doi.org/10.36721/PJPS.2020.33.5.REG.2161-2170.1

REPORT

Synthesis of promising antibacterial and antifungal agents: 2-[[(4-Chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-*N*-(un/substituted-phenyl)acetamides

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Abstract: In the presented work, 2,3-dihydro-1,4-benzodioxin-6-amine (1) was reacted with 4-chlorobenzenesulfonyl chloride (2) in presence of aqueous basic aqueous medium to obtain 4-chloro-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)benzenesulfonamide (3). In parallel, various un/substituted anilines (4a-l) were treated with bromoacetyl bromide (5) in basified aqueous medium to obtain corresponding 2-bromo-*N*-(un/substituted)phenylacetamides (6a-l) as electrophiles. Then the compound 3 was finally reacted with these electrophiles, 6a-l, in dimethylformamide (DMF) as solvent and lithium hydride as base and activator to synthesize a variety of 2-[[(4-chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-*N*-(un/substituted)phenylacetamides (7a-l). The synthesized compounds were corroborated by IR, ^{1}H-NMR and EI-MS spectral data for structural confirmations. These molecules were then evaluated for their antimicrobial and antifungal activities along with their %age hemolytic activity. Some compounds were found to have suitable antibacterial and antifungal potential, especially the compound 2-[[(4-chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-*N*-(3,5-dimethylphenyl)acetamide (7l) exhibited good antimicrobial potential with low value of % hemolytic activity.

Keywords: 2, 3-Dihydro-1, 4-benzodioxin-6-amine, 4-Chlorobenzenesulfonyl chloride, *N*-(un/substituted) phenylacetamides, spectral analysis, antibacterial, antifungal.

INTRODUCTION

The systematic progression of innovative methods for the synthesis of novel amides and related drugs derivatives are gaining wide importance in physical sciences because of their industrial and pharmaceutical applications and as potent intermediates in organic synthesis (Theodoroue *et al*., 2009). The synthesis of compounds containing multiple functional groups simultaneously has been raising now-a-days (Abbasi *et al*., 2016a). Sulfonamides, also called sulfa drugs, take the lead among all the well-used antibacterial medicines exploited in the drug engineering (Behmadi *et al*., 2009).

The characteristics functional group of Sulfonamides is SO_2NH_2, a sulfonyl moiety connected to an amine group, enhanced their medicinal importance as a drug and display wide varieties of biological activities. They are well known as anti-inflammatory and antiviral agents (Selvam *et al*., 2001; Abbasi *et al*., 2015).These compounds also find their application as antitumor, high ceiling diuretics, anti-thyroid, and anticonvulsants, because they have been proved to inhibit cancer cell growth and to cease tumor invasion (Ali *et al*., 2010). They have widespread clinical uses, as hypoglycemic and HIV protease inhibitors and potent cysteine protease inhibitors (Mirian *et al*., 2011). These sulfa compounds have wider remedial purposes in Alzheimer's and other mental disorders and malfunctioning (Abbasi *et al*., 2014), arthritis and cancer (Lahtinen *et al*., 2014). Sulfonamides possess structure similar to that of *para*-amino benzoic acid (PABA) which is a vital component for bacterial growth and thus act as a competitive inhibitor of PABA by coupling with dihydroproteasesynthetase to inhibit the conversion of folic acid into folate and thus inhibit the growth of bacterial cells (El-Sayed *et al*., 2011). The carbonic anhydrases catalyze the hydration of CO_2 to bicarbonate which is a critical chemical interconversion for various metabolic processes (Yong *et al*., 2014). Sulfonamides cut down the production of nucleotides by inhibiting the formation of bicarbonate and other precursors essential for cell components assemblies like membrane lipids and thus control the proliferation of tumor and cancerous cell (Pastorekova *et al*., 2004).

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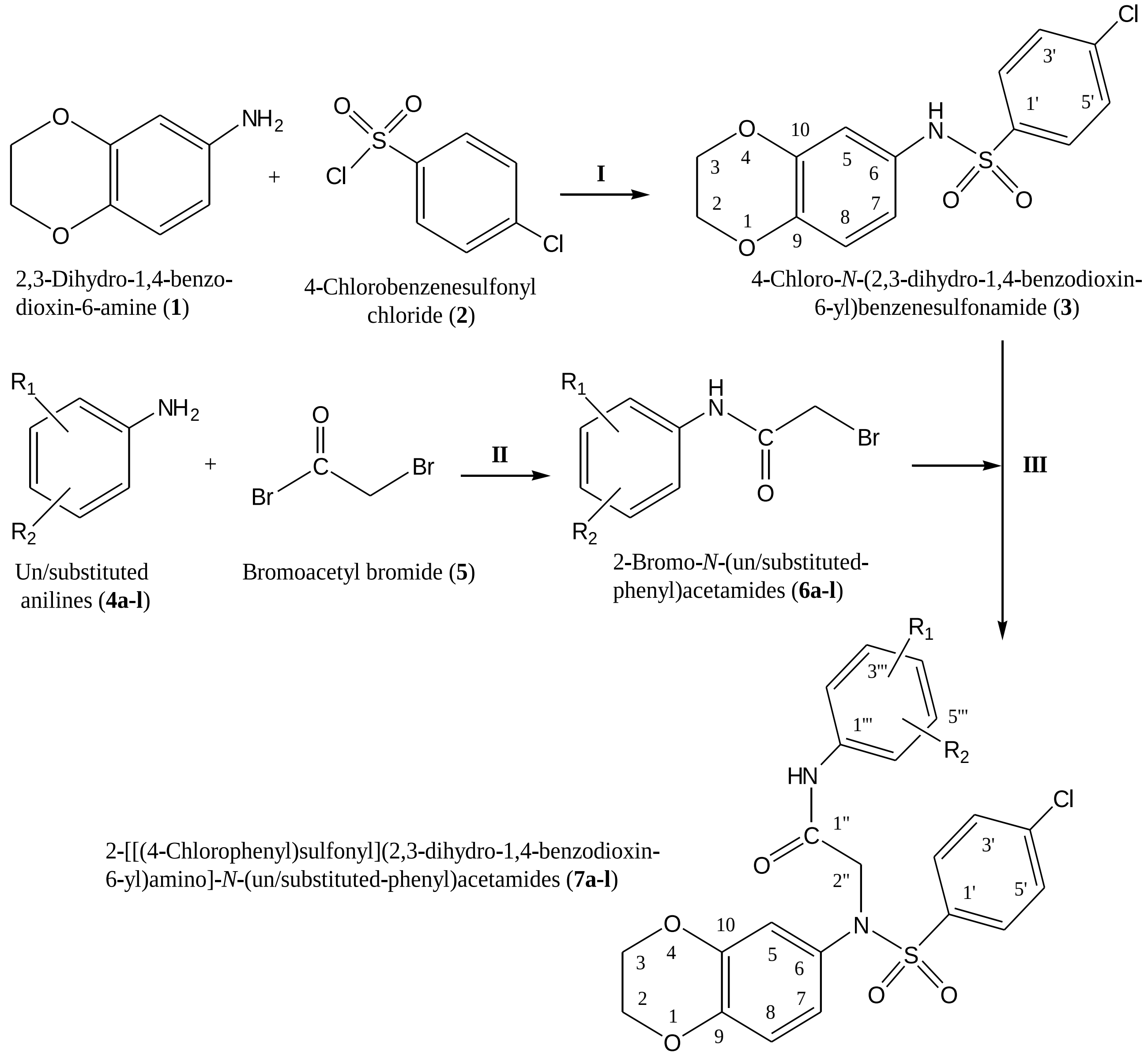

