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Synthesis, Biological Evaluation and Docking Studies of Sulfonyl Piperazine Derivatives: Part-A

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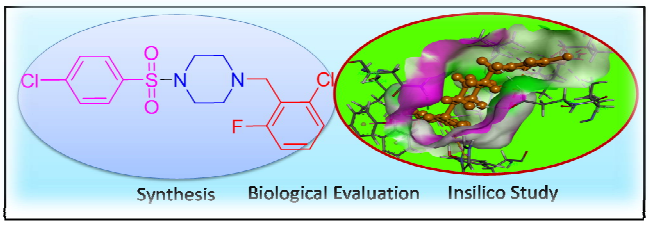
ABSTRACT

Twelve novel N-alkylated sulfonyl piperazine derivatives were prepared by condensation of chloro and bromo substituted 1-(phenylsulfonyl) piperazine with six differently substituted benzyl chlorides. Structures of the compounds were verified by IR, ^{1}HNMR, ^{13}CNMR and LCMS spectroscopic techniques. Compounds were screened for their in vitro antimicrobial activity against two bacterial strains S. aureus and E. coli and against two fungi C. albicans, A. flavus. Anthelmintic activity of compounds was also studied against Pheretima posthuma. Some of the sulfonyl piperazine derivatives showed comparatively good antimicrobial properties and anthelmintic activities compared to standard drug. The biological activity was supported by virtual screening using molecular docking study.

Graphical Abstract



Keywords: Piperazine, Antibacterial activity, Anthelmintic activity, Molecular docking.

INTRODUCTION

Diseases caused by microbial infection are a serious menace to the health of human beings and often have connection to some other diseases. The migration of people from one place to another contributes to the diffusion of infectious disease. A revolution came in the medicinal world with the discovery of antibiotics, for treatment of various bacterial infections. However their indiscriminate

use led to an alarming increase in antibiotics résistance among microorganisms, giving rise to multi resistant strain, which has become global concern [**1**].

Recently, many drug-resistant human pathogenic microbes have been reported [**2**]. The progressive increase of antimicrobial resistance is a challenging task to human beings to manage infectious diseases that are common and thus resulting in mortality and morbidity of individuals [**3**]. In absence of potent antibiotics, many standard medical treatments will become ineffective against multi-drug resistant microbes [4]. This calls for the immediate global united move to find potent antimicrobial agents, otherwise, the world will head towards an era where antibiotics have no longer function.

In order to find potent antimicrobial agents, Sulfonamide derivatives are important category of pharmacophores that have a wide spectrum of pharmaceutical accomplishments as antimalarial [5], anti-microbial [**6**], anti-bacterial [**7, 8**], anti-cancer [**9**], anti-fungal [**10**], anti-oxidant [**11**], anti-HIV [**12**], antiplasmodial [**13**], anti-neoplastic [**14**], anti-proliferative [15] activities and additionally known to act as 5-HT6, 5-HT7 receptor antagonists [**16, 17**], A2B and CXCR3 antagonists [**18, 19**], 11β-HSD [**20**], histone deacetylase (HDAC) inhibitors [**21**].

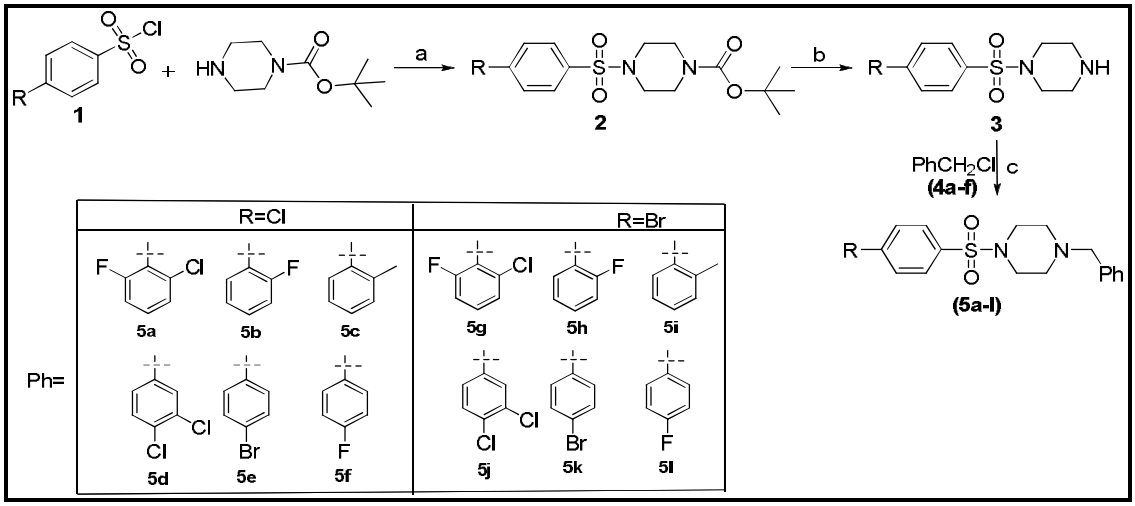
In addition piperazine nuclei have constituted an attractive pharmacological scaffold present in various potent marketed drugs. The inclusion of piperazine is an important synthetic strategy in drug discovery due to its easy modifiability, proper alkalinity, water solubility, the capacity for the formation of hydrogen bonds and adjustment of molecular physicochemical parameters. This dinitrogen moiety has been an inseparable component of plethora of drugs. A number of substituted piperazines possess significant pharmacological action such as antihistamic [**22, 23**], antibacterial [**24**], acetylcholinesterae inhibitors [**25**], antimalarial [**26**], dopamine transporter [**27, 28**], D2/D4 antagonist [**29**], MC4 Receptor [**30**] and HIV-protease inhibitor [**31**]. Piperazine linked to sulfonamides exhibit diverse therapeutic activities such as antibacterial, matrix metalloprotein-3 inhibition and carbonic anhydrase inhibition [**32**]. Intensive research has been carried out on the synthesis and analysis of pharmacological activities of these derivatives.

