}$, 260 $(C_{15}H_{15}ClNO)^+$, 199 $(C_8H_7O_4S)^+$, 135 $(C_8H_7O_2)^+$, 125 $(C_7H_6Cl)^+$, 95 $(C_6H_7O)^+$, 86 $(C_4H_3Cl)^+$.

N-4-Chlorobenzyl-N-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide (**5h**)

Dark brown semi-solid; Yield: 94 %; Molecular formula: $C_{23}H_{22}ClNO_5S$; Molecular weight: 459 $gmol^{-1}$; HR-MS: $[M]^+$ 459.9436 (calculated. For $C_{23}H_{22}ClNO_5S$; 459.9437); IR (KBr, cm^{-1}): ν_{max}: 3251(N-H stretching), 3049 (C-H stretching of aromatic ring), 2966 (-CH_2 stretching), 1635 (C=C stretching of aromatic ring), 1369 (-SO_2 stretching), 704 (C-Cl stretching); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 7.56 (d, J = 8.0 Hz, 2H, H-2" & H-6"), 7.32(d, J = 8.0 Hz, 2H, H-3" & H-5"), 6.92 (d, J= 8.8 Hz, 2H, H-3' & H-5'), 6.72 (d, J = 8.6 Hz, 2H, H-2' & H-6'), 6.58 (d, J = 2.0 Hz, 1H, H-5), 6.51 (dd, J = 1.2, 8.0 Hz, 1H, H-7), 6.31 (d, J = 7.4 Hz, 1H, H-8), 4.89 (s, 2H, CH_2-7"), 4.33-4.12 (m, 4H, CH_2-2 & CH_2-3), 3.69 (q, J = 8.4 Hz, 2H, -O$\underline{CH_2}$), 1.30 (t, J = 6.4 Hz, 3H, $OCH_2\underline{CH_3}$); EIMS (*m/z*): 459 $(C_{23}H_{22}ClNO_5S)^{\cdot+}$, 395 $(C_{23}H_{22}ClNO_3)^{\cdot+}$, 274 $(C_{15}H_{13}ClNO_2)^{\cdot+}$, 260 $(C_{15}H_{15}ClNO)^+$, 199 $(C_8H_7O_4S)^+$, 135 $(C_8H_7O_2)^+$, 125 $(C_7H_6Cl)^+$, 95 $(C_6H_7O)^+$, 86 $(C_4H_3Cl)^+$.

N-2-Bromobenzyl-N-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide (**5i**)

Light yellow semi-solid; Yield: 95 %; Molecular formula: $C_{23}H_{22}BrNO_5S$; Molecular weight: 504 $gmol^{-1}$; HR-MS: $[M]^+$ 504.3954 (calculated. For $C_{23}H_{22}BrNO_5S$; 504.3955); IR (KBr, cm^{-1}): ν_{max}: 3251 (N-H stretching), 3053 (C-H stretching of aromatic ring), 2987 (-CH_2 stretching), 1639 (C=C stretching of aromatic ring), 1391 (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 7.62 (br.d, J = 6.5 Hz, 1H, H-3"), 7.41 (d, J = 6.5 Hz, 1H, H-6"), 7.27 (br.s, 1H, H-5), 7.26 (br.d, J = 6.1 Hz, 1H, H-7), 7.15 (dd, J = 1.7, 7.0 Hz, 1H, H-5"), 7.08 (br.t, J = 6.4 Hz, 1H, H-4"), 7.00 (d, J = 7.4 Hz, 2H, H-2' & H-6'), 6.94 (d, J = 7.0 Hz, 1H, H-8), 6.73 (d, J = 7.4 Hz, 2H, H-3' & H-5'), 4.86 (s, 2H, CH_2-7"), 4.36-4.31 (m, 4H, CH_2-2 & CH_2-3), 3.99 (q, J = 5.8 Hz, 2H, -O$\underline{CH_2}$), 1.36 (t, J = 5.5 Hz, 3H, $OCH_2\underline{CH_3}$); EIMS (*m/z*): 504 $(C_{23}H_{22}BrNO_5S)^{\cdot+}$, 369 $(C_{15}H_{15}BrNO_3S)^{\cdot+}$, 319 $(C_{15}H_{13}BrNO_2)^{\cdot+}$, 305 $(C_{15}H_{15}BrNO)^+$, 199 $(C_8H_7O_4S)^+$, 170 $(C_7H_6Br)^+$, 135 $(C_8H_7O_2)^+$, 130 $(C_4H_3Br)^+$, 121 $(C_8H_9O)^+$, 95 $(C_7H_6O)^+$.