2,3-Dihydro-1,4-benzodioxin-6-amine (**1**)

4-Chlorobenzenesulfonyl chloride (**2**)

4-Chloro-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)benzenesulfonamide (**3**)

Un/substituted anilines (**4a-l**)

Bromoacetyl bromide (**5**)

2-Bromo-*N*-(un/substituted-phenyl)acetamides (**6a-l**)

2-[[(4-Chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-*N*-(un/substituted-phenyl)acetamides (**7a-l**)

Reagents & Conditions: (I) Aq. Na_2CO_3 soln./pH 9-10/stirring at RT for 3 hrs. (II) Aq. Na_2CO_3 soln./pH 9-10/stirring at RT for 2-3 hrs. (III) DMF/LiH/stirring at RT for 4-5 hrs.
Different substituents ($-R_1$& $-R_2$) in scheme 1.

Compd.	$-R_1$	$-R_2$
7a	-H	-H
7b	$2-OCH_3$	-H
7c	$4-OC_2H_5$	-H
7d	$2-CH_3$	-H
7e	$3-CH_3$	-H
7f	$4-CH_3$	-H

Compd.	$-R_1$	$-R_2$
7g	$2-CH_3$	$3-CH_3$
7h	$2-CH_3$	$4-CH_3$
7i	$2-CH_3$	$5-CH_3$
7j	$2-CH_3$	$6-CH_3$
7k	$3-CH_3$	$4-CH_3$
7l	$3-CH_3$	$5-CH_3$

Scheme-1: Outline for the synthesis of 2-[[(4-chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-*N*-(un/substituted-phenyl)acetamides (7a-l).

Table 1: Antibacterial activity of compounds, 7a-l.

Compd.	*B. subtillus*		*S.aureus*		*E.coli*		*P. multocida*	
	(ZOI mm) Mean ± S.D	MIC (µg/mL)	(ZOI mm) Mean ± S.D	MIC (µg/mL)	(ZOI mm) Mean ± S.D	MIC (µg/mL)	(ZOI mm) Mean ± S.D	MIC (µg/mL)
7a	5.67 ± 1.52	25	4.66 ± 0.58	50	1.67 ± 0.62	50	1.33 ± 1.15	50
7f	6.67 ± 1.15	25	2.33d ± 0.58	50	1.67 ± 0.57	50	3.33 ± 0.58	50
7g	11.00 ± 1.00	25	8.66 ± 1.15	25	4.67 ± 1.15	50	4.33 ± 0.58	50
7h	12.67 ± 1.52	25	5.66 ± 0.58	50	1.33 ± 1.15	50	4.67 ± 0.58	50
7i	4.67 ± 1.15	25	4.66 ± 1.15	50	3.00 ± 1.73	50	3.33 ± 1.15	50
7j	4.33 ± 1.15	25	2.33 ± 0.58	50	1.67 ± 0.57	50	1.33 ± 1.27	50
7k	4.67 ± 0.57	25	3.66 ± 0.58	50	1.67 ± 0.59	50	2.33 ± 0.62	50
7l	14.33 ± 2.08	25	7.66 ± 0.58	25	7.33 ± 0.57	25	9.33 ± 1.15	25
Rifampicin	24.33 ± 1.51	12.5	30.33 ± 1.73	12.5	26.67 ± 1.65	12.5	31.00 ± 2.0	12.5

Note: Compounds 7b-e remained inactive.

Table 2: Antifungal activity ofacetamides,7a-l.

Compd.	*A. alternata*		*R. solani*		*A.niger*		*F. solani*	
	(ZOI mm) Mean ± S.D	MIC (µg/mL)	(ZOI mm) Mean ± S.D	MIC (µg/mL)	(ZOI mm) Mean ± S.D	MIC (µg/mL)	(ZOI mm) Mean ± S.D	MIC (µg/mL)
7a	4.67 ± 1.15	50	3.33 ± 0.58	50	1.33 ± 0.58	50	1.33 ± 0.58	50
7f	6.67 ± 0.58	50	1.67 ± 1.15	50	1.33 ± 1.53	50	2.33 ± 0.58	50
7g	9.67 ± 0.58	25	7.33 ± 1.15	25	4.33 ± 1.15	50	2.67 ± 1.53	50
7h	8.33 ± 0.58	25	2.67 ± 1.15	50	1.67 ± 0.58	50	2.33 ± 0.58	50
7i	2.33 ± 0.58	50	2.67 ± 1.15	50	1.67 ± 1.15	50	3.33 ± 0.58	50
7j	3.33 ± 0.58	50	1.33 ± 0.58	50	1.33 ± 1.00	50	1.00 ± 0.58	50
7k	3.33 ± 1.53	50	2.33 ± 0.58	50	1.33 ± 0.58	50	1.67 ± 0.58	50
7l	10.33 ± 0.58	25	6.33 ± 0.58	25	4.33 ± 1.15	50	6.67 ± 0.58	25
Terbinafine	12.5 ± 1.50	12.5	28.00 ± 1.50	12.5	30.00 ± 1.35	12.5	30.00 ± 0.75	12.5

Note: Compounds 7b-e remained inactive.

Table 3: The percentage hemolytic activity of 7a-l.

Compound Code	%age Hemolytic Activity (Mean ± S.D)	Compound Code	%age Hemolytic activity (Mean ± S.D)	Compound Code	%age Hemolytic activity (Mean ± S.D)
7a	14.25 ± 0.97	7e	26.19 ± 2.30	7i	5.47 ± 0.69
7b	15.90 ± 0.23	7f	16.97 ± 0.46	7j	23.46 ± 1.89
7c	11.79 ± 0.24	7g	5.47 ± 0.69	7k	25.14 ± 1.01
7d	15.75 ± 0.29	7h	18.73 ± 0.92	7l	8.32 ± 0.96
PBS	00.00	Triton-X	97.65 ± 0.34		

Sulfonamides constitutes the most widely utilized antibacterial specialists in the world as a result of their low cost, ease, high efficiency low harmfulness and incredible action against common bacterial diseases (Kołaczek *et al.*, 2014).