Our group has previously reported the pharmacological activity of alkylated sulfonyl piperazines. In continuation of previous work, here we present the synthesis of new sulfonyl piperazine derivatives and screened them for their biological activity.

MATERIALS AND METHODS

Melting points reported were determined in open capillary. The structures of the newly synthesized compounds were established using IR, ^{1}HNMR, ^{13}CNMR and LC-MS data. FT-IR Spectra was recorded on Jasco FT-IR Spectrometer, ^{1}HNMR and ^{13}CNMR were recorded in DMSO-d_6 at 399.65 MHz and 100.40 MHz respectively. All the chemical shifts were reported in parts per million (ppm). LC-MS was recorded using Waters Alliance 2795 separations module and Waters Micromass LCT mass detector. Elemental analysis (C, H and N) was performed on Elementar vario MICRO cube. All the chemicals were purchased from Merck India, Spectrochem and Sigma-Aldrich. Solvents and Chemicals used were of LR grade. The purity of the compound was confirmed using TLC on pre-coated silica gel plate and further purification was done using column chromatography. All the bacterial strains were procured from CSIR-National Chemical Laboratory(NCL), Pune, India.

Preparation of 1-(tert-butoxy)-4-((4-substituted phenyl)sulfonyl)piperazine(2): N-Boc piperazine (0.11 mol) was dissolved in methylene dichloride to this added triethylamine (TEA) (0.33 mol) and substituted benzene sulfonyl chloride **(1)** (0.11 mol), reaction mixture was stirred for 5 h at room temperature. Reaction was monitored by TLC, after completion, organic layer was washed with water, separated, washed with brine, dried over Na_2SO_4 and crude compound was purified by column chromatography to obtain title compound **(2)**. m.p.: 190-191°C. Yield: 88%.



Scheme 1 Synthesis of Title compounds; Reagents and conditions:
(a) TEA, DCM, rt, 5 h; (b) TFA, DCM, 0 °C, 3-4 h; (c) $PhCH_2Cl$ (4a-f), K_2CO_3, MeCN, rt, 10 h.

Preparation of 1-((4- substituted phenyl)sulfonyl)piperazine(3): To a solution of compound **(2)** (0.2310 mol) in methylene dichloride, trifluoroacetic acid (2 mL) was added drop wise for about half an hour and the reaction mixture was stirred at 0°C for 3-4 h. Completion of the reaction was monitored by TLC. After completion, the excess trifluoroacetic acid was neutralized by sodium carbonate solution. Then it was extracted with methylene dichloride, organic layer was separated. The solvent was removed under reduced pressure to get compound **(3)** as white solid m.p.: 211-213°C; Yield 64 %.

General Procedure for the preparation of 1-(substituted benzyl)-4-((4-chloro/bromophenyl) sulfonyl)piperazine(5a-l): Equimolar quantities of 1-((4- substituted phenyl)sulfonyl)piperazine **(3)** (0.001 mol), different substituted benzylchloride **(4a-f)** (0.001 mol) in acetonitrile were stirred for 10h in the presence of K_2CO_3(0.003 mol). The completion of the reaction was checked by TLC. After the completion, it was poured into ice cold water; the organic layer was separated, washed with water and dried over anhydrous sodium sulphate. The organic layer was concentrated under reduced pressure to get alkylated piperazine containing sulfonyl nucleus as a solid **(5a-l)** in good yield. Physical data of all the final compounds are given in table 1**.**

Spectral Interpretation

Spectral data of 1-(2-chloro-6-fluorobenzyl)-4-((4-chlorophenyl)sulfonyl)piperazine(5a): IR (KBrcm^{-1}): 1329.4(S=O asymmetric), 1160.5(S=O symmetric).**^{1}HNMR** (400 MHz, $CDCl_3$, TMS)δ ppm: 2.61(t, 4H, -CH_2), 2.98(s, 4H, -CH_2), 3.6(s, 2H, -CH_2), 6.91-6.96 (m, 3H, Ar-H), 7.44-7.46 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.63-7.65 (d, *J* = 8.4 Hz, 2H, Ar-H). **^{13}CNMR** (100 MHz, $CDCl_3$, TMS) δ ppm**:** 46.03, 51.75, 77.02, 113.92, 123.11,125.46,129.4,134.08,136.4, 139.39, 160.6, 163.16. **Mass:** m/z 402 [M + 1].