N-4-Bromobenzyl-N-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide (**5j**)

Light yellow semi-solid; Yield: 93 %; Molecular formula: $C_{23}H_{22}BrNO_5S$; Molecular weight: 504 $gmol^{-1}$; HR-MS: $[M]^+$ 504.3954 (calculated. For $C_{23}H_{22}BrNO_5S$; 504.3955); IR (KBr, cm^{-1}): ν_{max}: 3251 (N-H stretching), 3053 (C-H stretching of aromatic ring), 2987 (-CH_2 stretching), 1639 (C=C stretching of aromatic ring), 1391 (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 7.37 (d, J = 8.3 Hz, 2H, H-2" & H-6"), 7.11 (d, J = 8.3 Hz, 2H, H-3" & H-5"), 7.28 (br.s, 1H, H-5), 7.24 (br.d, J = 8.1 Hz, 1H, H-7), 6.87 (d, J = 8.5 Hz, 1H, H-8), 6.82 (d, J = 8.9 Hz, 2H, H-2' & H-6'), 6.73 (d, J = 8.9 Hz, 2H, H-3' & H-5'), 4.63 (s, 2H, CH_2-7"), 4.36-4.31 (m, 4H, CH_2-2 & CH_2-3), 3.98 (q, J = 6.9 Hz, 2H, -O$\underline{CH_2}$), 1.39 (t, J = 6.9 Hz, 3H, $OCH_2\underline{CH_3}$); EIMS (*m/z*): 504 $(C_{23}H_{22}BrNO_5S)^{\cdot+}$, 369 $(C_{15}H_{15}BrNO_3S)^{\cdot+}$, 319 $(C_{15}H_{13}BrNO_2)^{\cdot+}$, 305 $(C_{15}H_{15}BrNO)^+$, 199 $(C_8H_7O_4S)^+$, 170 $(C_7H_6Br)^+$, 135 $(C_8H_7O_2)^+$, 130 $(C_4H_3Br)^+$, 121 $(C_8H_9O)^+$, 95 $(C_7H_6O)^+$.

N-2-Methylbenzyl-N-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide (**5k**)

Gray semi-solid; Yield: 89 %; Molecular formula: $C_{24}H_{25}NO_5S$; Molecular weight: 439 $gmol^{-1}$; HR-MS: $[M]^+$ 439.5256 (calculated. For $C_{24}H_{25}NO_5S$; 439.5257); IR (KBr, cm^{-1}): ν_{max}: 3049 (C-H stretching of aromatic ring), 2930 (-CH_2 stretching), 1629 (C=C stretching of aromatic ring), 1359 (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 7.22 (d, J = 2.5 Hz, 1H, H-5), 7.09 (dd, J = 2.0, 9.8 Hz, 1H, H-7), 7.05-7.02 (m, 2H, H-3" to H-5"), 6.98 (d, J = 10.0 Hz, 1H, H-8), 6.96 (d, J = 9.0

Hz, 1H, H-6"), 6.81 (d, *J* = 9.8 Hz, 2H, H-2' & H-6'), 6.64 (d, *J* = 9.8 Hz, 2H, H-3' & H-5'), 4.66 (s, 2H, CH_2-7"), 4.32-4.28 (m, 4H, CH_2-2 & CH_2-3), 3.91 (q, *J* = 9.0 Hz, 2H, -O$\underline{CH_2}$), 2.31 (s, 3H, CH_3), 1.34 (t, *J* = 8.5 Hz, 3H, $OCH_2\underline{CH_3}$); EIMS: (*m/z)* $[M]^+$ 439 $(C_{24}H_{25}NO_5S)^{\cdot+}$, 411 $(C_{22}H_{21}NO_5S)^{\cdot+}$, 375 $(C_{24}H_{25}NO_3)^{\cdot+}$, 318 $(C_{16}H_{16}NO_4S)^{\cdot+}$, 254 $(C_{16}H_{16}NO_2)^+$, 240 $(C_{16}H_{18}NO)^+$, 105 $(C_8H_9)^+$, 199 $(C_8H_7O_4S)^+$, 135 $(C_8H_7O_2)^+$, 121 $(C_8H_9O)^+$, 91 $(C_7H_7)^+$, 95 $(C_6H_7O)^+$, 65 $(C_5H_5)^+$.