Acetamide linkage is found to be very dynamic in various imperative medications and numerous bioactive acetamide derivatives have been synthesized since then. Acetamide moiety is known to possess anthelmintic (Sawant and Kawade, 2011), anti-arthritic (Szilagyi *et al.*, 1990), anticonvulsant, anticancer, antibacterial and antifungal potentialities (Siddiqui *et al.*, 2007). 1,4-Benzodioxane template occurs as a valuable synthetic intermediates in various pharmaceuticals syntheses displaying a broad spectrum of biological activity hence, widely employed in the design of therapeutic agents with, antigastric, spasmolytic, antipsychotic and anxiolytic properties. The compounds bearing this moiety are well-known antihypertensive, antidepressant and anti-inflammatory drugs (Vaidya *et al.*, 2009).

The inclusion of poly functional groups i.e. sulfonamide, acetamide and 1,4-benzodioxane moiety in a single compound may boost all the inherited biological properties and thus may prove to be very effective to combat the increasing microorganism resistance against the drugs (Abbasi *et al.*, 2015). By exploring the evidences present in the literature survey and in continuation of previous studies of our research group

(Abbasi *et al*., 2016b). Hereby a new series of various 2-[[(4-chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-*N*-(un/substituted-phenyl)acetamideshas been synthesized. These compounds were then evaluated for their antibacterial and antifungal activities in search of valuable therapeutic agents.

MATERIALS AND METHODS

From local suppliers of Sigma Aldrich and Alfa Aesar (Germany), all the chemicals and solvents of analytical grade were purchased. To access the progress of reaction completion the pre-coated silica gel plates were utilized. The TLC was run in the solvent system comprising of ethyl acetate and *n*-hexane in 80:20 % as gradient solvent system. The detection was done at λ_{max} of UV_{254}. In open capillary tubes the melting points of the synthesized molecules was done on Gallonkamp apparatus. On the Jasco-320-A spectrophotometer, the IR spectra (ν, cm^{-1}) were conducted by making KBr pellet of target molecules. The Bruker Advance III 600 As- cend spectrometer was utilized to do the ^{1}H-NMR spectra (δ, ppm). The instrument was run on 600 MHz for ^{1}H-NMR and at 150 MHz for ^{13}C-NMR spectra, in $CDCl_3$ using BBO probe for later analysis. On a JEOL JMS-600H instrument the EI-MS spectra were recorded with data processing system.

Procedure for the synthesis of 4-chloro-N-(2,3-dihydro-1,4-benzodioxin-6-yl)benzenesulfonamide (3)

2,3-Dihydro-1,4-benzodioxin-6-amine (5g; 0.033 mol; 1) was taken in round bottom flask and suspended in 100mL distilled water and stirred shortly at room temperature. The pH was maintained at 9-10 by aqueous solution of Na_2CO_3. 4-Chlorobenzenesulfonyl chloride (6.96g; 0.033 mol; 2) was added in the reaction flask and further stirred for 3 hrs. Progress of the reaction was monitored with TLC. On completion, conc. HCl was added slowly to adjust the pH around 2-3. The reaction mixture was kept at room temperature for 15 min to allow the precipitates to get settled andthen were filtered and washed with distilled water to afford 4-chloro-*N*-(2,3-dihydro-1,4-benzodioxin- 6-yl)benzenesulfonamide (3) in 91% yield.

Procedure for the synthesis of 2-bromo-N-(un/substituted-phenyl)acetamides(6a-l)

Synthesis of 2-bromo-*N*-(un/substituted-phenyl) acetamides was carried out by reaction of various un/substituted anilines (4a-l) with 2-bromoacetyl bromide (5) in equimolar quantities (0.001mol) under vigorous manual shaking in basic (10% Na_2CO_3) aqueous medium under dynamic pH control. The reaction contents were kept under stirring afterwards, for 2-3 hrs. Solid precipitates were formed and were filtered and washed with cold distilled water to obtain the desired electrophiles (6a-l).

Procedure for the synthesis of 2-[[(4-chlorophenyl) sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-N-(un/substituted-phenyl)acetamides (7a-l)

Equimolar quantities of 4-chloro-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)benzenesulfonamide (0.2g; 0.0006 mol; 3) and 2-bromo-*N*-(un/substituted-phenyl)acetamides (0.0006 mol; 6a-l) in *N-N*-dimethylformamide (DMF; 7 mL) and LiH were reacted in a 50mL round bottomed flask. The reaction mixture was allowed to stir for 4-5 hrs at room temperature. On complete reactants conversion to the desired product, the contents of reaction were poured on finely crushed ice. Immediate precipitation was observed. The ppts were filtered and rinsed with distilled water; drying yielded pure 2-[[(4-chlorophenyl)sulfonyl] (2,3-dihydro-1,4-benzodioxin-6-yl)amino]-*N*-(un/ substituted-phenyl)acetamides (7a-l) in good yields. The synthetic pathway is illustrated in fig. 1 and table 1.

Structural Characterization

2-[[(4-Chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-N-phenylacetamide (*7a*)

Dark brown solid; Yield: 81%; m.p. 156-158^{o}C; Molecular formula: $C_{22}H_{19}N_2O_5SCl$; Molecular weight: 283g/mol; IR (KBr, υ, cm^{-1}): 3343 (N-H, stretching), 3080 (C-H, str. of aromatic ring), 1658 (C=O str.), 1554 (C=C, aromatic str.), 1240 (S=O streching); ^{1}H-NMR (500 MHz, $CDCl_{3,}$ *δ, ppm*); 8.27 (s, 1H, NH), 7.64 (d, *J* = 8.5 Hz, 2H, H-2′ & H-6′), 7.56 (br.d, *J* = 8.5 Hz, 2H, H-2‴ & H-6‴), 7.48 (br. t, *J* = 9.0 Hz, 2H, H-3‴ & H-5‴), 7.38 (d, *J* = 8.5 Hz, 2H, H-3′ & H-5′), 7.31 (br.t, *J* = 8.0 Hz, 1H, H-4‴), 6.69 (d, *J* = 8.5 Hz, 1H, H-8), 6.61 (d, *J* = 2.5 Hz, 1H, H-5), 6.47 (dd, *J*=2.0 ,8.5 Hz, 1H, H-7), 4.23-4.21 (m, 4H, CH_2-2 & CH_2-3), 4.19 (s, 2H, CH_2-2″); EI-MS: *m/z* 462 (M + 4]$^+$, 460 [M + 2]$^+$, 458 $(C_{22}H_{19}N_2O_5SCl)^{\cdot+}$ [M]$^+$, 283 $(C_{16}H_{15}N_2O_3)^+$, 179 $(C_6H_4O_2SCl+4)^+$, 177 $(C_6H_4O_2SCl+2)^+$, 175 $(C_6H_4O_2SCl)^+$, 135 $(C_8H_7O_2)^+$, 120 $(C_7H_6NO)^+$, 113 $(C_6H_4Cl+2)^+$, 111$(C_6H_4Cl)^+$, 92 $(C_6H_6N)^+$, 77 $(C_6H_5)^+$.