Spectral data of 1-((4-chlorophenyl)sulfonyl)-4-(2-fluorobenzyl)piperazine(5b): IR (KBr, cm^{-1}) 1312.5(S=O asymmetric), 1149.5(S=O symmetric).**^{1}HNMR** (400 MHz, $CDCl_3$, TMS)δ ppm: 2.54(s, 4H, -CH_2), 3.01(s, 4H, CH_2), 3.54(s, 2H, -CH_2), 6.97-7.06 (m, 2H, Ar-H), 7.2-7.24 (m, 2H, Ar-H). 7.46-7.48 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.64-7.66 (d, *J* = 8.4 Hz, 2H, Ar-H). **^{13}CNMR** (100 MHz, $CDCl_3$,TMS) δ ppm**:** 45.99, 51.7, 54.96, 77.04, 115.29, 123.92, 129.1, 131.4, 133.9, 139.4, 160.09, 162.5. **Mass:** m/z 369 [M + 1].

Spectral data of 1-((4-chlorophenyl)sulfonyl)-4-(2-methylbenzyl)piperazin(5c): IR(KBr, cm^{-1}): 1342.8 (S=O asymmetric), 1182.4 (S=O symmetric); 2918.89 (Ar-H) **^{1}HNMR** (400 MHz, $CDCl_3$,

TMS)δ ppm: 2.27 (s, 3H, -CH_3), 2.51 (t, 4H, -CH_2), 2.99 (s, 4H, CH_2), 3.44 (s, 2H, -CH_2), 7.07-7.16 (m, 4H, Ar-H), 7.47-7.50 (m, 2H, Ar-H) 7.65-7.68 (m, 2H, Ar-H). **^{13}CNMR** (100 MHz, $CDCl_3$, TMS) δ ppm**:** 19.18, 46.15, 52.05, 60.30, 77.57, 125.57, 127.34,129.3, 134.16,135.5,137.4, 139.38. **Mass:** m/z 365 [M + 1].

Spectral data of1-((4-chlorophenyl)sulfonyl)-4-(3,4-dichlorobenzyl)piperazine (5d): IR (KBr, cm^{-1}) 1352.5 (S=O asymmetric), 1189.3 (S=O symmetric). **^{1}HNMR** (400 MHz, $CDCl_3$, TMS)δ ppm: 2.48 (t, 4H, -CH_2), 3.00 (s, 4H, CH_2), 3.4 (s, 2H, -CH_2), 7.04-7.06 (d, *J* = 6.8 Hz, 1H, Ar-H), 7.31-7.33 (d, *J* = 8 Hz, 2H, Ar-H), 7.48-7.5 (d, *J* = 8 Hz, 2H, Ar-H). 7.65-7.67 (d, *J* = 8 Hz, 2H, Ar-H). **^{13}CNMR** (100 MHz, $CDCl_3$, TMS) δ ppm**:** 45.96, 51.97, 61.18, 77.01, 128.1, 129.13, 130.25, 131.2, 132.4, 134.2, 137.9, 139.47. **Mass:** m/z 420 [M+ 1].

Spectral data of 1-(4-bromobenzyl)-4-((4-chlorophenyl)sulphonyl)piperazine(5e): IR (KBr, cm^{-1}) 1352.9(S=O asymmetric), 1158.8(S=O symmetric).**^{1}HNMR** (400 MHz, $CDCl_3$, TMS)δ ppm: 2.49-2.50(t, 4H, -CH_2), 3.00(s, 4H, CH_2), 3.4(s, 2H, -CH_2), 7.09-7.11 (d, *J* = 6.4 Hz, 2H, Ar-H), 7.37-7.39 (d, *J* = 8 Hz, 2H, Ar-H), 7.48-7.50 (d, *J* = 8 Hz, 2H, Ar-H), 7.65-7.67 (d, *J* = 8 Hz, 2H, Ar-H), **^{13}C NMR** (100 MHz, $CDCl_3$, TMS)δ ppm**:** 45.9, 51.9, 61.34, 77.02, 121.17, 129.17, 130.63, 131.45, 133.9, 36.45,139.4. **Mass:** m/z 430 [M + 1].

Spectral data of 1-((4-chlorophenyl)sulfonyl)-4-(4-fluorobenzyl)piperazine(5f): IR (KBr, cm^{-1}) 1301.5(S=O asymmetric), 1149.5(S=O symmetric).**^{1}HNMR** (400 MHz, $CDCl_3$, TMS)δ ppm: 2.54(s, 4H, -CH_2), 3.01(s, 4H, CH_2), 3.54(s, 2H, -CH_2), 6.97-7.06 (m, 2H, Ar-H), 7.2-7.24 (m, 2H, Ar-H). 7.46-7.48 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.64-7.66 (d, *J* = 8.4 Hz, 2H, Ar-H). **^{13}CNMR** (100 MHz, $CDCl_3$, TMS) δ ppm**:** 45.19, 51.7, 54.46, 77.24, 115.29, 121.92, 125.1, 125.4, 132.1, 134.49, 159.04, 161.1. **Mass:** m/z 369 [M + 1].

