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Synthesis, Bacterial biofilm inhibition and cytotoxicity of new *N*-Alkyl/aralkyl-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-nitrobenzenesulfonamides

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Abstract: The current research was commenced by reaction of 1,4-benzodioxane-6-amine (**1**) with 4-nitrobenzenesulfonyl chloride (**2**) in the presence of aqueous base under dynamic pH control at 9 to yield *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-nitrobenzenesulfonamide (**3**) which was further reacted with a series of alkyl/aralkyl halides (**4a-i**) in polar aprotic solvent using catalytic amount of lithium hydride which acts as base to afford some new *N*-alkyl/aralkyl-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-nitrobenzenesulfonamides (**5a-i**). The projected structures of all the synthesized derivatives were characterized by contemporary techniques i.e., IR, ^{1}H-NMR and EIMS. The biofilm Inhibitory action of all synthesized molecules was carried out against *Escherichia coli* and *Bacillus subtilis*. It was inferred from their results that 5f and 5e exhibited suitable inhibitory action against the biofilms of these bacterial strains. Moreover, their cytotoxicity was also checked and it was concluded that these synthesized molecules displayed docile cytotoxicity.

Keywords: *N*-(2,3-Dihydro-1,4-benzodioxin-6-yl)-4-nitrobenzenesulfonamide, alkyl/ aralkyl halides, spectral analysis, biofilm inhibition, antibacterial, cytotoxicity.

INTRODUCTION

Sulfonamides are compounds bearing -$NHSO_2$ group and represent an important class of medicinally effective drugs. They are largely employed and systematically used because of their low cost, less toxicity and excellent bioactivities against various infections and bacterial diseases. Some important derivatives of sulfonamide are known to possess anti-microbial, anti-cancer, anti-convulsant and diuretic properties (Connor, 1998). Sulfa drugs have shown multiple applications in medical field as good antiviral HIV and protease inhibitors e.g. Amprenavir utilized in treatment Alzheimer's disease (Kołaczek *et al*., 2014). Furthermore, they exhibit powerful inhibition against carbonic anhydrase and cysteine protease (Brendan *et al*., 2007). Advanced studies revealed that aryl sulfonamides work as anti-tumor agents during the distribution of microtubules in G1 phase of cell cycle (Kamil *et al*., 2009). Some sulfonamide derivatives exhibit CNS activity. Sulfonamides bearing 1,4-benzodioxane core exhibited various biological activities like anti-hepatotoxic (Ahmed *et al*., 2003), anti-inflammatory (Vázquez *et al*., 1997) and α-adrenergic blocking agents (Chapleo *et al*., 1983). Silymarin isolated from *Silybum marianum* exhibit potent antihepato-toxic potential against several 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, contains 1,4-benodioxane ring system and possesses remarkable anti-hepatotoxic activity (Searles *et al*., 2005). Aryl sulfonamides having benzodioxane moiety have been identified as ExoU inhibitors (*Irshad et al*., 2014). So, owing to the broad applicability and remarkable pharmacological activity of sulfonamides, we synthesized a new series of sulfonamides bearing 1,4-benzodioxane heterocyclic core to explore their biofilm inhibitory potential against *Escherichia coli* and *Bacillus subtilis*.

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MATERIALS AND METHODS

General

All the chemicals/solvents utilized were of analytical grade and procured from Sigma Aldrich/Fluka. Reactions were monitored on pre-coated silica gel G-25-UV254 plates using various percentages of *n*-hexane and ethyl acetate as a mobile phase and visualized on UV lamp at 254 nm. Gallenkamp melting point apparatus was used for measuring melting points. IR peaks were recorded on MIDAC M 2000 spectrometer with wave number in cm^{-1}. ^{1}H-NMR signals were noted in $CDCl_3$ on Burker spectrometer operating at 500 MHz. Chemical shifts (δ) are depicted in ppm and coupling constant (J) in hertz

(Hz). Finnigan MAT-312 instrument was used to measure mass spectra (EIMS).

Synthesis

Synthesis of N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-nitrobenzenesulfonamide (3).

1,4-Benzodioxane-6-amine (2mL; 0.02mol; 1) was suspended in 50mL distilled water and the 10mL 10% aqueous Na_2CO_3 was poured into reaction mixture, stirred well up to 0.5 hour, then 4-nitrobenzenesulfonyl chloride (3.61 g; 0.016 mol; 2) was added in the reaction media. Reaction mixture was further stirred for 4 hours along with monitoring by TLC till single spot which show the completion of reaction. After the end of reaction, product was collected by filtration, washed in distilled water and dried to afford pure *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-nitrobenzenesulfonamide (3) as greenish amorphous powder in 95% yield having m.p. 122˚C, molecular formula $C_{14}H_{12}N_2O_6S$ and molecular weight 336 $gmol^{-1}$; IR (KBr, cm^{-1}): v_{max}: 3252 (N-H stretching), 3047 (C-H stretching of aromatic ring), 1642 (C=C stretching of aromatic ring), 1387 (-SO_2 stretching), 1330 (-NO_2 stretching), 1158 (C-O-C stretching of ether); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 9.95 (s, -NH, 1H), 8.35 (d, *J* = 8.8 Hz, H-3' & H-5', 2H), 7.86 (d, *J*=8.8 Hz, H-2' & H-6', 2H), 6.82 (d, *J*=8.5 Hz, H-8, 1H), 6.59 (d, *J*=2.5 Hz, H-5,1H), 6.49 (dd, *J*=2.5, 8.5 Hz, H-7,1H), 4.29-4.26 (m, CH_2-2 & CH_2-3, 4H).