N-4-Fluorobenzyl-N-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide (**5l**)

Gray solid; Yield: 95 %; m.p: 125 °C; Molecular formula: $C_{23}H_{22}FNO_5S$; Molecular weight: 443 $gmol^{-1}$; HR-MS: $[M]^+$ 443.4898 (calculated. For $C_{23}H_{22}FNO_5S$; 443.4899); IR (KBr, cm^{-1}): ν_{max}: 3041 (C-H stretching of aromatic ring), 2987 (-CH_2 stretching), 1633 (C=C stretching of aromatic ring), 1381 (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 7.28 (br.s, 1H, H-5), 7.26 (d, *J* = 7.5 Hz, 1H, H-8), 7.14 (dd, *J* = 1.6, 6.4 Hz, 1H, H-7), 7.19 (d, *J* = 6.7 Hz, 2H, H-2" & H-6"), 6.92 (d, *J* = 6.7 Hz, 2H, H-3" & H-5"), 6.87 (d, *J* = 7.3 Hz, 2H, H-2' & H-6'), 6.72 (d, *J* = 7.3 Hz, 2H, H-3' & H-5'), 4.66 (s, 2H, CH_2-7"), 4.35-4.31 (m, 4H, CH_2-2 & CH_2-3), 3.97 (q, *J* = 6.0 Hz, 2H, -O$\underline{CH_2}$), 1.39 (t, *J* = 6.0 Hz, 3H, $OCH_2\underline{CH_3}$); EIMS: (*m/z)* $[M]^+$ 443 $(C_{23}H_{22}FNO_5S)^{\cdot+}$, 415 $(C_{21}H_{18}FNO_5S)^+$, 379 $(C_{23}H_{22}FNO_3)^{\cdot+}$, 322 $(C_{15}H_{13}FNO_4S)^{\cdot+}$, 258 $(C_{15}H_{13}FNO_2)^+$, 244 $(C_{15}H_{15}FNO)^+$, 199 $(C_8H_7O_4S)^+$, 135 $(C_8H_7O_2)^+$, 121 $(C_8H_9O)^+$, 109 $(C_7H_6F)^+$.

Enzyme Inhibition Assays:

α-Glucosidase Assay

The α-glucosidase inhibition activity was performed according to the slightly modified method [25,26]. Total volume of the reaction mixture was made 100 µL by 70 µL phosphate buffer saline (50 mM, pH 6.8), 10 µL test compound (0.5 mM) and 10 µL enzyme (0.057 $unitswell^{-1}$). The contents were mixed, preincubated for 10 min at 37 ºC and pre-read at 400 nm. The reaction was initiated by the addition of 10 µL of substrate (0.5 mM, *p*-nitrophenylglucopyranoside). Acarbose was used as positive control. After 30 min of incubation at 37 ºC, absorbance was measured at 400 nm using Synergy HT microplate reader. All experiments were carried out in duplicates. The percent inhibition was calculated by the following equation:

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

IC_{50} values (concentration at which there is 50 % in enzyme catalyzed reaction) compounds were calculated using EZ-Fit Enzyme Kinetics Software.

Urease Assay

The urease inhibition assay was performed following the cited methods [27]. Reaction mixtures comprising 25 μL of urease enzyme solution and 55 μL of buffers containing urea (2-24 mM) were incubated with 5 μL of each studied compound, separately, at 30^{o}C for 15 min in DMSO in 96-well plates. The increased absorbance at 560 nm was measured after 10 min., using a microplate reader (Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200 μL. The results (change in absorbance per min) were processed by using SoftMax Pro software (Molecular Device, USA). All the assays were performed at pH 6.8 (3 mM sodium phosphate buffer) and 7 µg of phenol red per ml as indicator. Percentage inhibitions were calculated from 100-($OD_{testwell}/OD_{control}$) × 100. Thiourea was used as the standard inhibitor of urease.