Spectral data of 1-((4-bromophenyl)sulfonyl)-4-(2-chloro-6-fluorobenzyl) piperazine (5g): IR (KBr, cm^{-1}) :1322.2(S=O asymmetric), 1170.3(S=O symmetric). **^{1}HNMR** (400 MHz, $CDCl_3$, TMS)δ ppm: 2.61 (t, 4H, CH_2), 2.98(s, 4H, -CH_2), 3.66(s, 2H, -CH_2), 6.92-6.96 (m, 3H, ArH), 7.15 (t,2H, Ar-H), 7.55-7.57 (d, *J* = 8 Hz, 2H, Ar-H), 7.61-7.63 (d, *J* = 8 Hz, 2H, Ar-H), **^{13}CNMR** (100 MHz, $CDCl_3$, TMS) δ ppm**:** 46.03,51.75, 77.03, 113.9,123.08,125.45, 127.8, 129.25,134.5,136.4, 160.67, 163.1. **Mass**: m/z 448 [M + 1].

Spectral data of 1-((4-bromophenyl)sulfonyl)-4-(2-fluorobenzyl)piperazine(5h): IR (KBrcm^{-1}): 1340.0(S=O asymmetric), 1178.5(S=O symmetric).**^{1}H-NMR** (400 MHz, $CDCl_3$, TMS)δ ppm: 2.54 (t, 4H, -CH_2), 3.00(s, 4H, CH_2), 3.54(s, 2H, -CH_2), 6.96.-7.06(m, 4H, Ar-H), 7.1 (t, 2H, Ar-H), 7.56-7.58 (d, *J* = 8 Hz, 2H, Ar-H). 7.62-7.64 (d, *J* = 8 Hz, 2H, Ar-H). **^{13}C NMR** (100 MHz, $CDCl_3$, TMS) δ ppm**:** 45.99, 51.74, 54.93, 77.05, 115.26, 123.77, 127.88, 129.11, 131.45, 132.3, 134.69, 160.09, 162.54. **Mass:** m/z 414 [M + 1].

Spectral data of 1-((4-bromophenyl)sulfonyl)-4-(2-methylbenzyl)piperazine(5i): IR(KBrcm^{-1}): 1335.4(S=O asymmetric), 1155.8(S=O symmetric). **^{1}HNMR** (400 MHz, $CDCl_3$, TMS)δ ppm: 2.27 (s, 3H, -CH_3), 2.51(t, 4H, -CH_2), 2.99(s, 4H, CH_2), 3.43(s, 2H, -CH_2), 7.09-7.24 (m, 4H, Ar-H), 7.57-7.59 (d, *J* = 8 Hz, 2H, Ar-H), 7.64-7.67 (d, *J* = 8.8 Hz, 2H, Ar-H). **^{13}CNMR** (100 MHz, $CDCl_3$, TMS) δ ppm**:** 19.18, 46.15, 52.05, 60.30, 77.03, 125.5, 127.3, 129.25, 130.3, 132.34, 134.6, 135.5, 137.4. **Mass:** m/z 411 [M + 1].

Spectral data of1-((4-bromophenyl)sulfonyl)-4-(3,4-dichlorobenzyl)piperazine(5j): IR (KBrcm^{-1}) 1329.3(S=O asymmetric), 1164.5(S=O symmetric). **^{1}HNMR** (400 MHz, $CDCl_3$, TMS)δ ppm:2.48 (t, 4H, -CH_2), 3.00(s, 4H, CH_2), 3.41(s, 2H, -CH_2), 7.05 (t, 1H, Ar-H), 7.31-7.33 (d, 2H, *J* = 8 Hz, Ar-H), 7.66-7.68 (d, *J* = 8 Hz, 2H, Ar-H). **^{13}CNMR** (100 MHz, $CDCl_3$, TMS) δ ppm**:** 45.9, 51.9, 61.19, 77.03, 128.02, 129.25, 130.25, 131.2, 132.4, 137.96. **Mass:** m/z 404 [M + 1].

Spectral data of 1-(4-bromobenzyl)-4-((4-bromophenyl)sulphonyl)piperazine(5k): IR (KBrcm^{-1}) 1337.5(S=O asymmetric), 1149.1(S=O symmetric).^{1}**HNMR (CDCl$_3$) δ ppm:** 2.48(t, 4H, -CH_2), 3.00(s, 4H, CH_2), 3.4(s, 2H, -CH_2), 7.09-7.11 (d, *J* = 8 Hz, 2H, Ar-H), 7.37-7.40 (d, *J* = 12Hz, 2H, Ar-H) 7.57-7.59 (d, *J* = 8 Hz, 2H, 2H, Ar-H), 7.65-7.67 (d, *J* = 8 Hz, 2H 2H, Ar-H) ^{13}**CNMR(CDCl$_3$) δ ppm:** 46.01**,** 51.9, 61.7, 77.0, 121.14, 127.9, 129.2,130.6, 131.4, 132.3,134.4, 136.6. **Mass:** m/z 402 [M + 1].