2-[[(4-Chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-N-(2-methoxyphenyl)acetamide (***7b***)

Grey brown powder; Yield: 84%; m.p. 136-138°C; Molecular formula: $C_{23}H_{21}N_2O_6SCl$; Molecular weight: 488g/mol; IR (KBr, υ, cm^{-1}): 3345 (N-H, stretching), 3084 (C-H, str. of aromatic ring), 1656 (C=O str.), 1557 (C=C, aromatic str.), 1244 (S=O streching); ^{1}H-NMR (600 MHz, $CDCl_{3,}$ *δ*, ppm); 8.15 (s, 1H, NH), 7.66 (d, *J* = 8.4 Hz, 2H, H-2′ & H-6′), 7.45 (d, *J* = 8.2 Hz, 2H, H-3′ & H-5′), 7.26 (d, *J* = 7.6 Hz, 1H, H-6‴), 7.01-6.89 (m, 3H, H-3‴ to H-5‴), 6.69 (d, *J* = 8.5 Hz, 1H, H-8), 6.65 (d, *J* = 2.3 Hz, 1H, H-5), 6.47 (dd, *J* =2.5, 8.5 Hz, 1H, H-7), 4.24-4.20 (m, 4H, CH_2-2 & CH_2-3), 4.23 (s, 2H, CH_2-2″), 3.88 (s, 3H, 2-OCH_3); EI-MS: *m/z* 492 [M+4]$^+$, 460 [M+2]$^+$, 458 $(C_{23}H_{21}N_2O_6SCl)^{\cdot+}$ [M]$^+$, 313 $(C_{17}H_{17}N_2O_4)^+$, 179 $(C_6H_4O_2SCl+4)^+$, 177 $(C_6H_4O_2SCl+2)^+$, 175

$(C_6H_4O_2SCl)^+$, 135 $(C_8H_7O_2)^+$, 122 $(C_7H_8NO)^+$, 113 $(C_6H_4Cl+2)^+$, 111$(C_6H_4Cl)^+$, 107 $(C_7H_7O)^+$.

***2-[[(4-Chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-N-(4-ethoxyphenyl)acetamide* (*7c*)**

Mudy whitescaly solid; Yield: 80%; m.p. 148-149°C; Molecular formula: $C_{24}H_{23}N_2O_6SCl$; Molecular weight: 502g/mol; IR (KBr, υ, cm^{-1}): 3342 (N-H, stretching), 3079 (C-H, str. of aromatic ring), 1657 (C=O str.), 1558 (C=C, aromatic str.), 1242 (S=O streching); ^{1}H-NMR (600MHz, $CDCl_3$, *δ*, ppm); 8.16 (s, 1H, NH), 7.65 (d, *J* = 8.4 Hz, 2H, H-2′ & H-6′), 7.41 (d, *J* = 8.4 Hz, 2H, H-3′& H-5′), 7.38 (d, *J* = 8.9Hz, 2H, H-2‴ & H-6‴), 6.85 (d, *J* = 8.9Hz, 2H, H-3‴ & H-5‴), 6.71 (d, *J* = 8.5 Hz, 1H, H-8), 6.63 (d, *J* = 2.3Hz, 1H, H-5), 6.49 (dd, *J* = 2.5, 8.5 Hz, 1H, H-7), 4.25-4.23 (m, 4H, CH_2-2 & CH_2-3), 4.22 (s, 2H, CH_2-2″), 4.0 (q, *J* = 7.0 Hz, 2H, 4-O$\underline{CH_2}$-CH_3), 1.40 (t, *J* = 7.0 Hz, 3H, 4-OCH_2-$\underline{CH_3}$); EI-MS: *m/z* 506 $[M+4]^+$, 504 $[M+2]^+$, 502 $(C_{24}H_{23}N_2O_6SCl)^{\cdot+}$ $[M]^+$, 327 $(C_{18}H_{19}N_2O_4)^+$, 179 $(C_6H_4O_2SCl+4)^+$, 177 $(C_6H_4O_2SCl+2)^+$, 175 $(C_6H_4O_2SCl)^+$, 164 $(C_9H_{10}NO_2)^+$, 136 $(C_8H_{10}NO)^+$,135 $(C_8H_7O_2)^+$, 121 $(C_8H_9O)^+$, 113 $(C_6H_4Cl+2)^+$, 111 $(C_6H_4Cl)^+$.

***2-[[(4-Chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-N-(2-methylphenyl)acetamide* (*7d*)**

Mustard color solid; Yield: 78%; m.p. 138-139°C; Molecular formula: $C_{23}H_{21}N_2O_5SCl$; Molecular weight: 472g/mol; IR (KBr, υ, cm^{-1}): 3343 (N-H, stretching), 3081 (C-H, str. of aromatic ring), 1658 (C=O str.), 1553 (C=C, aromatic str.), 1240 (S=O streching); ^{1}H-NMR (500MHz, $CDCl_3$, *δ*, ppm); 8.26 (s, 1H, NH), 7.83 (br.d, *J* = 8.5 Hz, 1H, H-6‴), 7.20-7.18 (m, 1H, H-5‴), 7.56 (d, *J* = 8.5 Hz, 2H, H-2′ & H-6′), 7.51 (br.d, *J* = 8.5 Hz, 1H, H-3‴), 7.47 (d, *J* =8.5 Hz, 2H, H-3′ & H-5′), 7.08-7.05 (m, 1H, H-4‴), 6.78 (d, *J* = 9.0 Hz, 1H, H-8), 6.68 (d, *J* = 2.5 Hz, 1H, H-5), 6.54 (dd, *J* = 2.5, 8.5 Hz, 1H, H-7), 4.24-4.19 (m, 6H, CH_2-2, CH_2-3 & CH_2-2″), 2.30 (s, 3H, 2-CH_3); EI-MS: *m/z* 476 $[M+4]^+$, 474 $[M+2]^+$, 472 $(C_{23}H_{21}N_2O_5SCl)^{\cdot+}$ $[M]^+$, 297 $(C_{17}H_{17}N_2O_3)^+$, 179 $(C_6H_4O_2SCl+4)^+$, 177 $(C_6H_4O_2SCl+2)^+$, 175 $(C_6H_4O_2SCl)^+$, 134 $(C_8H_9NO)^+$, 122 $(C_7H_8NO)^+$, 113 $(C_6H_4Cl+2)^+$, 111 $(C_6H_4Cl)^+$, 106 $(C_7H_8O)^+$, 91 $(C_7H_7)^+$.