General procedure for the N-Alkyl/aralkyl-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-nitrobenzenesulfonamides (5a-i)

N-(2,3-Dihydro-1,4-benzodioxin-6-yl-4-nitrobenzenesulfonamide (0.1g; 0.30 mmol; 3) solubilized in dimethyl formamide DMF (10.0 mL) was taken in 50 mL round bottomed flask, followed by the addition of lithium hydride (0.1mmol) to the reaction mixture which was stirred for 30 minutes at 25°C. Different alkyl/aralkyl halides (4a-i; 0.07mol) were added in reaction mixture which was further stirred for 4-5 h. The reaction was monitored by TLC till single spot. After completion the reaction mixture was poured in crushed ice and precipitates were filtered, washed and air-dried to get pure products.

Spectral characterization of synthesized compounds

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-N-ethyl-4-nitrobenzenesulfonamide (5a)

Peach colored solid; Yield: 85%; m.p. 162°C; Molecular formula: $C_{16}H_{16}N_2O_6S$; Molecular weight: 364 $gmol^{-1}$; IR (KBr, cm^{-1}): *v* 3056 (C-H str. of aromatic ring), 2981 (-CH_2 str.), 1625 (C=C aromatic str.), 1383 (-SO_2 stretching), 1341 (-NO_2 stretching), 1147 (C-O-C stretching of ether); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 8.35 (d, *J*=8.5 Hz, H-3' & H-5', 2H), 7.89 (d, *J* = 8.5 Hz, H-2' & H-6', 2H), 6.67 (d, *J*=8.5 Hz, H-8, 1H), 6.52 (d, *J*=2.5 Hz, H-5, 1H), 6.42 (dd, *J*=2.5, 8.5 Hz, H-7, 1H), 4.29-4.26 (m, CH_2-2 & CH_2-3, 4H), 3.10 (q, *J*=7.0 Hz, CH_2-1″, 2H). 1.15 (t, *J*=7.5 Hz, CH_3-2″, 3H); EIMS (*m/z*): 364 [M; $C_{16}H_{16}N_2O_6S]^{\cdot+}$, 336 $[C_{14}H_{12}N_2O_6S]^+$, 335 $[C_{14}H_{11}N_2O_6S]^+$, 300 $[C_{16}H_{16}N_2O_4]^+$, 242 $[C_{10}H_{12}NO_4S]^+$, 186 $[C_6H_4NO_4S]^+$, 178 $[C_{10}H_{12}NO_2]^+$, 135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 107 $[C_6H_3O_2]^+$, 96 $[C_4H_2NO_2]^+$, 81 $[C_4HO_2]^+$, 75 $[C_6H_3]^+$, 51 $[C_4H_3]^+$, 29 $[C_2H_5]^+$.

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-4-nitro-N-(1-propyl)benzenesulfonamide (5b)

Brown solid; Yield: 92%; m.p: 159°C; Molecular formula: $C_{17}H_{18}N_2O_6S$; Molecular weight: 378 $gmol^{-1}$; IR (KBr, cm^{-1}): *v* 3066 (C-H str. of aromatic ring), 2985 (-CH_2 stretching), 1665 (C=C aromatic str.), 1370 (-SO_2 stretching), 1310 (-NO_2 stretching), 1151 (C-O-C stretching of ether); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 8.35 (d, *J*=8.5 Hz, H-3' & H-5', 2H), 7.89 (d, *J* = 8.5 Hz, H-2' & H-6', 2H), 6.67 (d, *J*=8.5 Hz, H-8, 1H), 6.52 (d, *J*=2.5 Hz, H-5, 1H), 6.42 (dd, *J*=2.5, 8.5 Hz, H-7, 1H), 4.55 (sep, *J* =6.5 Hz, H-1″,1H), 4.29-4.26 (m, CH_2-2 & CH_2-3, 4H), 3.16 (t, *J*=7.0 Hz, CH_2-1″, 2H), 1.57 (sext., *J*=7.2 Hz, CH_2-2″, 2H). 0.90 (t, *J*=7.6 Hz, CH_3-3″, 3H); EIMS (*m/z*): 378 [M; $C_{17}H_{18}N_2O_6S]^{\cdot+}$, 350 $[C_{15}H_{14}N_2O_6S]^+$, 335 $[C_{14}H_{11}N_2O_6S]^+$, 314 $[C_{17}H_{18}N_2O_4]^+$, 256 $[C_{11}H_{14}NO_4S]^+$, 192 $[C_{11}H_{14}NO_2]^+$, 186 $[C_6H_4NO_4S]^+$, 135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 107 $[C_6H_3O_2]^+$, 96 $[C_4H_2NO_2]^+$, 81 $[C_4HO_2]^+$, 75 $[C_6H_3]^+$, 51 $[C_4H_3]^+$, 43 $[C_3H_7]^+$.