Spectral data of 1-((4-bromophenyl)sulfonyl)-4-(4-fluorobenzyl)piperazine(5l): IR (KBr, cm^{-1}): 1341.0(S=O asymmetric), 1162.5(S=O symmetric).^{1}**HNMR** (400 MHz, $CDCl_3$, TMS)δ ppm: 2.54 (t, 4H, -CH_2), 3.00(s, 4H, CH_2), 3.54(s, 2H, -CH_2), 6.96.-7.06(m, 4H, Ar-H), 7.1 (t, 2H, Ar-H), 7.56-7.58 (d, *J* = 8 Hz, 2H, Ar-H). 7.62-7.64 (d, *J* = 8 Hz, 2H, Ar-H). ^{13}**CNMR** (100 MHz, $CDCl_3$, TMS) δ ppm**:** 45.92, 51.61, 54.13, 77.15, 115.16, 123.00, 127.18, 129.31, 131.15, 132.3, 1438.69, 159.09, 160.54. **Mass:** m/z 414 [M + 1].

Table 1. Physical data of compounds (5a-l)

Comp. Code	Molecular Formula	R	Ph	Mol. Wt.	Calculated (Found) %			Melting Point (^{o}C)	Yield (%)
					C	H	N		
5a	$C_{17}H_{17}Cl_2FN_2O_2S$	Cl	2-Cl,6-F	403.30	50.63 (50.22)	4.25 (4.03)	6.95 (7.00)	138	82
5b	$C_{17}H_{18}ClFN_2O_2S$	Cl	2-F	368.85	55.36 (65.23)	4.92 (4.99)	7.59 (7.31)	156	81
5c	$C_{18}H_{21}ClN_2O_2S$	Cl	2-CH_3	364.89	59.25 (59.24)	5.80 (5.31)	7.68 (6.63)	168	82
5d	$C_{17}H_{17}Cl_3N_2O_2S$	Cl	2,4-Cl_2	419.75	48.64 (48.21)	4.08 (4.77)	6.67 (6.66)	147	86
5e	$C_{17}H_{18}BrClN_2O_2S$	Cl	4-Br	429.76	47.51 (47.22)	4.22 (4.74)	6.52 (6.65)	125	75
5f	$C_{17}H_{18}ClFN_2O_2S$	Cl	4-F	368.85	55.36 (55.23)	4.92 (4.48)	7.59 (7.21)	155	79
5g	$C_{17}H_{17}BrClFN_2O_2S$	Br	2-Cl,6-F	447.75	45.60 (45.20)	3.83 (3.46)	6.26 (6.62)	156	82
5h	$C_{17}H_{18}BrFN_2O_2S$	Br	2-F	413.30	49.40 (49.22)	4.39 (4.88)	7.68 (7.66)	143	87
5i	$C_{18}H_{21}BrN_2O_2S$	Br	2-CH_3	409.34	52.81 (52.67)	5.17 (5.26)	6.84 (6.62)	148	85
5j	$C_{17}H_{17}BrCl_2N_2O_2S$	Br	2,4-Cl_2	464.20	58.99 (58.67)	3.69 (3.26)	6.03 (6.62)	167	85
5k	$C_{17}H_{18}Br_2N_2O_2S$	Br	4-Br	474.21	43.06 (43.28)	3.83 (3.69)	5.91 (5.75)	132	88
5l	$C_{17}H_{18}BrFN_2O_2S$	Br	4-F	413.30	49.40 (49.22)	4.39 (4.88)	7.68 (7.66)	167	90

Antibacterial activity: Compounds (**5a-l)** were evaluated for antibacterial activity. Agar well diffusion method was adopted for antibacterial activity using known literature procedure [33]. Experiment was performed in triplicates; the mean values are given in table 2.

Antifungal Activity: Antifungal activities of all piperazine derivatives towards two mold fungi were studied, viz. *Candida albicans* (human pathogen) *Aspergillus flavus* (mold). Poisoned food technique method [34] was used to assess the antifungal activity of the synthesized compounds, Nystatin (10 µg disc^{-1}) was used as standard fungicide. The experiment was performed in triplicate the average values were reported in table 3.

Anthelmintic activity: Anthelmintic activity of compounds **5a-l** was evaluated using *Pheretima posthuma* (Indian Earthworm), anthelmintic activity of the compounds were evaluated as per the standard method reported [35]. Time taken for paralysis and time taken for death as objective and was documented in minutes. Total loss of motility with faded body colour was concluded as death based on observation and the results are shown in table 4.