Results and Discussion

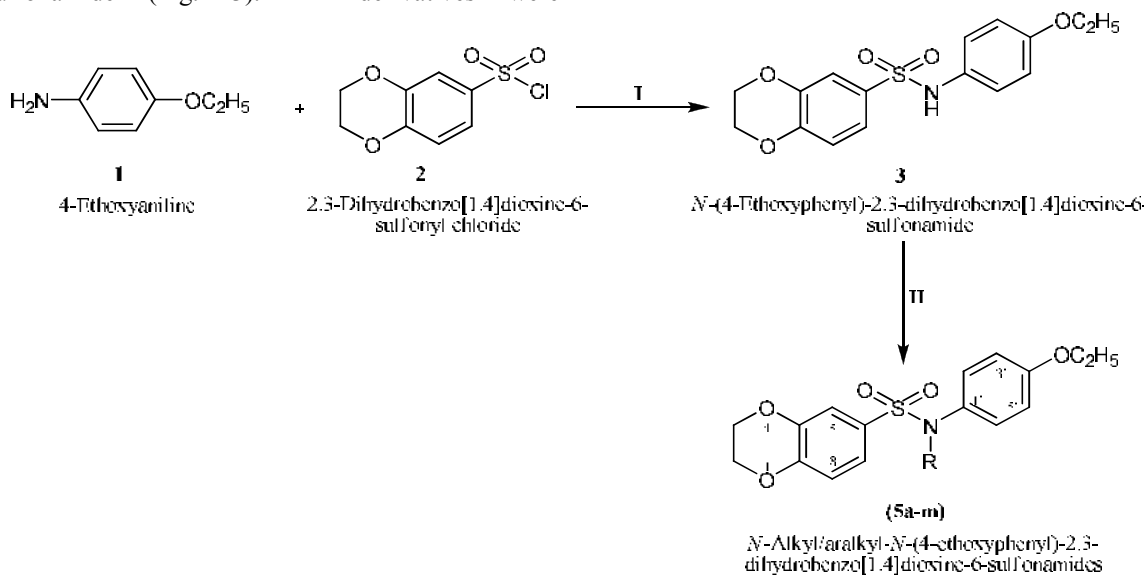
Chemistry

N-Alkyl/aralkylated-*N*-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]dioxin-6-sulfonamides were synthesized according to the outline illustrated in (**5a-l**; Scheme-1 & Table-1). The procedures and reaction conditions are elaborated in experimental portion. The synthesis took place in two steps, the first step involved the reaction of 2,3-dihydrobenzo[1,4]dioxine-6-sulfonyl chloride (**1**) with 4-ethoxyaniline (**2**) in aqueous alkali at pH 9 under stirring at room temperature for 2 h to afford *N*-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]dioxin-6-sulfonamide (**3**) which was obtained as pure product under acidic condition generated with few aliquots of concentrated HCl at pH 2. The second step involved the coupling of **3** with a series of alkyl/aralkyl halides (**4a-l**; Table-1) in DMF; a polar aprotic solvent and lithium hydride which was added in catalytic amount as a base [28] to achieve *N*-alkyl/aralkyl-*N*-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]dioxin-6-sulfonamides (**5a-l**). The plausible structures of synthesized derivatives were explicated by contemporary structural techniques e.g. ^{1}H-NMR, IR and EIMS. For example compound **5c** was obtained as a white amorphous solid in 89 % yield, m.p. 145 °C having molecular formula, $C_{23}H_{23}NO_5S$ and molecular weight 425 $gmol^{-1}$. The functionalities in the compound were confirmed appearance of absorption bands in IR spectra at 3055 cm^{-1} (C-H stretching of aromatic ring), 2928 cm^{-1} (-CH_2

stretching), 1620 cm^{-1} (C=C stretching of aromatic ring) and 1305 cm^{-1} (-SO_2 stretching). The molecular formula was established by appearance of molecular ion peak at *m/z* 425 $[M]^+$ (Fig. 2) and by counting the number of protons in the ^{1}H-NMR spectrum. In aromatic region of spectrum the peaks for benzodioxane ring appeared as a doublet at δ 7.28 having *meta* coupling of 2.0 Hz for proton positioned at 5, a doublet of doublet appeared at δ 7.15 having an *ortho* and a *meta* coupling of 2.0 and 8.4 Hz respectively for proton at 7and finally a doublet for H-8 proton appeared at δ 6.94 having *J* of 8.4 Hz. The attachment of 4-ethoxyphenyl group was confirmed by appearance of an A_2B_2 system as di-*ortho* coupled doublets at δ 6.90 and δ 6.72 for protons positioned at 3' & 5' and 2' & 6' respectively. The signal of benzyl ring resonated in form of multiplet at δ 7.25-7.22 having integration of 5Hs positioned at 2"-6". In the aliphatic region of the spectrum a singlet appeared at δ 4.69 for methylene protons of benzyl group positioned at 7", a multiplet resonated at δ 4.38-4.32 having integration of 4Hs positioned for CH_2-2 & CH_2-3 protons. The presence of ethoxy linkage was further confirmed by appearance of a quartet at δ 3.97 for 2Hs of methylene protons attached to oxygen atom. Finally a triplet appeared at δ 1.39 for three protons of CH_3 group. On the basis of aforementioned data compound **5c** was designated as *N*-benzyl-*N*-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide (Fig. 3). All derivatives were characterized in a similar pattern and characteristic pattern of 1,4-benzodioxane moiety was observed in all of the *N*-substituted derivatives. Moreover, the appearance of singlet of methylene protons which is an important peak in incorporation of alkyl/aralkyl group onto parent sulfonamide further confirmed the attachment. The 4-ethoxy group was confirmed by appearance of signals as quartet and triplet. A_2B_2 spins system was observed as di-*ortho*coupled doublets in all the *para*-substituted aralkyl groups. The alkyl groups were confirmed by appearance of signals as quartet and triplet in case of ethyl group and as septet and doublet in case of *iso*-propyl group.