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-4-nitro-N-(2-propyl)benzenesulfonamide (5c)

Brown solid; Yield: 90%; m.p: 157°C; Molecular formula: $C_{17}H_{18}N_2O_6S$; Molecular weight: 378 $gmol^{-1}$; IR (KBr, cm^{-1}): *v* 3067 (C-H str. of aromatic ring), 2983 (-CH_2 stretching), 1665 (C=C aromatic str.), 1375 (-SO_2 stretching), 1345 (-NO_2 stretching), 1152 (C-O-C stretching of ether); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 8.35 (d, *J*=8.5 Hz, H-3' & H-5', 2H), 7.89 (d, *J*= 8.5 Hz, H-2' & H-6', 2H), 6.67 (d, *J*=8.5 Hz, H-8, 1H), 6.52 (d, *J*=2.5 Hz, H-5, 1H), 6.42 (dd, *J*=2.5, 8.5 Hz, H-7, 1H), 4.55 (sept., *J*=6.5 Hz, H-2″,1H), 4.29-4.26 (m, CH_2-2 & CH_2-3, 4H), 1.04 (d, *J*=6.7 Hz, CH_3-1″ & CH_3-3″, 6H); EIMS (*m/z*): 378 [M; $C_{17}H_{18}N_2O_6S]^{\cdot+}$, 350 $[C_{15}H_{14}N_2O_6S]^+$, 335 $[C_{14}H_{11}N_2O_6S]^+$, 314 $[C_{17}H_{18}N_2O_4]^+$, 256 $[C_{11}H_{14}NO_4S]^+$, 192 $[C_{11}H_{14}NO_2]^+$, 186 $[C_6H_4NO_4S]^+$, 135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$ 107 $[C_6H_3O_2]^+$, 96 $[C_4H_2NO_2]^+$, 81 $[C_4HO_2]^+$, 75 $[C_6H_3]^+$, 51 $[C_4H_3]^+$, 43 $[C_3H_7]^+$.

N-(1-Butyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-nitrobenzenesulfonamide (5d)

Brown solid; Yield: 87%; m.p: 95°C; Molecular formula: $C_{18}H_{20}N_2O_6S$; Molecular weight: 392 $gmol^{-1}$; IR (KBr, cm^{-1}): *v* 3072 (C-H str. of aromatic), 3012 (-CH_2 stretching), 1666 (C=C str. of aromatic), 1342 (-SO_2 stretching), 1305 (-NO_2 stretching), 1162 (C-O-C stretching of ether); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 8.35 (d, *J*=8.5 Hz, H-3' & H-5', 2H), 7.89 (d, *J*= 8.5

Hz, H-2' & H-6', 2H), 6.67 (d, *J*=8.5 Hz, H-8, 1H), 6.52 (d, *J*=2.5 Hz, H-5, 1H), 6.42 (dd, *J*=2.5, 8.5 Hz, H-7, 1H), 4.29-4.26 (m, CH_2-2 & CH_2-3, 4H), 3.16 (t, *J*=7.6 Hz, 2H, CH_2-1", 2H), 1.49-1.31 (m, CH_2-2" & CH_2-3", 4H,), 0.90 (t, *J*=6.8 Hz, CH_3-4", 3H); EIMS (*m/z*): 392 [M; $C_{18}H_{20}N_2O_6S]^{\cdot+}$, 364$[C_{16}H_{16}N_2O_6S]^+$, 335$[C_{14}H_{11}N_2O_6S]^+$, 328$[C_{18}H_{20}N_2O_4]^+$, 270$[C_{12}H_{16}NO_4S]^+$, 206 $[C_{12}H_{16}NO_2]^+$, 186 $[C_6H_4NO_4S]^+$, 135 $[C_8H_7O_2]^+$, 107 $[C_6H_3O_2]^+$, 122 $[C_6H_4NO_2]^+$, 96 $[C_4H_2NO_2]^+$, 81 $[C_4HO_2]^+$, 75 $[C_6H_3]^+$, 57 $[C_4H_9]^+$ 51 $[C_4H_3]^+$.

N-(2-Butyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-nitrobenzenesulfonamide (5e)

Golden yellow solid; Yield: 87%; m.p: 151°C; Molecular formula: $C_{18}H_{20}N_2O_6S$; Molecular weight: 392 $gmol^{-1}$; IR (KBr, cm^{-1}): *v* 3067 (C-H str. of aromatic ring), 2985 (-CH_2 stretching), 1665 (C=C str. of aromatic), 1373 (-SO_2 stretching), 1320 (-NO_2 stretching), 1151 (C-O-C stretching of ether); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 8.35 (d, *J*=8.5 Hz, H-3' & H-5', 2H), 7.89 (d, *J*=8.5 Hz, H-2' & H-6', 2H), 6.67 (d, *J*=8.5 Hz, H-8, 1H), 6.52 (d, *J*=2.5 Hz, H-5, 1H), 6.42 (dd, *J*=2.5, 8.5 Hz, H-7, 1H), 4.31-4.27 (m, CH_2-2, CH_2-3, & H-2″, 5H), 1.59-1.39 & 1.33-1.26 (m, H_a-3" & H_b-3", 2H, diastereotopic), 1.06 (d, *J* = 6.8 Hz, CH_3-1", 3H,), 0.90 (br.t, *J* = 7.5 Hz, CH_3-4", 3H); EIMS (*m/z*): 392 [M; $C_{18}H_{20}N_2O_6S]^{\cdot+}$, 364 $[C_{16}H_{16}N_2O_6S]^+$, 335$[C_{14}H_{11}N_2O_6S]^+$, 328$[C_{18}H_{20}N_2O_4]^+$, 270$[C_{12}H_{16}NO_4S]^+$, 206$[C_{12}H_{16}NO_2]^+$, 186 $[C_6H_4NO_4S]^+$, 135 $[C_8H_7O_2]^+$, 107 $[C_6H_3O_2]^+$, 122 $[C_6H_4NO_2]^+$, 96 $[C_4H_2NO_2]^+$, 81 $[C_4HO_2]^+$, 75 $[C_6H_3]^+$, 57 $[C_4H_9]^+$, 51 $[C_4H_3]^+$.