Molecular docking: Four target proteins namely Antibacterial dehydrosqualene synthase (3ACX) [**36, 37**], N-myristoyltranferase (PDB ID:1IYK) [**38, 39**] and Anthelmintic Tubulin (1SAO) [**40, 41**] were selected to assess the binding efficacy and inhibitory effects of compounds (5a-l) against them in in-silico method. The protein structures were downloaded from protein data bank [**42**] and cleaned by deleting water molecules and any other heterogeneous molecules that came from crystallographic preparations. Energy minimization of the targets was performed with the help of standard dynamics cascade protocol of Discovery Studio 3.5 (DS 3.5) by 20,000 steps of steepest descent minimization protocol and a standard relaxation procedure using restrained molecular dynamics [**43**]. The active sites were identified by receptor cavity method using DS 3.5[**44, 45**]. 2D structure of ligands was drawn in Chemdraw ultra 11.0 and exported as mol file for further processing in DS 3.5. Using Prepare Ligand tool in the DS, all possible conformers were generated based on the flexibility of bonds in the molecule. The generated conformers were optimized using CHARMm force field and then minimized through 500 steps of steepest descent minimization algorithm with RMS gradient kept at 0.1. The lowest energy ligand conformation was selected among the generated conformations and subjected to docking. Molecular docking provides preliminary information about the binding modes of ligand molecule in and around receptor cavities as well as predicts possible intermolecular non-covalent bonding. Binding modes may then be used to predict the strength of association between the ligand and receptors using certain scoring functions that include number and types of bonds, hysteric clashes, and electronic repulsions as parameters. The flex docking score correlates with the binding affinity of the molecule with the targets [**46-49**] and are given in table 5.

RESULTS AND DISCUSSION

Compounds were synthesized as per Scheme 1, Initially N-Boc piperazine was made to react with 4–substituted–benzene sulfonyl chloride **(1)** to give 1-(tert-butoxy)-4-((4-substituted phenyl) sulfonyl) piperazine **(2)**. On deprotection of Boc yielded 1-((4- substituted phenyl)sulfonyl)piperazine **(3)**. Then it was treated with different substituted benzyl chlorides to get alkylated piperazine sulfonyl derivatives **(5a-l)** in good yield. In the ^{1}HNMR spectrum of compound **5a** a singlet at δ 3.6 ppm was observed due to the presence of methylene protons. The protons of p-chloro benzene sulfonyl moiety resonate as two doublets at δ7.44-7.46 ppm and 7.63-7.65 ppm. Similar type of two triplets at δ 2.60-2.62 ppm and δ 2.9 ppm were noticeable due to the CH_2 protons of piperazine moiety (Figure 1). Further evidence for the formation of compound **5a** was confirmed by recording its LCMS spectrum shows m/z 402 [M+1]. Thus the structures of compounds were successfully confirmed without any ambiguity.

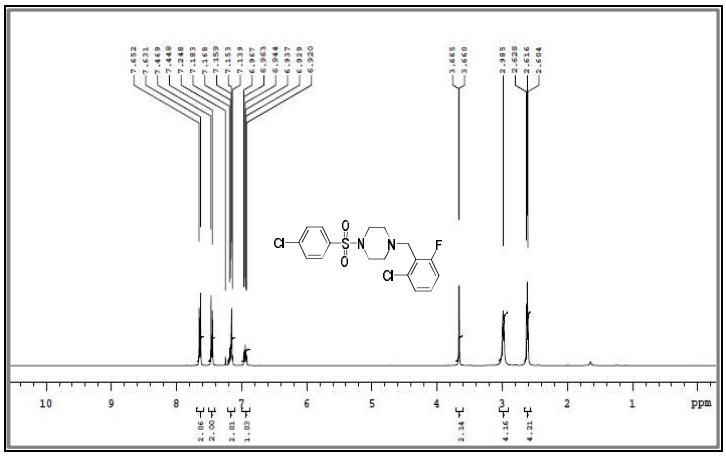


Figure 1. ^{1}HNMR spectrum of compound 5a.

Compounds **5a-l** was screened for their antibacterial activity. All the derivatives showed inhibition against *Escherichia coli* and *Staphylococcus aureus* and none of the derivative showed inhibition against *P. aerogenosa*. Initially different concentrations of 2.5µg, 5 µg, 10 µg, 15 µg, 20 µg 20 μL^{-1}

have been tried. At 10µg 10µL^{-1} minimum inhibitory concentration (MIC) was observed. Data present in table showed that the derivatives are active at and above 10µg 10µL^{-1}. Among all derivatives, **5f**, **5g** and **5j** were substantially active against *E. coli* and *S. aureus* and all others were moderately active against tested strains compared to ciprofloxacin (Table 2).

Table 2. Antifungal activity Zone of Inhibition of compounds (5a-l)

Samples	Treatment (µg µL^{-1})	*Escherichia coli* (Mean±SE)	*Staphylococcus aureus* (Mean±SE)
5a	10	16.09±0.55	11.73±0.30
5b	10	08.42±0.22	5.04±0.43
5c	10	15.62±0.62	6.41±0.15
5d	10	16.42±0.23	11.13±0.41
5e	10	09.01±0.31	5.90±0.32
5f	10	17.02±0.10*	12.98±0.16*
5g	10	17.43±0.41*	13.47±0.10*
5h	10	08.16±0.37	5.61±0.78
5i	10	15.56±0.76	6.61±0.25
5j	10	17.29±0.41*	13.23±0.16*
5k	10	15.00±0.73	6.11±0.17
5l	10	16.89±0.17	11.91±0.12
CIPRO	10	20.0±0.5	25.0±0.5

Compounds **5a-l** showed less inhibitory action against *C. albicans*and *A. flavus* as compared to standard Nystatin**.** Initially different concentrations of 2.5µg, 5 µg, 10 µg, 15 µg and 20 µg 20 µL^{-1} have been tried. At 10µg 10µL^{-1} minimum inhibitory concentration (MIC) was observed for both *C. albicans* and *A. flavus*. Data present in table showed that the derivatives are active at and above 10µg 10 µL^{-1}. Among all derivatives **5c** and **5i** showed significant activity against *C. albicans and A. flavus.* Whereas all other showed moderate activity against organisms compared to standard Nystatin (Table 3).