Enzyme Inhibition Assays

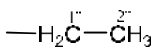
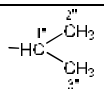
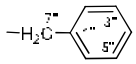
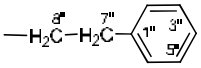
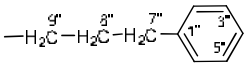
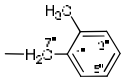
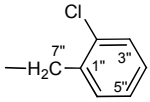
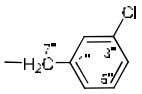
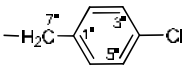
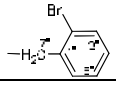
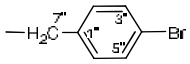
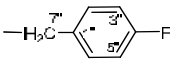
The synthesized *N*-alkyl/aralkylated-*N*-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamides (**5a-l**) displayed moderate to weak enzyme inhibition activity as tabulated in (Table-2). (*N*-Benzyl-*N*-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide; **5c**) displayed decent activity as evident from the IC_{50} values i.e. 227.35±0.18 µM and 123.54±0.12 µM against α-glucosidase and urease respectively as compared to acarbose (38.25±0.12 µM) and thiourea (21.25±0.15 µM). This can be interpreted that insertion of benzyl moiety at *N*-position of the parent *N*-4-ethoxyphenyl-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide.



Scheme-1**:** Schematic outline for the synthesis of *N*-Alkyl/aralkylated-*N*-(4-ethoxyphenyl)-2,3,-dihydrobenzo[1,4]dioxine-6-sulfonamides (**5a-l**).

Reagents & Conditions: (**I**) 2,3-Dihydrobenzo[1,4]dioxine-6-sulfonyl chloride **1**/10 % Na_2CO_3/distilled water/4-ethoxyaniline **2**/stirring/2 hours. (**II**) *N*-(4-Ethoxyphenyl)-2,3-dihydrobenzo-[1,4]dioxin-6-sulfonamide **3**/Alkyl/Aralkyl halides (**4a-l**) /DMF/LiH/stirring/RT/3 hours.

Table-1: Different Alkyl/aralkyl halides utilized in the synthesis of *N*-Alkyl/aralkylated-*N*-(4-ethoxyphenyl)-2,3-dihydrobenzo[1,4]dioxin-6-sulfonamides (**4a-l)**.

Code	R	Code	R	Code	R
4a		**4b**		**4c**	
4d		**4e**		**4f**	
4g		**4h**		**4i**	
4j		**4k**		**4l**	

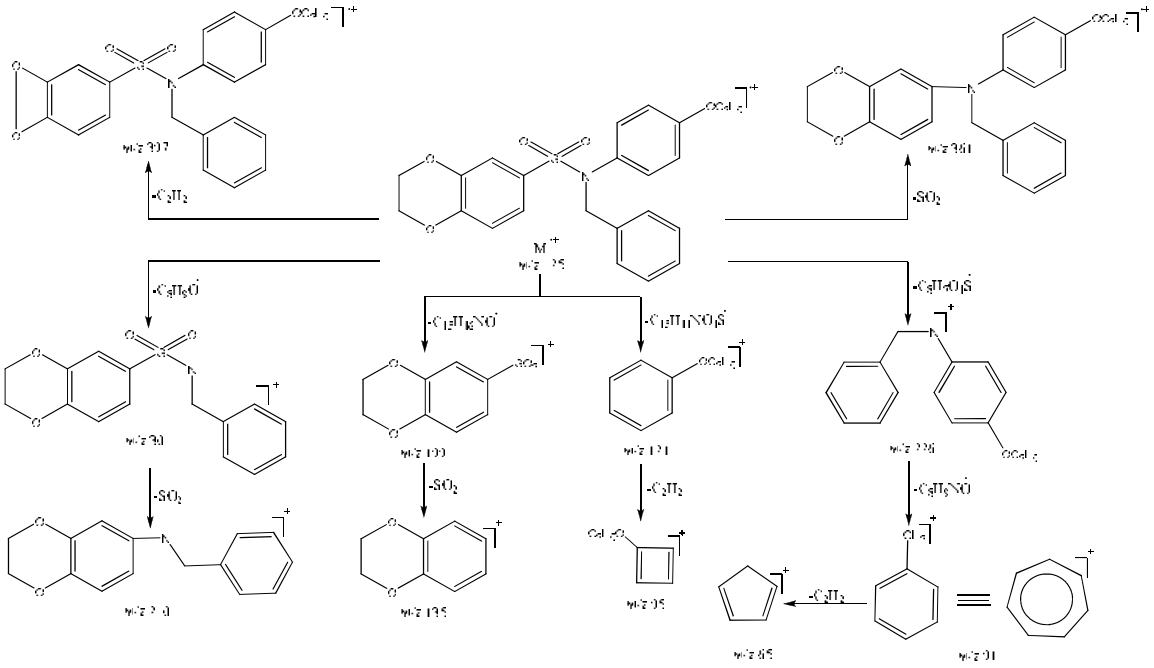


Fig. 2: Proposed mass fragmentation pattern of *N*-Benzyl-*N*-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide **5c**.