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-4-nitro-N-(1-pentyl)benzenesulfonamide (5f)

Brown solid; Yield: 87%; m.p: 116°C; Molecular formula: $C_{19}H_{22}N_2O_6S$; Molecular weight: 406 $gmol^{-1}$; IR (KBr, cm^{-1}): *v* 3077 (C-H str. of aromatic ring), 3011 (-CH_2 stretching), 1656 (C=C str. of aromatic), 1359 (-SO_2 stretching), 1325 (-NO_2 stretching), 1163 (C-O-C stretching of ether); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 8.35 (d, *J*=8.5 Hz, H-3' & H-5', 2H), 7.89 (d, *J*=8.5 Hz, H-2' & H-6', 2H), 6.67 (d, *J*=8.5 Hz, H-8, 1H), 6.52 (d, *J*=2.5 Hz, H-5, 1H), 6.42 (dd, *J*=2.5, 8.5 Hz, H-7, 1H), 4.29-4.26 (m, CH_2-2 & CH_2-3, 4H), 3.65 (t, *J*=7.6 Hz, 2H, CH_2-1"), 1.34-1.16 (m, 6H CH_2-2" to CH_2-4", 6H), 0.80 (t, *J*=6.8 Hz, 3H, CH_3-5", 3H); EIMS (*m/z*): 406 [M; $C_{19}H_{22}N_2O_6S]^{\cdot+}$, 378 $[C_{17}H_{18}N_2O_6S]^+$, 342 $[C_{19}H_{22}N_2O_4]^+$, 335 $[C_{14}H_{11}N_2O_6S]^+$, 284 $[C_{13}H_{18}NO_4S]^+$, 220 $[C_{13}H_{18}NO_2]^+$, 186 $[C_6H_4NO_4S]^+$, 135 $[C_8H_7O_2]^+$, 107 $[C_6H_3O_2]^+$, 122 $[C_6H_4NO_2]^+$, 96 $[C_4H_2NO_2]^+$, 81 $[C_4HO_2]^+$, 75 $[C_6H_3]^+$, 71 $[C_5H_{11}]^+$, 51 $[C_4H_3]^+$.

N-Benzyl-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-nitrobenzenesulfonamide (5g)

Brown amorphous solid; Yield: 88%; m.p: 153°C; Molecular formula: $C_{21}H_{18}N_2O_6S$; Molecular weight: 426 $gmol^{-1}$, IR (KBr, cm^{-1}): *v* 3022 (C-H str. of aromatic ring), 2975 (-CH_2 stretching), 1631 (C=C str. of aromatic), 1409 (-SO_2 stretching), 1350 (-NO_2 stretching), 1144 (C-O-C stretching of ether); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 8.35 (d, *J*=8.8 Hz, H-3' & H-5', 2H), 7.86 (d, *J*=8.8 Hz, H-2' & H-6', 2H), 7.28-7.23 (m, H-2″ to H-6″, 5H), 6.66 (d, *J*=8.5 Hz, H-8, 1H), 6.52 (d, *J*=2.5 Hz, H-5, 1H), 6.41 (dd, *J*=2.5, 8.5 Hz, H-7, 1H), 4.42 (s, CH_2-7″, 2H), 4.29-4.26 (m, CH_2-2 & CH_2-3, 4H); EIMS (*m/z*): 426 [M; $C_{21}H_{18}N_2O_6S]^{\cdot+}$, 398 $[C_{19}H_{14}N_2O_6S]^+$, 388 $[C_{18}H_{16}N_2O_6S]^+$, 380 $[C_{21}H_{18}NO_4S]^+$, 316 $[C_{21}H_{18}NO_2]^+$, 240 $[C_{15}H_{14}NO_2]^+$, 186 $[C_6H_4NO_4S]^+$,135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 107 $[C_6H_3O_2]^+$, 91 $[C_7H_7]^+$, 81 $[C_4HO_2]^+$, 75 $[C_6H_3]^+$, 65 $[C_5H_5]^+$.