Table 3. Antifungal activities Zone of Inhibition of compounds (5a-l)

Samples	Treatment (µg µL^{-1})	*Candida albicans* (Mean±SE)	*Aspergillus flavus* (Mean±SE)
5a	10	10.85±0.19	08.94±0.46
5b	10	10.12±0.42	09.56±0.52
5c	10	17.01±0.22*	12.97±0.42*
5d	10	10.24±0.22	08.91±0.13
5e	10	10.09±0.89	08.17±0.18
5f	10	14.21±0.27	12.86±0.12
5g	10	10.91±0.25	09.84±0.42
5h	10	10.07±0.36	09.13±0.26
5i	10	17.47±0.32*	13.42±0.41*
5j	10	10.06±0.29	08.01±0.21
5k	10	16.82±0.31	11.76±0.80
5l	10	14.01±0.37	08.86±0.23
NYS	10	29.33±0.13	21.33±0.23

Compounds **5a-l** showed less results against *Pheretima posthuma* (Indian earthworm) with that of standard Piperazine citrate. Earthworms belonging to control group showed paralysis time at 142.33±0.49 min and death time at 167.17±0.87 min. The test samples **5a-l** at the concentrations 10 mg/mL showed the time in min of paralysis and death is reported in table 4. Standard drug piperazine citrate 10 mg mL^{-1} exhibited 47.00±0.58 and 49.17±0.48 min for paralysis and death. Examination of

anthelmintic activity revealed that alkylated piperazine sulfonamide derivative **5a** and **5d** showed significant (**p<0.01) activity against *P. posthuma*.

Table 4. Anthelmintic activities of compounds (5a-l)

Test Samples	Concentration (mg mL^{-1})	Time taken for paralysis (min)	Time taken for death(min)
Control	-	142.33±0.49	167.17±0.87
5a	10	42.06±1.23	46.07±1.16
5b	10	57.34±0.61	59.91±1.26
5c	10	67.72±2.63	69.23±0.60
5d	10	43.01±0.86	47.76±1.16
5e	10	67.72±0.47	69.91±1.02
5f	10	58.41±2.02*	62.95±1.22*
5g	10	63.12±0.27	65.03±1.23
5h	10	54.21±0.44	58.21±0.76
5i	10	67.03±0.27	68.76±0.28
5j	10	54.73±0.24	56.98±0.73
5k	10	57.79±0.29	59.83±0.41
5l	10	58.88±1.46*	62.27±0.60*
PC	10	47.00±0.58	49.17±0.48

The molecular docking studies were carried out using Lead IT for antibacterial, antifungal and anthelmintic activity. Docking energy of the synthesized molecules along with standards was tabulated in table 5. The Molecular Docking result shows that all the standards have higher docking energy compared with the newly synthesized compounds. All the molecules were docked in the active sites of respective proteins with effectively. The fitting of the most potent compounds **5f**, **5g** and **5j** against bacteria, **5c** and **5i** against fungi, and the anthelmintic activity of **5a** and **5d** are shown in figures 2 to 4. Interactions shows hydrogen bonding, Hydrophobic, Pi-Pi stacked and Pi-alkyl interactions.

Table 5. Docking energy in Kcal $mole^{-1}$ of compounds (5a-l)

Compound Code	Antibacterial	Antifungal	Anthelmintic
	PDB ID:3ACX	PDB ID:1IYK	PDB ID:1SAO
5a	-3.148	-2.672	-6.619
5b	-1.308	-2.083	-6.136
5c	-2.408	-4.469	-6.251
5d	-3.098	-2.641	-6.519
5e	-1.913	-2.576	-6.280
5f	-3.230	-3.03	-6.322
5g	-3.509	-3.03	-6.423
5h	-1.286	-2.059	-5.592
5i	-2.238	-4.532	-6.155
5j	-3.568	-2.641	-5.774
5k	-2.014	-2.03	-5.230
5l	-2.313	-2.13	-5.012
Ciprofloxacin	-9.185	--	--
Nystatin	--	-23.499	--
Piperizine citrate	--	--	-8.254