Table-2: Enzyme Inhibition Activity of *N*-Alkyl/aralkylated-*N*-(4-ethoxyphenyl)-2,3-dihydrobenzo[1,4]dioxin-6-sulfonamides (**5a-l)**.

Enzymes	*α*-Glucosidase		Urease	
Codes	**% age Inhibition 0.5 mM**	**IC_{50} (µM)**	**% age Inhibition 0.5 mM**	**IC_{50} (µM)**
5a	**35.98±0.16**	**243.61±0.14**	**56.71±0.35**	**363.65±0.21**
5b	**39.54±0.12**	**-**	**48.14±0.15**	**-**
5c	**16.45±0.23**	**227.35±0.18**	**57.71±0.29**	**123.54±0.12**
5d	**71.98±0.24**	**-**	**41.71±0.13**	**-**
5e	**12.52±0.15**	**-**	**46.58±0.12**	**-**
5f	**84.17±0.37**	**-**	**45.12±0.16**	**-**
5g	**32.52±0.11**	**-**	**35.48±0.12**	**-**
5h	**89.17±0.18**	**-**	**46.71±0.24**	**-**
5i	**36.27±0.13**	**-**	**85.84±0.18**	**375.87±0.24**
5j	**39.72±0.24**	**-**	**58.64±0.18**	**364.76±0.12**
5k	**41.98±0.26**	**364.65±0.17**	**54.71±0.24**	**396.74±0.19**
5l	**78.89±0.13**	**369.69±0.18**	**55.78±0.19**	**387.82±0.13**
Control	**Acarbose 92.23±0.14**	**38.25±0.12**	**Thiourea 98.45±0.87**	**21.25±0.15**

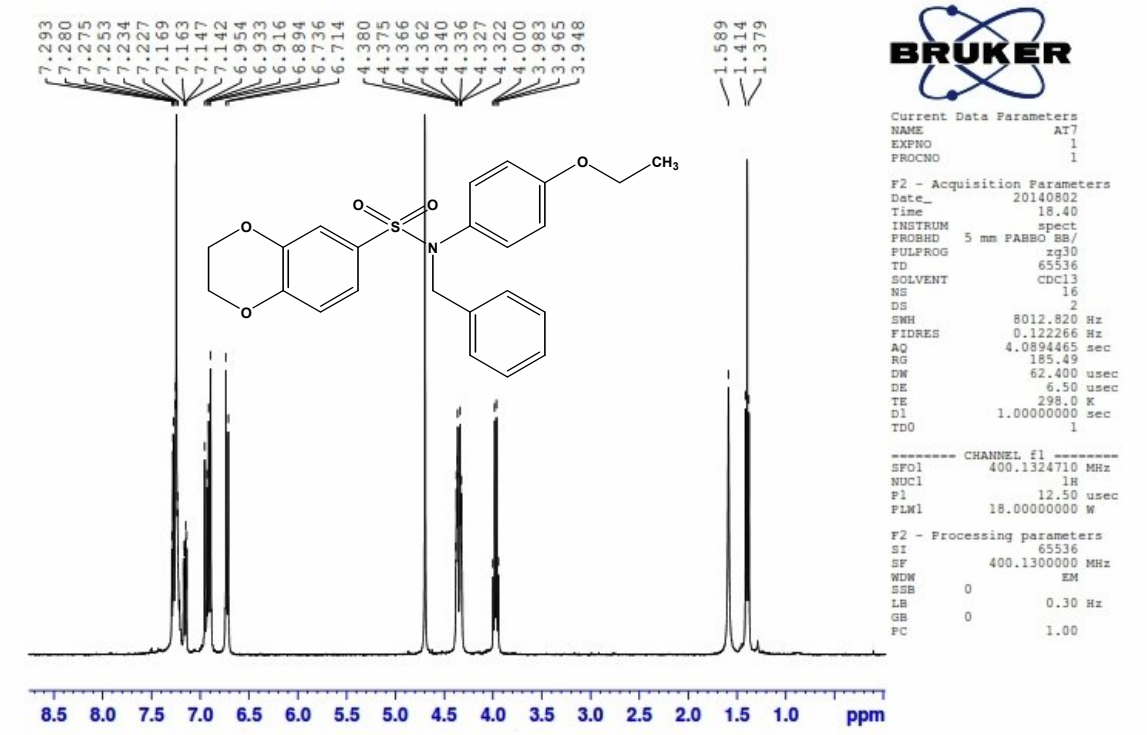


Fig. 3: ^{1}H-NMR of *N*-Benzyl-*N*-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide **5c**.