***2-[[(4-Chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-N-(3-methylphenyl)acetamide* (*7e*)**

Brown solid; Yield: 82%; m.p. 114-115 °C; Molecular formula: $C_{21}H_{20}N_4O_2S$; Molecular weight: 472 g/mol; IR (KBr, υ, cm^{-1}): 3345 (N-H, stretching), 3082 (C-H, str. of aromatic ring), 1659 (C=O str.), 1554 (C=C, aromatic str.), 1244 (S=O streching); ^{1}H-NMR (600 MHz, $CDCl_3$, *δ*, ppm): 8.22 (s, 1H, NH), 7.65 (d, *J* = 8.5 Hz, 2H, H-2′ & H-6′), 7.49 (d, *J* = 8.5 Hz, 2H, H-3′ & H-5′), 7.33 (br.s, 1H, H-2‴), 7.31 (br.d, *J* = 8.7 Hz, 1H, H-6‴), 7.22-7.20 (m, 2H, H-3‴ & H-4‴), 6.79 (d, *J* = 8.6 Hz, 1H, H-8), 6.63 (d, *J* = 2.6 Hz, 1H, H-5), 6.55 (dd, *J* = 2.6, 8.6 Hz, 1H, H-7), 4.28-4.19 (m, 6H, CH_2-2, CH_2-3 & CH_2-2″), 2.34 (s, 3H, 3-CH_3); EI-MS: *m/z* 476 $[M+4]^+$, 474 $[M+2]^+$, 472 $(C_{23}H_{21}N_2O_5SCl)^{\cdot+}$ $[M]^+$, 297 $(C_{17}H_{17}N_2O_3)^+$, 179 $(C_6H_4O_2SCl+4)^+$, 177 $(C_6H_4O_2SCl+2)^+$, 175 $(C_6H_4O_2SCl)^+$, 134 $(C_8H_9NO)^+$, 122 $(C_7H_8NO)^+$, 113 $(C_6H_4Cl+2)^+$, 111 $(C_6H_4Cl)^+$, 106 $(C_7H_8O)^+$, 91 $(C_7H_7)^+$.

***2-[[(4-Chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-N-(4-methylphenyl)acetamide* (*7f*)**

Light brown solid; Yield: 81%; m.p. 143-145^{o}C; Molecular formula: $C_{23}H_{21}N_2O_5SCl$; Molecular weight: 472g/mol; IR (KBr, υ, cm^{-1}): 3343 (N-H, stretching), 3085 (C-H, str. of aromatic ring), 1654 (C=O str.), 1559 (C=C, aromatic str.), 1241 (S=O streching); ^{1}H-NMR (400 MHz, $CDCl_3$, *δ*, ppm): 8.15 (s, 1H, NH), 7.87 (d, *J* = 8.5Hz, 2H, H-2′ & H-6′), 7.51 (d, *J* = 8.5 Hz, 2H, H-3′ & H-5′), 7.37 (d, *J* = 7.6 Hz, 2H, H-2‴ & H-6‴), 7.13 (d, *J* = 7.6 Hz, 2H, H-3‴ & H-5‴), 6.79 (d, *J* = 9.0 Hz, 1H, H-8), 6.55 (d, *J* = 2.5 Hz,1H, H-5), 6.45 (dd, *J* = 2.5, 9.0 Hz, 1H, H-7), 4.48 (s, 2H, CH_2-2″), 4.27-4.23 (m, 4H, CH_2-2 & CH_2-3), 2.35 (s, 3H, 4-CH_3); EI-MS: *m/z* 476 $[M+4]^+$, 474 $[M+2]^+$, 472 $(C_{23}H_{21}N_2O_5SCl)^{\cdot+}$ $[M]^+$, 297 $(C_{17}H_{17}N_2O_3)^+$, 179 $(C_6H_4O_2SCl+4)^+$, 177 $(C_6H_4O_2SCl+2)^+$, 175 $(C_6H_4O_2SCl)^+$, 134 $(C_8H_9NO)^+$, 122 $(C_7H_8NO)^+$, 113 $(C_6H_4Cl+2)^+$, 111 $(C_6H_4Cl)^+$, 106 $(C_7H_8O)^+$, 91 $(C_7H_7)^+$.

***2-[[(4-Chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-N-(2,3-dimethylphenyl)acetamide* (*7g*)**

Grey white solid; Yield: 80%; m.p. 93-94°C; Molecular formula: $C_{24}H_{23}N_2O_5SCl$; Molecular weight: 486g/mol; IR (KBr, υ, cm^{-1}): 3343 (N-H, stretching), 3082 (C-H, str. of aromatic ring), 1658 (C=O str.), 1559 (C=C, aromatic str.), 1244 (S=O streching); ^{1}H-NMR (400 MHz, $CDCl_3$, *δ, ppm*): 8.20 (br.s, 1H, NH), 7.56 (d, *J* = 8.4 Hz, 2H, H-2′ & H-6′), 7.49 (br.d, *J* = 7.2 Hz, 1H, H-4≡≠), 7.46 (d, *J* = 8.8 Hz, 2H, H-3′ & H-5′), 7.07 (br.t, *J* = 7.6 Hz, 1H, H-5≡≠), 7.01 (br.d, *J* = 7.6 Hz, 1H, H-6≡≠), 6.78 (d, *J* = 8.8 Hz, 1H, H-8), 6.69 (d, *J* = 2.4 Hz, 1H, H-5), 6.55 (dd, *J* = 2.4, 8.8 Hz, 1H, H-7), 4.24-4.19 (m, 6H, CH_2-2, CH_2-3 & CH_2-2≠′), 2.28 (s, 3H, 3-CH_3), 2.15 (s, 3H, 2-CH_3); EI-MS: *m/z* 490 $[M+4]^+$, 488 $[M+2]^+$, 486 $(C_{24}H_{23}N_2O_5SCl)^{\cdot+}$ $[M]^+$, 311$(C_{18}H_{19}N_2O_3)^+$, 179 $(C_6H_4O_2SCl+4)^+$, 177 $(C_6H_4O_2SCl+2)^+$, 175 $(C_6H_4O_2SCl)^+$, 148 $(C_9H_{10}NO)^+$, 120 $(C_8H_{10}N)^+$, 113 $(C_6H_4Cl+2)^+$, 111 $(C_6H_4Cl)^+$, 105 $(C_8H_9)^+$.

***2-[[(4-Chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-N-(2,4-dimethylphenyl)acetamide* (*7h*)**

Grey white solid; Yield: 76%; m.p. 110-112°C; Molecular formula: $C_{24}H_{23}N_2O_5SCl$; Molecular weight: 486g/mol;

IR (KBr, υ, cm^{-1}): 3346 (N-H, stretching), 3080 (C-H, str. of aromatic ring), 1658 (C=O str.), 1552 (C=C, aromatic str.), 1247 (S=O streching); ^{1}H-NMR (400 MHz, $CDCl_3$, *δ*, ppm): 8.15 (br.s, 1H, NH), 7.64 (br.d, *J* = 8.0 Hz, 1H, H-6≡≠), 7.56 (d, *J* = 8.4 Hz, 2H, H-2′ & H-6′), 7.46 (d, *J* = 8.4 Hz, 2H, H-3′ & H-5′), 6.99-6.97 (m, 2H, H-3≡≠ & H-5≡≠), 6.77 (d, *J* = 8.4 Hz, 1H, H-8), 6.68 (d, *J* = 2.4 Hz, 1H, H-5), 6.54 (dd, *J* = 2.4, 8.8 Hz, 1H, H-7), 4.26-4.20 (m, 6H, CH_2-2, CH_2-3 & CH_2-2≠′), 2.27 (s, 3H, 4-CH_3), 2.23 (s, 3H, 2-CH_3). EI-MS: *m/z* 490 $[M + 4]^+$, 488 $[M + 2]^+$, 486 $(C_{24}H_{23}N_2O_5SCl)^{\cdot+}$ $[M]^+$, 311 $(C_{18}H_{19}N_2O_3)^+$, 179 $(C_6H_4O_2SCl+4)^+$, 177 $(C_6H_4O_2SCl+2)^+$, 175 $(C_6H_4O_2SCl)^+$, 148 $(C_9H_{10}NO)^+$, 120 $(C_8H_{10}N)^+$, 113 $(C_6H_4Cl+2)^+$, 111 $(C_6H_4Cl)^+$, 105 $(C_8H_9)^+$.