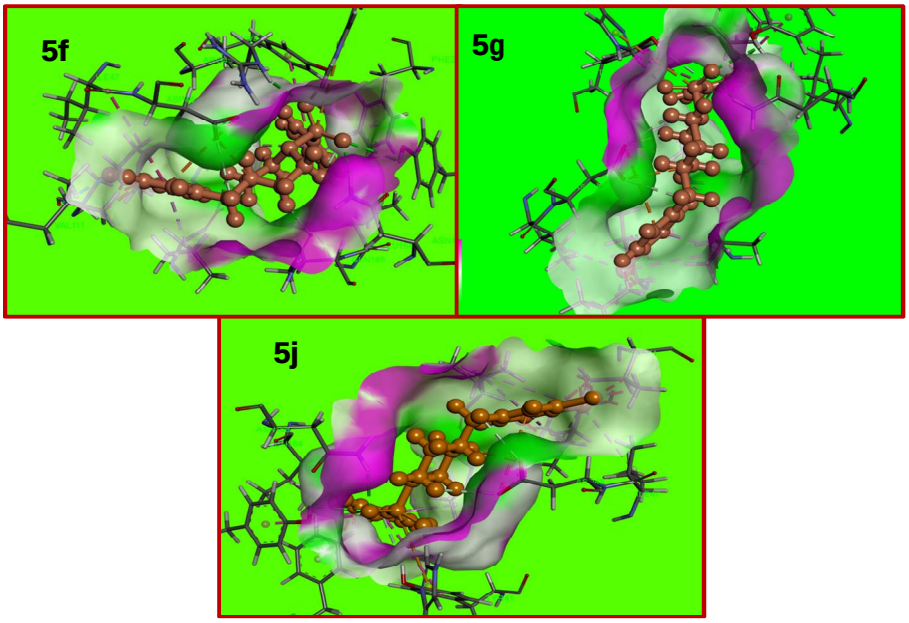
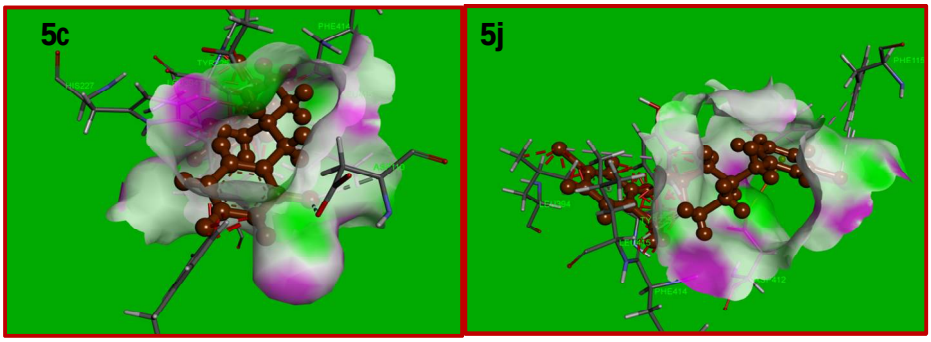


Figure 2. Docking poses of 5f, 5g and 5j with protein 3ACX



Fiure 3. Docking poses of 5c and 5j with protein 1IYK

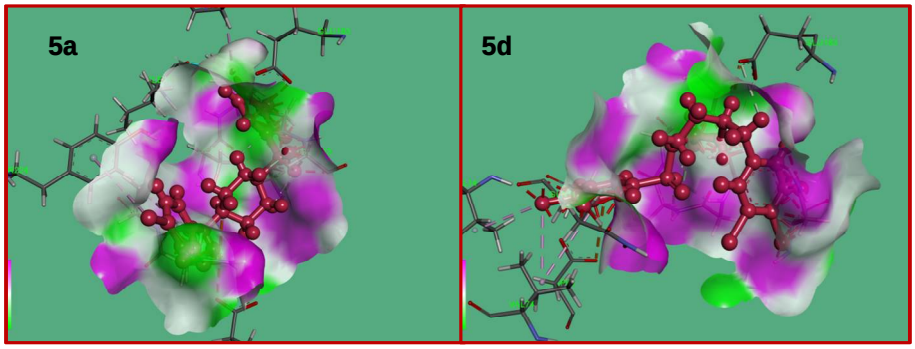


Figure 4. Docking poses of 5a and 5d with protein 1SAO

APPLICATION

The investigation of antibacterial, antifungal and anthelmintic screening studies revealed that all the tested compounds **(5a-l)** showed moderate to good inhibition in DMSO. The compounds **5f**, **5g** and **5j** showed comparatively good activity against all the bacterial strains. The compounds **5c** and **5i** showed significant activity against all the tested fungal strains. Further compounds **5a** and **5d** showed significant anthelmintic activity against *P. posthuma*.

The Molecular Docking result shows that compounds **5f**, **5g** and **5j** have high docking energy compared with antibacterial standard ciprofloxacin. Compounds **5c** and **5i** have moderate docking score compared to antifungal standard Nystatin, but in case of anthelmintic activity most of the compounds have docking score which are more comparable to piperazine citrate. Studies like SAR may be studied on these molecules in future.

CONCLUSION

In this study new series of compounds were synthesized and screened fortheir antibacterial, antifungal, anthelmintic. Out of all tested compounds **5f, 5g** and **5j** shows noteworthy antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* and **5c** and **5i** showed antifungal and other piperazine derivatives **5a** and **5d** showed comparable anthelmintic activity compared to standard. The antimicrobial activity results indicated that some of the tested compounds showed the most promising antibacterial and antifungal activities.

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