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-4-nitro-N-(phenethyl)benzenesulfonamide (5h)

Dark brown solid; Yield: 79%; m.p: 112°C; Molecular formula: $C_{22}H_{20}N_2O_6S$; Molecular weight: 440 $gmol^{-1}$; IR (KBr, cm^{-1}): *v* 3023 (C-H str. of aromatic ring), 2980 (-CH_2 stretching), 1637 (C=C str. of aromatic), 1377 (-SO_2 stretching), 1340 (-NO_2 stretching), 1153 (C-O-C stretching of ether); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 8.35 (d, *J*=8.5 Hz, H-3' & H-5', 2H), 7.88 (d, *J*=8.8 Hz, H-2' & H-6', 2H), 7.35-7.28 (m, H-2″ to H-6″), 6.69 (d, *J*=8.5 Hz, H-8, 1H), 6.52 (d, *J*=2.5 Hz, H-5,1H), 6.42 (dd, *J*=2.5, 8.5 Hz, H-7,1H), 4.29-4.26 (m, CH_2-2 & CH_2-3, 4H), 3.49 (t, *J*=7.0 Hz, CH_2-8″, 2H), 2.28 (t, *J*=7.0 Hz, CH_2-7″, 2H); EIMS (*m/z*): 440 [M; $C_{22}H_{20}N_2O_6S]^{\cdot+}$, 412 $[C_{20}H_{16}N_2O_6S]^+$, 404$[C_{19}H_{20}N_2O_6S]^+$, 394 $[C_{22}H_{20}NO_4S]^+$, 330$[C_{22}H_{20}NO_2]^+$, 254$[C_{16}H_{16}NO_2]^+$, 186$[C_6H_4NO_4S]^+$, 135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 107 $[C_6H_3O_2]^+$, 105 $[C_8H_9]^+$, 81$[C_4HO_2]^+$, 77$[C_6H_5]^+$, 75$[C_6H_3]^+$, 51$[C_4H_3]^+$.

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-4-nitro-N-(3-phenylpropyl)benzenesulfonamide (5i)

Golden brown solid; Yield: 89%; m.p: 128°C; Molecular formula: $C_{23}H_{22}N_2O_6S$; Molecular weight: 454 $gmol^{-1}$; IR (KBr, cm^{-1}): *v* 2913 (C-H str. of aromatic ring), 2992 (-CH_2 stretching), 1627 (C=C str. of aromatic), 1371 (-SO_2 stretching), 1338 (-NO_2 stretching), 1163 (C-O-C stretching of ether); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 8.35 (d, *J*=8.5 Hz, H-3' & H-5', 2H), 7.88 (d, *J*=8.8 Hz, H-2' & H-6', 2H), 7.40-7.29 (m, H-2″ to H-6″), 6.69 (d, *J*=8.5 Hz, H-8, 1H), 6.52 (d, *J*=2.5 Hz, H-5,1H), 6.42 (dd, *J*=2.5, 8.5 Hz, H-7,1H), 4.29-4.26 (m, CH_2-2 & CH_2-3, 4H), 3.16 (t, *J*=7.0 Hz, CH_2-9″, 2H), 2.62 (t, *J*=7.0 Hz, CH_2-7″, 2H), 2.10-2.06 (m, CH_2-8″, 2H); EIMS (*m/z*): 454 [M; $C_{23}H_{22}N_2O_6S]^{\cdot+}$, 426 $[C_{21}H_{18}N_2O_6S]^+$, 416 $[C_{20}H_{20}N_2O_6S]^+$,408 $[C_{23}H_{22}NO_4S]^+$, 344 $[C_{23}H_{22}NO_2]^+$, 268 $[C_{17}H_{18}NO_2]^+$, 186 $[C_6H_4NO_4S]^+$,135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 119 $[C_9H_{11}]^+$,107 $[C_6H_3O_2]^+$, 91 $[C_7H_7]^+$, 81 $[C_4HO_2]^+$, 75 $[C_6H_3]^+$, 51 $[C_4H_3]^+$.

Biological studies

Assessment of bacterial biofilm inhibition

Micro titer-plate method was used for the assessment of the inhibition of bacterial (*Bacillus subtilis/ Escherichia*

coli) biofilm formation as described by Stepanovic *et al.* (2000). Biofilm formation was performed in 96-well micro titer plates, with 100µL of nutrient broth (Oxoid, UK). Concentrations, which were 2.5 and 5.0µg of the testing samples (dissolved in 1mL of DMSO), were added in different wells. At last, 20µL of the bacterial suspension containing 1×10^9 CFU/mL was inoculated. The well of positive control was contained with Ampicillin and nutrient broth (Oxoid, UK) whereas the well of negative control contained nutrient broth and microbial strain. After that, plates were covered and aerobically incubated for 24 hours at 37°C. Subsequently, using sterile phosphate buffer (pH: 7.2) of 220 µL the contents of each well were beheld thrice. Plates were vigorously shaken to remove all non-adherent bacteria. Then the bacteria which attached on plates were fixed with 220 mL of 99% methanol per well. After every 15 min, the plates were emptied and left to dry. Then, by using 220 mL of 50% crystal violet per well the plates were stained for 5 min. Surplus stain was rinsed of using distilled water. Then plates were re-solubilized with 220 µL of 33% (v/v) glacial acetic acid per well after air-dried and the bound dye. By using 630 nm micro-plate reader (Biotek, USA) the optical density (OD) of each well was measured. Against selected bacterial strains all the tests carried thrice and the result were averaged. The bacterial growth inhibition (Inhibition %) was calculated using the following formula:

$$\text{Inhibition \%} = 100 \quad \frac{(OD_{630\ \text{of sample}} \times 100)}{OD_{630\ \text{control}}}$$