2-[[(4-Chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-N-(2,5-dimethylphenyl)acetamide (*7i*)

Pinkish white solid; Yield: 79%; m.p. 93-94°C; Molecular formula: $C_{24}H_{23}N_2O_5SCl$; Molecular weight: 486g/mol; IR (KBr, υ, cm^{-1}): 3347 (N-H, stretching), 3084 (C-H, str. of aromatic ring), 1659 (C=O str.), 1558 (C=C, aromatic str.), 1243 (S=O streching); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ*, ppm); 8.15 (brs, 1H, NH), 7.62 (br.d, *J* = 8.0 Hz, 1H, H-3≡≠), 7.56 (d, *J* = 8.5 Hz, 2H, H-2′ & H-6′), 7.46 (d, *J* = 8.5 Hz, 2H, H-3′ & H-5′), 6.99-6.97 (m, 2H, H-4≡≠ & H-6≡≠), 6.77 (d, *J* = 9.0 Hz, 1H, H-8), 6.68 (d, *J* = 2.5 Hz, 1H, H-5), 6.54 (dd, *J* = 2.5, 8.5 Hz, 1H, H-7), 4.24-4.21 (m, 6H, CH_2-2, CH_2-3 & CH_2-2≠′), 2.26 (s, 3H, 5-CH_3), 2.23 (s, 3H, 2-CH_3); EI-MS: *m/z* 490 $[M + 4]^+$, 488 $[M + 2]^+$, 486 $(C_{24}H_{23}N_2O_5SCl)^{\cdot+}$ $[M]^+$, 311$(C_{18}H_{19}N_2O_3)^+$, 179 $(C_6H_4O_2SCl+4)^+$, 177 $(C_6H_4O_2SCl+2)^+$, 175 $(C_6H_4O_2SCl)^+$, 148 $(C_9H_{10}NO)^+$, 120 $(C_8H_{10}N)^+$, 113 $(C_6H_4Cl+2)^+$, 111 $(C_6H_4Cl)^+$, 105 $(C_8H_9)^+$.

2-[[(4-Chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-N-(2,6-dimethylphenyl)acetamide (*7j*)

Dark brown solid; Yield: 75%; m.p. 93-95°C; Molecular formula: $C_{24}H_{23}N_2O_5SCl$; Molecular weight: 486g/mol; IR (KBr, υ, cm^{-1}): 3345 (N-H, stretching), 3081 (C-H, str. of aromatic ring), 1659 (C=O str.), 1559 (C=C, aromatic str.), 1246 (S=O streching); ^{1}H-NMR (600 MHz, $CDCl_3$, *δ*, ppm): 7.91 (s, 1H, NH), 7.65 (d, *J* = 8.2 Hz, 2H, H-2′ & H-6′), 7.39 (d, *J* = 8.2 Hz, 2H, H-3′ & H-5′), 7.11-7.05 (m, 3H, H-3≠≡, H-4≠≡ & H-5≠≡), 6.81 (d, *J* = 8.6 Hz, 1H, H-8), 6.73 (d, *J* = 2.5 Hz, 1H, H-5), 6.48 (dd, *J* = 2.5, 8.6 Hz, 1H, H-7), 4.32 (s, 2H, CH_2-2≠′), 4.27-4.20 (m, 4H, CH_2-2 & CH_2-3), 2.11 (s, 6H, 2-CH_3 & 6-CH_3); EI-MS: *m/z* 490 $[M + 4]^+$, 488 $[M + 2]^+$, 486 $(C_{24}H_{23}N_2O_5SCl)^{\cdot+}$ $[M]^+$, 311$(C_{18}H_{19}N_2O_3)^+$, 179 $(C_6H_4O_2SCl+4)^+$, 177 $(C_6H_4O_2SCl+2)^+$, 175 $(C_6H_4O_2SCl)^+$, 148 $(C_9H_{10}NO)^+$, 120 $(C_8H_{10}N)^+$, 113 $(C_6H_4Cl+2)^+$, 111 $(C_6H_4Cl)^+$, 105 $(C_8H_9)^+$.

2-[[(4-Chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-N-(3,4-dimethylphenyl)acetamide (*7k*)

Cream colored solid; Yield: 81%; m.p. 173-175°C; Molecular formula: $C_{24}H_{23}N_2O_5SCl$; Molecular weight: 486g/mol; IR (KBr, υ, cm^{-1}): 3342 (N-H, stretching), 3083 (C-H, str. of aromatic ring), 1658 (C=O str.), 1556 (C=C, aromatic str.), 1247 (S=O streching); ^{1}H-NMR (400 MHz, $CDCl_3$, *δ*, ppm): 8.19 (s, 1H, NH), 7.57 (d, *J* = 8.4 Hz, 2H, H-2′ & H-6′)., 7.46 (d, *J* = 8.8 Hz, 2H, H-3′ & H-5′), 7.23-7.20 (m, 2H (Merged in the signal of $CDCl_3$), H-2≠≡, H-5≠≡) 7.06 (br.d, *J* = 8.0 Hz, 1H, H-6≠≡), 6.76 (d, *J* = 8.4 Hz, 1H, H-8), 6.67 (d, *J* = 2.4 Hz, 1H, H-5), 6.54 (dd, *J* = 2.4, 8.8, 1H, H-7), 4.22-4.20 (m, 6H, CH_2-2, CH_2- 3 & CH_2-2≠′), 2.23 (s, 3H, 4-CH_3), 2.20 (s, 3H, 3-CH_3); EI-MS: *m/z* 490 $[M+4]^+$, 488 $[M+2]^+$, 486 $(C_{24}H_{23}N_2O_5SCl)^{\cdot+}$ $[M]^+$, 311$(C_{18}H_{19}N_2O_3)^+$, 179 $(C_6H_4O_2SCl+4)^+$, 177 $(C_6H_4O_2SCl+2)^+$, 175 $(C_6H_4O_2SCl)^+$, 148 $(C_9H_{10}NO)^+$, 120 $(C_8H_{10}N)^+$, 113 $(C_6H_4Cl+2)^+$, 111 $(C_6H_4Cl)^+$, 105 $(C_8H_9)^+$.