Conclusion

N-Alkyl/aralkylated-*N*-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamides (**5a-l**) were synthesized in good yield and their spectral data able-bodied the proposed structures. It can be concluded that all synthesized compounds possess moderate to weak enzyme inhibitory potential against *α*-glucosidase and urease, especially *N*-benzyl-*N*-(4-ethoxyphenyl)-2,3-dihydrobenzo-[1,4]-dioxine-6-sulfonamide; **5c** demonstrated fair activity against *α*-glucosidase and urease as compared to standards; acarbose and thiourea respectively which may be manifested by the incorporation of benzyl moiety at *N*-position of the parent sulfonamide.

Acknowledgement

The authors are thankful to Higher Education Commission (HEC) of Pakistan for providing financial grant for this study.

References

1. P. Dauban and R. H. Dodd, Synthesis of Cyclic Sulfonamides via Intramolecular Copper-Catalyzed Reaction of Unsaturated Iminoiodinanes *Org. Lett*., **2**, 2327 (2000).
2. W. R. Roush, J. Cheng, B. Knapp-Reed, A. Alvarez-Hernandez, J. H. McKerrow, E. Hansell and J. C. Engel, Vinyl Sulfonate Esters and Vinyl Sulfonamides: Potent, Irreversible Inhibitors of Cysteine Proteases, *J. Am. Chem. Soc.*, **120**, 10994 (1998).
3. V. Tougu, Acetylcholinesterase: Mechanism of Catalysis and Inhibition, *Curr. Med. Chem-Central Nervous System Agents,* **1**, 155 (2001).
4. G. D. Hartman, W. Halczenko, J. D. Prugh, R. L. Smith, M. F. Sugrue, P. Mallorga, S. R.Michelson, W. C. Randall, H. Schwam and J. M. Sondey, Thieno[2,3-B]Furan-2-Sulfonamides As Topical Carbonic Anhydrase Inhibitors, *J. Med. Chem.,* **35**, 3027 (1992).
5. R. Cremlyn, *An Introduction to Sulphur Chemistry*, Wiley, New York, p. 224 (1996).
6. O. Lukin, V. Gramlich, R. Kandre, I. Zhun, T. Felder, C. A. Schalley and G. Dolgonos, Designer Dendrimers: Branched Oligosulfonimides with Controllable Molecular Architectures, *J. Am. Chem. Soc*., **128**, 8964 (2008).
7. K. Isik, and F. O. Kocak, Antimicrobial Activity Screening of Some Sulfonamide Derivatives on Some *Nocardia* Species and Isolates, *Microbio. Res.,* **164**, 49 (2009).
8. D. Caballero, J. R. Torres-Lapasio, J. J. Baeza-Baeza and M. C. Garcia-Alvarez-Coque, Micellar Chromatographic Procedure with Direct Injection for the Determination of Sulfonamides in Milk

and Honey Samples, *J. Liq. Chromatogr. Rel. Technol*., **24**, 117 (2001).