Hemolytic activity

Bovine blood samples were collected in EDTA that was diluted with saline (0.9% NaCl) and centrifuge at 1000xg for 10min. The erythrocytes separated diluted in phosphate buffer saline of pH 7.4 and a suspension was made. Add 20µL of synthetic compounds solution (10 mg/mL) in 180µL of RBCs suspension and incubate for 30 min at room temperature. PBS was used as negative control and Triton 100-X was taken as positive control (Sharma *et al.* 2001; Powell *et al.,* 2000). The %age of hemolysis was taken as by using formula:

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

Code	-R	Code	-R
4a, 5a	—CH_2—CH_3 (1" 2")	**4e, 5e**	—CH(1"CH_3)—CH_2—CH_3 (2" 3" 4")
4b, 5b	—CH_2–CH_2–CH_3 (1" 2" 3")	**4f, 5f**	—CH_2–CH_2–CH_2–CH_2–CH_3 (1" 2" 3" 4" 5")
4c, 5c	—HC(2"; 1"CH_3; 3"CH_3)	**4g, 5g**	—H_2C— (7") benzene (1" 3" 5")
4d, 5d	—CH_2–CH_2–CH_2–CH_3 (1" 2" 3" 4")	**4h, 5h**	—H_2C—H_2C— (8" 7") benzene (1" 3" 5")
4i, 5i	—H_2C—H_2C—H_2C— (9" 8" 7") benzene (1" 3" 5")		

Table 2: Biofilm inhibition against *Escherichia coli.*

Compound	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**5i**
% Inhibition	65.84	40.17	50.72	66.87	55.49	69.15	61.08	42.03	54.55

Table 3: Biofilm inhibition against *Bacillus subtilis*

Compound	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**5i**
% Inhibition	43.21	56.48	40.55	22.81	61.57	58.39	67.09	52.12	67.07

Note: Ampicillin was used as a positive control. (% Inhibition) = 77.49.

Table 4: Cytotoxic potential through hemolytic activity.

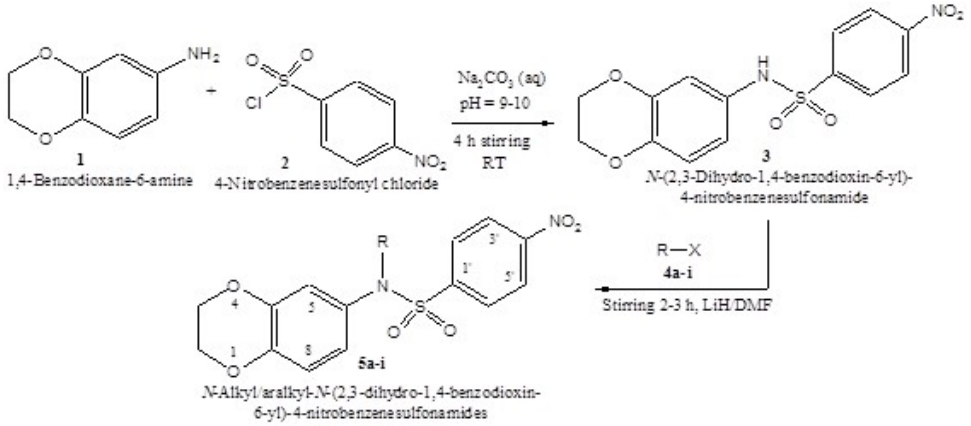
Compound	5a	5b	5c	5d	5e	5f	5g	5h	5i	Triton-X
% Hemolysis	7.37	9.58	6.11	6.21	7.05	8.32	7.58	8.21	12.21	89.00

Note: PBS (% Hemolysis) = 0.54.

$$(\%)\ \text{of Hemolysis} = \frac{\text{Absorbance of Sample} - \text{Absorbance of Negative Control}}{\text{Absorbance of Positive Control}} \times 100$$

STATISTICAL ANALYSIS

Statistical analysis was performed by Microsoft Excel 2010 for all the thrice measured values.



Scheme 1: Outline for the synthesis of *N*-alkyl/aralkyl-*N*-2,3-dihydro-1,4-benzodioxin-6-yl)-4-nitrobenzenesulfonamides (**5a-i**).