2-[[(4-Chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-N-(3,5-dimethylphenyl)acetamide (*7l*)

Light brown solid; Yield: 77%; m.p. 138-139°C; Molecular formula: $C_{24}H_{23}N_2O_5SCl$; Molecular weight: 486g/mol; IR (KBr, υ, cm^{-1}): 3345 (N-H, stretching), 3081 (C-H, str. of aromatic ring), 1658 (C=O str.), 1555 (C=C, aromatic str.), 1243 (S=O streching); ^{1}H-NMR (400 MHz, $CDCl_3$, *δ*, ppm); 8.13 (s, 1H, NH), 7.57 (d, *J* = 8.4 Hz, 2H, H-2′ & H-6′), 7.47 (d, *J* = 8.4 Hz, 2H, H-3′ & H-5′), 7.11 (br.s, 2H, H-2≠≡, H-6≠≡), 6.78 (d, *J* = 8.4 Hz, 1H, H-8), 6.67 (d, = 2.4 Hz, 1H, H-5), 6.54 (dd, *J* = 2.4, 8.4 Hz, 1H, H-7), 4.22-4.21 (m, 4H, CH_2-2 & CH_2-3), 4.19 (s, 2H, CH_2-2≠′), 2.28 (s, 6H, 3-CH_3 & 5-CH_3); EI-MS: *m/z* 490 $[M+4]^+$, 488 $[M+2]^+$, 486 $(C_{24}H_{23}N_2O_5SCl)^{\cdot+}$ $[M]^+$, 311$(C_{18}H_{19}N_2O_3)^+$, 179 $(C_6H_4O_2SCl+4)^+$, 177 $(C_6H_4O_2SCl+2)^+$, 175 $(C_6H_4O_2SCl)^+$, 148 $(C_9H_{10}NO)^+$, 120 $(C_8H_{10}N)^+$, 113 $(C_6H_4Cl+2)^+$, 111 $(C_6H_4Cl)^+$, 105 $(C_8H_9)^+$.

Antibacterial assay by well diffusion method

The sulfonamide derivatives were undergone antimicrobial screening by well diffusion method (CLSI, 2007). For this purpose selected bacterial strains like *E.coli, S.aureus, B. subtillus, P. multocida* and fungal species of *Aspergillus niger, Alternaria alternate, Rhizopous solani* and *Fusarium solani* were employed. 28.08 g/L of nutrient agar was dispensed in number of petri plates and inoculated with the culture of bacterial strains. This combination of agar medium was given time span to solidify. The wells were prepared by a sterile borer in solidified nutrient agar gel medium and subsequently diluted test samples were transferred in them. For positive and negative control; we have used Rifampicin and DMSO respectively. For proficient growth of bacteria the petri plates were incubated for 24 hours at 37°C. The growth of bacterial strains was observed to be inhibited by the synthesized compounds and revealed the clear zones around them; the clear zones were measured with the help of zone reader (Narasaiah *et al.,* 2012).

Antifungal assay by well diffusion method

For antifungal assay about 39.06 g/L of Potato Dextrose Agar (PDA) was transferred into petri plates and afterwards inoculated with the different fungal species. The setup was let to solidify. The wells were prepared by a sterile borer in solidified nutrient agar gel medium and subsequently diluted test samples were transferred in them. Fluconazole and DMSO were used as positive and negative controls respectively. The incubation of petri plates was done at 28ºC for 48 hours; the rest of descriptions for assessing the antifungal activity were same as elaborated above (Hayam *et al.,* 2013).

Minimum inhibitory concentrations (MIC)

100µL of nutrient broth was poured in all wells of 96 well plates (micro dilution plates). Subsequently added 100µL of samples in the first well and used its two-fold diluted form by making the use of dilution method. Later on all the wells were cultured with 20µL of bacterial strains and incubation at 37°C for 24 hours were carried out. Resazurine was used as bacterial growth indicator and color changes from blue to pink scored as bacterial growth initiation.

Hemolytic assay

Bovine blood sample was collected and stored in EDTA. The collected sample was diluted with 0.9% NaCl solution (saline), and centrifuged at 1000xg for 10 min. The blood erythrocytes get separated and diluted in H_3PO_4 buffer saline bearing pH 7.4. The buffer treatment resulted in suspension formation. Synthetic compounds (20µL of solution; one in each experiment) were added (made by 10mg/mL) in 180µL of RBCs suspension and incubated for 30 min at room temperature. PBS and Triton 100-X were used as negative and positive controls respectively (Shahid *et al.,* 2013). The %age of hemolysis was taken as by using formula:

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

RESULTS

The synthesis of designed molecules (7a-l), has been delineated in Scheme-1. The detailed procedures are given in the experimental section. The structural characterization of the targeted molecules was substantiated through spectral analysis. All these molecules were evaluated for their antibacterial and antifungal potential and the subsequent results are tabulated in table 1 and table 2. Moreover, the cytotoxicity of these derivatives was also evaluated through hemolytic assay and these results are presented in table 3.

DISCUSSION

The synthesis was initiated by the reaction of 2,3-dihydro-1,4-benzodioxin-6-amine (1) with 4-chlorobenzene-sulfonyl chloride (2) in presence of basic aqueous medium (pH 9-10) to form 4-chloro-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)benzenesulfonamide (3) on one hand, and on the other hand, various un/substituted anilines (4a-l) were reacted with bromoacetyl bromide (5) in equimolar quantities in Na_2CO_3 solution under dynamic pH (9-10) control to obtain corresponding 2-bromo-*N*-(un/substituted)phenylacetamides (6a-l) as electrophiles. Then, the compound 3 was coupled with these electrophiles (6a-l) in aprotic solvent (DMF) and lithium hydride (LiH) used in a pinch; behaves as an activator and base for proton abstraction. The net results were the synthesis of 2-[[(4-Chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-*N*-(un/substituted-phenyl) acetamides (7a-l, Scheme-1). The structural verification of the synthesized compounds were done by IR, ^{1}H-NMR and EI-MS spectral data.

We have chosen one representative compound (7h) from the series for the structural description. The purpose was to enhance the reader's feasibility in understanding the spectral data. The IR spectrum of 7h confirmed different functionalities, where the absorption bands were observed at 3346 cm^{-1}for N-H stretching and at 3080 cm^{-}1for aromatic C-H stretching. A peak at 1658 cm^{-1} was of C=O stretching and that at 1552 cm^{-1}was characteristic for aromatic C=C stretching. A stretching band at 1247 cm^{-1}confirmed the S=O functionality in the molecule. Molecular formula of this compound was ascertained by the molecular ion peak $[M]^+$ at *m/z* 486. Other fragmented peaks in the EI-MS spectrum appeared at *m/z* 311, 175, 148, 120,113, 11 and 105, also collectively helped to deduce the structure of 7h. The assignment of molecular formula was also augmented by adding up all the magnetically equivalent & non-equivalent protons in its ^{1}H-NMR spectrum. A broad singlet at chemical shift value of 8.15 was observed in the ^{1}H-NMR spectrum. It was assigned to a hetero-atom proton (-N-H) of an amide group; which are frequently appeared in least shielded and downfield region of spectrum. A 4-chlorobenzenesulofonyl moiety was clearly corroborated by two *ortho*-coupled doublets in aromatic region at δ 7.56 (J = 8.4 Hz, 2H, H-2′ & H-6′), and δ 7.46 (d, J = 8.4 Hz, 2H, H-3′ & H-5′). An AMX spin system, for a 6-amino-benzodioxane moiety in this molecule, was identified by an *ortho*-coupled doublet at δ 6.77 (J = 8.4 Hz, 1H, H-8), a *meta*-coupled doublet at δ 6.68 (J = 2.4 Hz, 1H, H-5), and a reciprocal doublet of doublet at δ 6.54 (J = 2.4, 8.8 Hz, 1H, H-7). The 2,4-dimethylphenyl unit in this molecule was ascertained by two resonances in aromatic region at δ 7.64 (br.d, J = 8.0 Hz, 1H, H-6′′′), and 6.99-6.97 (m, 2H, H-3′′′ & H-5′′′), along with two methyl signals in aliphatic region at δ 2.27 (s, 3H, 4-CH_3), and 2.23 (s, 3H, 2-CH_3). The two methylene groups (CH_2-2 & CH_2-3) of 1,4-benzodioxane moiety and a methylene group of (substituted)acetamido group were overlapped as a multiplet at δ 4.26-4.20 (m, 6H, CH_2-2, CH_2-3 & CH_2-2′′). So, based on all these cumulative evidences, the structure of 7h was confirmed and it was given the name as 2-[[(4-chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-*N*-(2,4-dimethylphenyl) acetamide. The structures of other derivatives were also confirmed in a similar pattern.