9. L. Mallesha and K. N. Mohana, Synthesis, Antimicrobial and Antioxidant Activities of Novel 4-aryl-1-(1,4- benzodioxane-2-carbonyl)piperazines, *Eur. J. Chem*. **2**, 193 (2011).
10. M. T. Vazquez, G. Rosell and M. D. Pujol, Synthesis and Anti-Inflammatory Activity of Rac-2-(2,3-dihydro-1,4-benzodioxin)propionic acid and its *R* and *S* Enantiomers, *Eur. J. Med. Chem.,* **32**, 529 (1997).
11. A. Ahmed, S. A. Khan and T. Alam, Synthesis and Anti-Hepatotoxic Activity of Some Heterocyclic Compounds Containing the 1,4-dioxane Ring System, *Pharmazie*., **58**, 173 (2003).
12. C. B. Chapleo, P. L. Myers, C. M. Butler, J. C. Doxey, A. G. Roach and C. F. C. Smith, Alpha.-Adrenoreceptor Reagents. Synthesis of some 1,4-benzodioxans as selective presynaptic alpha 2-Adrenoreceptor Antagonists and Potential Antidepressants, *J. Med. Chem.,* **26**, 823 (1983).
13. S. A. Khan, B. Ahmed and T. Alam, Synthesis and Anti-Hepatotoxic Activity of Some New Chalcones Containing 1,4-dioxane Ring System, *Pak. J. Pharm. Sci*., **19**, 290 (2006).
14. P. R. Davis-Searles, Y. Nakanishi, N. C. Kim, T. N. Graf, N. H. Oberlies and M. C. Wani, Milk Thistle and Prostate Cancer: Differential Effects of Pure Flavonolignans from *Silybum marianum* on Anti-Proliferative End Points in Human Prostate Carcinoma Cells, *Cancer Res*., **65**, 4448 (2005).
15. A. Arai, M. Kessler and P. Xiao, A Centrally Active Drug that Modulates AMPA Receptor Gated Currents, *Brain Res.,* **638**, 343 (1994).
16. M. J. O. Neill, D. Bleakman and D. M. Zimmerman, AMPA Receptor Potentiators for the Treatment of CNS Disorders, *Curr. Drug Targets: CNS Neurol. Disord*., **3**, 181 (2004).
17. Y. Avihai, O. Rachel, K. Tzofit, M. Sima, S. Daniel, A. Alex, S. Alina, B. Z. Efrat, S. Nili, B. K. Osnat, K. Alexander, B. Vered and M. B. Oren, 1-(Sulfonyl)-5-(arylsulfonyl)indoline as Activators of the Tumor Cell Specific M2 Isoform of Pyruvate Kinase, *Bioorg. Med. Chem. Lett.,* **22**, 6460 (2012).
18. J. W. Martin, R. B. Kyle, V. Henrike, M. B. James, D. Thomas, L. William, C. C. Lewis, J. I. William, G. V. H. Matthew, S. Min, S. A. Douglas, J. T. Craig and B. B. Matthew, 2-Oxo-*N*-aryl-1,2,3,4-tetrahydroquinoline-6-sulfonamides as Activators of the Tumor Cell Specific M2 Isoform of Pyruvate Kinase, *Bioorg. Med. Chem. Lett.,* **21**, 6322 (2011).
19. M. Irshad, M. A. Abbasi, Aziz-Ur-Rehman, S. Z. Siddiqui, M. Ashraf, S. A. Ejaz, M. A. Lodhi and S. B. Jamal, Synthesis, Characterization, and Biological Screening of Some New Sulfonamide Derivatives of 1,4-benzodioxane-6-amine, *J. Chem. Soc. Pak.,* **36**, 660 (2014).
20. K. Doran, B. Jihae, S. Jiho, B. Hyeyoung, M. Hyeyoung and H. M. Kyung, Identification of Arylsulfonamides as ExoU Inhibitors, *Bioorg. Med. Chem. Lett*., **24**, 3823 (2014).
21. N. S. EI-Sayed, E. R. EI-Bendary, S. M. EI-Ashry and M. EI-Kerdawy, Synthesis and Antitumor Activity of New Sulfonamide Derivatives of Thiadiazolo[3,2-*a*]pyrimidines, *Eur. J. Med. Chem*., **46**, 3714 (2011).
22. M. J. Garcia-Galan, M. Silvia Diaz-Cruz and D. Barcelo, Identification and Determination of Metabolites and Degradation Products of Sulfonamide Antibiotics, *TrAC Trends Anal. Chem*., **27**, 1008 (2008).
23. G. L. Perlovich, N. N. Strakhova, V. P. Kazachenko, T. V. Volkova, V. V. Tkachev, K. J. Schaper and O. A. Raevsky, Sulfonamides as a Subject to Study Molecular Interactions in Crystals and Solutions: Sublimation, Solubility, Solvation, Distribution and Crystal Structure, *Int. J. Pharm*., **349**, 300 (2008).
24. M. K. Parai, G. Panda, K. Srivastava and S. K. Puri, Design, Synthesis and Anti-Malarial Activity of Benzene and Isoquinoline Sulfonamide Derivatives, *Bioorg. Med. Chem. Lett*., **18**, 776 (2008).
25. P. Chapdelaine, R. R. Tremblay, and J. Y. Dubè., *p*-Nitrophenol-*á*-D-glucopyranoside as Substrate for Measurement of Maltase Activity in Human Semen. *Clin. Chem*., **24**, 208 (1978).
26. M. A. Abbasi, V. U. Ahmad, M. Zubair, M. A. Rashid, S. N. Khan, U. Farooq, M. I. Choudhary and K. P. Zeller, A New α-Glucosidase Inhibiting Dithiadiazetidin Derivative from *Symplocos racemosa*, *Heterocycles,* 65, 1837 (2005).
27. M. A. Abbasi, M. A. Lodhi, Aziz-ur-Rehman, V. U. Ahmad and M. I. Chaudhary, Kinetics Studies on Symplocomoside: A Urease Inhibitor, *J. Chem. Soc. Pak.*, **34**, 43 (2012).
28. M. A. Abbasi, A. Saeed, Aziz-ur-Rehman, K. M. Khan, M. Ashraf and S. A. Ejaz, Synthesis of Brominated 2-phenitidine Derivatives as Valuable Inhibitors of Cholinestersaes for the Treatment of Alzheimer's disease, *Iran. J. Pharm. Res.*, **13**, 87 (2014).