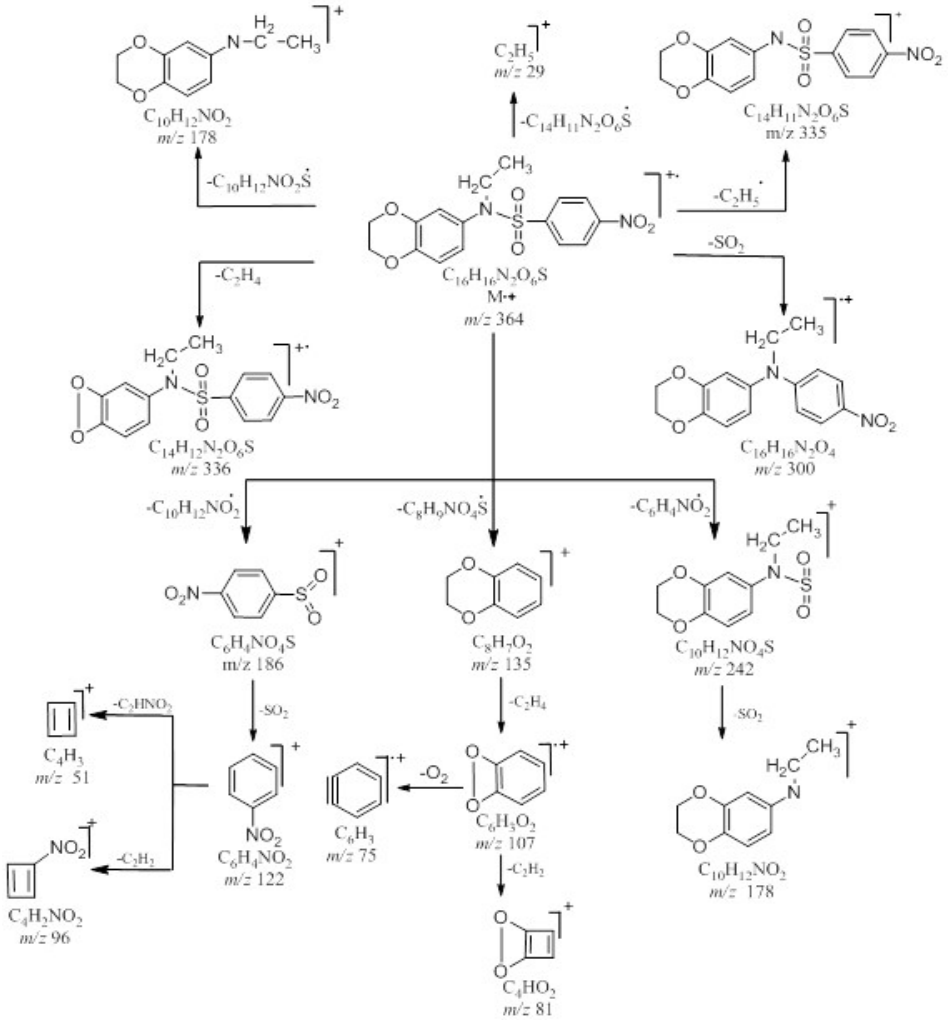


Fig. 1: Suggested mass fragmentation pattern of *N*-(2,3-Dihydro-1,4-benzodioxin-6-yl)- *N*-ethyl-4-nitrobenzenesulfonamide (**5a**).

RESULTS

N-Alkyl/Aralkyl-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-nitrobenzenesulfonamides (**5a-i**) were synthesized according to the protocol outlined in Scheme 1 and different substituents (-R) have been listed in table 1. In first step, 1,4-benzodioxane-6-amine (**1**) and 4-nitrobenzenesulfonyl chloride (**2**) were stirred in 10% Na_2CO_3 solution for 4 hours at room temperature to yield the parent molecule, *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-nitrobenzenesulfonamide (**3**) which further in second step was coupled with different alkyl/aralkyl halides (**4a-i**) in DMF and LiH as base to achieve the target compounds (**5a-i**). The projected structures of synthesized compounds were confirmed through their spectral data. The suggested mass fragmentation of **5a** has been sketched in fig 1 and its ^{1}H-NMR spectrum is shown in fig. 2.

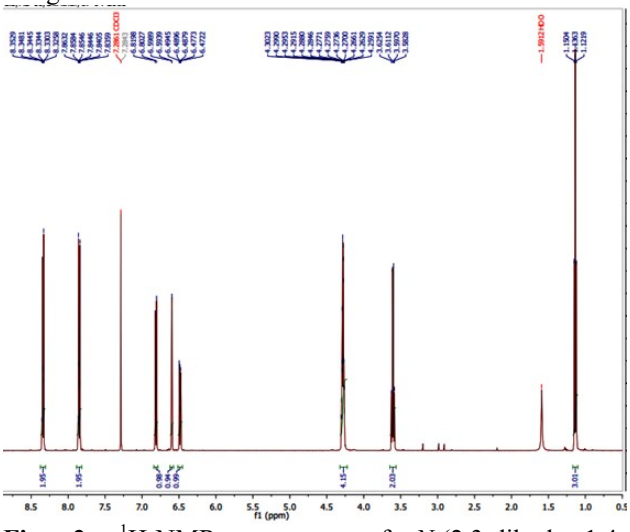


Fig. 2: ^{1}H-NMR spectrum of *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-*N*-ethyl-4-nitrobenzenesulfonamide (**5a**).

The phase contrast microscopic view of inhibition of *Escherichia coli* biofilm is displayed in fig. 3 while that of *Bacillus subtilis* biofilm is exhibited in fig. 4.