Reagents & Conditions

(I) Aq. Na_2CO_3 soln./pH 9-10/stirring at RT for 3 hrs. (II) Aq. Na_2CO_3 soln./pH 9-10/stirring at RT for 2-3 hrs. (III) DMF/LiH/stirring at RT for 4-5 hrs.

Antibacterial activity

The compounds were accessed by four bacterial pathogenic strains in an antibacterial assay. *Bacillus subtilis* and *Staphylococcus aureus* were the gram positive while *Escherichia coli* and *Pasteurella multocida* were among gram negative pathogenic strains. Rifampicin was the standard drug utilized for the antibacterial activity in all evaluations. Results depicting antibacterial activity and MIC values have been provided in table 1. Here it was noted that maximum antibacterial activity was shown by the compound 7l against *B. subtilis* having 14.33±2.08 mm zone of inhibition with MIC of 25 µg/mL. The compounds 7g and 7h also showed good activity against *B. subtilis having* 11.00±1.00 mm and 12.67±1.52 mm zone of inhibition respectively. The MIC value was 25 µg/mL in each case. Remaining compounds exhibited weak activity. Compounds, 7b-e, were found to be inactive and did not inhibit the growth of any bacterial strains used in this study.

Antifungal activity

Standard antifungal drug terbinafine was used as fungicidal reference drug. All compounds of sulfonamide series were screened against four selected fungal strains (*Aspergillus niger, Fusarium solani, Rhizopus solani, Alternaria alternata*) and results of antifungal activity are given in table 2. The compound 7l showed good activity against *A. alternata* with 10.33±0.58 mm zone of inhibition and MIC of 25 µg/mL. This compound has also shown better antifungal activity against *R. solani* and *F. solani* fungal strains. The compounds 7f, 7g and 7h exhibited moderate activity against *A. alternate* having zone of inhibition 6.67±0.58, 9.67±0.58 and 8.33±0.58 mm respectively. The compound 7g also showed moderate activity against *R. solani*. The compounds 7b-e remained inactive against all the fungal strains used in this study.

Hemolytic activity

All the synthesized compounds, 7a-l, were subjected to hemolytic assay. Results of percentage hemolysis are shown in table 3. The study demonstrated that nearly all of the compounds underlying these series were almost non-toxic in disruption of the red blood cell membrane. The compound 7e displayed maximum membrane toxicity (26.19±2.30%) and 7k (25.14±1.01%), while minimum toxicity was recorded in compounds 7g and 7i, both having same value (5.47±0.69%).

Structure activity relationship

The variable part in our synthesized multi-functional 2-[[(4-chlorophenyl)sulfonyl](2,3-dihydro-1,4-benzodioxin-6-yl)amino]-*N*-(un/substituted-phenyl)acetamides (7a-l) was the acetamidic *N*-aryl ring. It was an unsubstituted phenyl ring in only one molecule (7a) while bearing differently substituted methoxy, ethoxy or methyl groups in other molecules. From the bioactivity results, it was noticed that the change in position of -CH_3 group on phenyl ring of acetamidic functionality; resulted in boosting or lessening the antibacterial trend for the respective compounds. Antibacterial activity results have shown that maximum activity was revealed by the compound 7l having methyl groups substituted at 3 & 5 positions. It was observed that antibacterial activity of the compound was lost if methoxy or ethoxy group was present as substitution, as well depicted from the results of compounds 7b and 7c. In the same way the presence of a single -CH_3 group at 2 & 3 positions on phenyl ring have resulted in diminishing the antibacterial activity, particularly for compound 7d and 7e respectively. Its general observation that the electron withdrawing functionalities like halogens, oxygen, sulfur and nitrogen might be capable enough to integrate the antibacterial activity in the synthetic compounds.

Our results of antifungal activity have shown that compound 7l exhibited maximum antifungal activity against selected fungal strains. The compounds having 2-methoxy (7b), 4-ethoxy (7c), 2-methyl (7d) and 3-methyl (7e) substitution at acetamidic phenyl ring manifested no antifungal activity, although other antimicrobial functional groups are also present in these molecules, like sulfonamide and acetamidic group. In the current study it was observed that dimethyl substitution at different positions of acetamidic phenyl ring attributed considerable antifungal activity in synthetic compounds as in case of compounds 7g-l.

The mechanism of membrane toxicity is not clear here but our observations revealed that the presence of -CH_3 group at *ortho* positions of acetamidic phenyl ring might be responsible to incorporate the membrane toxicity action into the sample. These findings were well shown in case of compound 7j having 25.14 % toxicity; in which both of the -CH_3 groups were connected to the *ortho* positions.

Similar trend was illustrated by compound 7e which exhibit 26.19 % toxicity bearing -CH_3 groups on position 3rd on a ring. The absence of substitution at any position of acetamidic phenyl ring resulted in decreasing of toxicity; as shown by the compound 7a displaying 15.75 % toxicity. But we have observed that di-*meta* substitution of -CH_3 groups at benzene ring resulted in diminishing the toxicity level to 8.32 % as in case of compound 7l. While in compounds 7b and 7c the incorporation of -OCH_3 and -OC_2H_5 group did not show any remarkable change in membrane toxicity level.

CONCLUSION

It was concluded from the present study that targeted multi-functional acetamides were successfully synthesized in good yields. Some of the synthesized compounds, particularly bearing electron donating methyl groups in acetamidic *N*-aryl part, have exhibited considerable antibacterial and antifungal potentials along with mild cytotoxicity. So, such molecules can be regarded as valuable medicinal scaffolds in drug discovery and development program.

ACKNOWLEDGEMENT

The financial grant from the Higher Education Commission (HEC) of Pakistan is highly acknowledged for present study.

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