DISCUSSION

Chemistry

The synthesis of targeted *N*-sulfonated derivatives (**5a-i**) was accomplished by a facile strategy (Abbasi *et al*., 2016) and all these new compounds were obtained in very good yields. The structures of the products were verified via investigation of their spectral data. For example, compound 5a was obtained as peach colored solid in 85% yield having m.p. of 162˚C. The molecular formula, $C_{16}H_{16}N_2O_6S$, was ascertained through EIMS which showed $[M]^+$ peak at *m/z* 364 (fig. 1) and by counting the number of protons in the ^{1}H-NMR spectrum. The functionalities in the molecule were affirmed by IR spectral data showing absorption bands at 3056 cm^{-1} (C-H stretching of aromatic ring), 2981 cm^{-1} (-CH_2 stretching), 1625 cm^{-1} (C=C stretching of aromatic ring), 1383 cm^{-1} (-SO_2 stretching), 1341 cm^{-1} (-NO_2 stretching) and 1147 cm^{-1} (C-O-C stretching of ether). In aromatic region ^{1}H-NMR spectrum of (fig. 2), an A_2B_2 spin system was observed for a 4-nitrobenzenesulfonyl moiety which appeared as *diortho*-coupled doublets at δ 8.35 (H-3' & H-5') and δ 7.85 (H-2' & H-6') having integration of two protons each. A benzodioxane moiety in the molecule was confirmed from the emergence of two doublets and a doublet of doublet at δ 6.81 for H-8 and δ 6.59 for H-5 and δ 6.49 for H-7, respectively. A multiplet resonating at δ 4.29-4.26 display two methylene (CH-2 & CH-3) groups of the benzodioxane moiety. One quartet for a methylene (CH_2-1") at δ 3.62 and one triplet at δ 1.13 for a methyl group (CH_3-2″), inveterate the *N*-substitution of ethyl group in compound. On the bases of these combined indications molecule **5a** was named as *N*-ethyl-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-nitrobenzenesulfonamide. All other *N*-substituted derivatives were characterized on similar pattern.

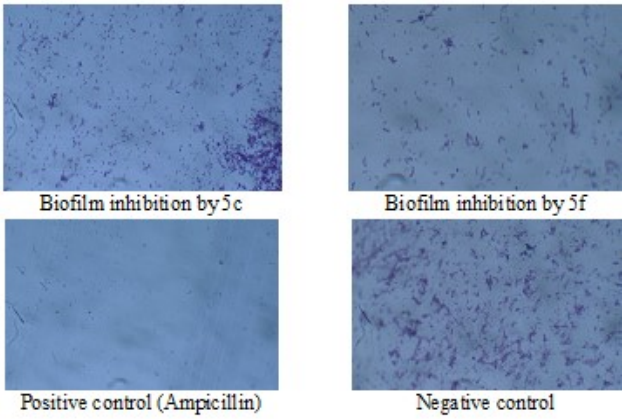


Fig. 3: Phase contrast microscopic view of inhibition of *Escherichia coli* biofilm.

Antibacterial activity via biofilm inhibition

The antibacterial activity of the derivatives, **5a-i,** was checked by biofilm inhibition method using bacterial pathogenic strains i.e. *Escherichia coli* and *Bacillus subtilis*. Ampicillin was used as a standard drug in this assay to compare the antibacterial potential of the synthesized molecules. From their results (table 2 & table 3), it was observed that among the series, maximum inhibition (69.15%) was given by **5f** against *Escherichia coli*, relative to ampicillin used as a standard and its percentage inhibition was noted as 78.88%. The phase contrast microscopic views of inhibition of *Escherichia coli* biofilm by **5c** and **5f** are shown in fig. 3. Against *Bacillus subtilis*, the molecule 5g and 5i were found to be more appropriate antibacterial agents with percentage inhibition of 67.09% and 67.07%, respectively. The better activity of these molecules was possibly because of the substitution of benzyl group. The compound **5e** also showed a reasonable inhibition (61.57%) against this

strain, relative to ampicillin (77.49%). The phase contrast microscopic view of inhibition of *Bacillus subtilis* biofilm by **5g** is shown in fig. 4.

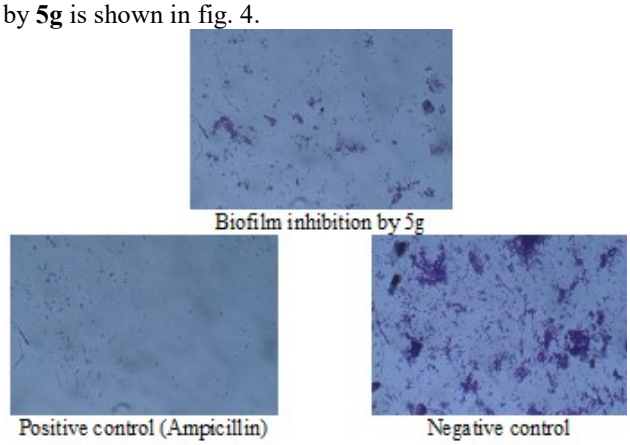


Fig. 4: Phase contrast microscopic view of inhibition of *Bacillus subtilis* biofilm.

Cytotoxicity via hemolysis

All these new synthesized molecules, **5a-i**, were also subjected to hemolytic assay to find out their cytotoxicity profile. Results of percentage hemolysis are shown in table 4, which revealed that all the tested compounds exhibited mild activity. Maximum toxicity was shown by the compound **5i** (12.21%), relative to Triton-X having % hemolysis of 89.00%.

CONCLUSION

N-Alkyl/aralkyl-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-nitrobenzenesulfonamides (**5a-i**) were prepared in a better yield and their proposed structures were corroborated by spectral data i.e. IR, ^{1}H-NMR and EI-MS spectra. On the basis of biofilm inhibition study, it was concluded that out of these synthesized molecules, **5f** exhibited relatively good antibacterial potential against *Escherichia* coli and **5g** and **5i** against *Bacillus subtilis,* so these molecules can be utilized further as safe curative candidates in drug designing.

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