Design and synthesis of new 4-(tert-butyl)-N-(3-(4-((4-phenyl) sulfonyl) piperazine-1-carbonyl)-2-methylphenyl) benzamide as antimicrobial agents.

Sadhana Mahajan[a], Dr. Ankit Singh[a], Dr. Vinod Bairagi[b]

[a] Department of Pharmacy, Shri Jagadishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan, India

[b] Department of Pharmacy, K.B.H.S.S. Trust's Institute of Pharmacy, Malegaon, Nashik, Maharashtra, India.

Corresponding Author: Dr. Ankit Singh

Abstract:

A series of new 4-(tert-butyl)-N-(3-(4-((4-phenyl) sulfonyl) piperazine-1-carbonyl)-2-methylphenyl) benzamide was synthesized. The catalytic reactions were employed in a sequence of processes to create the targets. The synthesized derivatives were evaluated using 1H NMR, 13C NMR, and mass spectral analytical methods. The anti-microbial activities of the new compounds were assessed in vitro. Many compounds demonstrated moderate to good activity. Four bacterial strains and two fungi were used to test the in vitro antimicrobial activity of the synthesized compounds. Among these, SM14 and SM16 derivatives of sulfonyl-piperazine exhibited relatively good antimicrobial and antifungal activities when measured against standard drugs. Other derivatives showed moderate to lower inhibition for all the strains.

Keywords: Phenyl, sulfonyl, Piperazine, antimicrobial activity.

Introduction

Heterocycles are of immense importance biologically and industrially for functioning any compounds with pharmacological activities. Researchers have been engaged in extensive efforts to produce novel heterocyclic compounds with broad spectrum of pharmacological activities along with minimal toxicity. This fact can be utilized for synthesis of novel heterocyclic compounds for desired and potent bioactivity. The cyclic organic compounds that contain at least one heteroatom are termed for heterocyclic compounds. The atoms like nitrogen, oxygen, and sulfur incorporated in the ring make the heterocyclic compounds which varied medicinal properties depending on the heteroatom. Heterocyclic motifs play an important part in medicinal chemistry research due to their wide spectrum of biological activities and have always emerged with higher therapeutic efficiency. Nitrogen-bearing heterocyclic play importance with a good track record of therapeutic advances in the recent drug discovery as lead molecules. Antimicrobial resistance is an alarming concern for

humankind as it emerges due to excess use of medicines. WHO had taken new initiatives towards control of antimicrobial resistance by celebrating Antimicrobial Awareness Week, prevention of antimicrobial resistance, spreading awareness globally, and avoiding excess use of medicine to avoid drug resistance etc. since 2015 in worldwide [1-2]. WHO alerted about deaths of 350 million humans by 2050 by antimicrobial drug resistance occurred from infections like methicillin-resistant staphylococcus aureus (MRSA), vancomycin-resistant enterococcus (VRE), multi-drug-resistant mycobacterium tuberculosis (MDR-TB), carbapenem-resistant Enterobacteriaceae (CRE), multidrug-resistant Neisseria gonorrhoeae and Escherichia coli etc. The diseases like cancer, HIV, parkinsons, Alzheimer, chronic diseases, diabetic, and central nervous system, etc required longer duration for treatments and there emerge the high possibility of drug resistance. These drug-resistance occurs mainly due to physical health of human, weaker immunity, etc which develops infections and later resulted in weaker immunity [3-4]. There is always a competition between bacterial developments towards the drugs, as it is never ending. Recently we have witnessed severe infections like COVID-19 pandemic and many humans lost their lives globally. Along with that in last 10 years we have witnessed Zika virus, Ebola virus, Swine flu, etc causes many deaths to specific parts of world which affected morbidity and mortality of human kind worldwide. Research needed to align with global threat of infectious diseases and to tacking the rapidly increasing newer strains of viruses [5]. Nitrogen heterocyclic compounds containing piperazine sulfonamide have been of significant interest in medicinal chemistry research for scientists due to their varied pharmacological activities [6].

The molecules compromising piperazine and sulfonamide are well known in drug discovery for their medicinal activities. The piperazine and sulfonamide act as antimicrobial, anticancer, anti-diabetic, sigma receptor ligands, and antibacterial [7-10]. The piperazine nuclei form a key component of many biologically active molecules and drugs, as the basic nature of nitrogen involved in the piperazine ring plays an important role in biological activity. The drugs containing Oxatomide used for the treatment of muscular dystrophy; Almitrine used for the treatment of respiratory disorders; Hydroxyzine, Buclizine, and Meclizine used as antihistamine drugs; Lomerizine used for the treatment of migraines all piperazine core [11]
The derivatization of cinnamic acids like their esters, amides, alcohols, aldehydes, hydroxyl amines etc reported for their varied biological activities like anticancer, antimicrobial, antifungal, antioxidant, anti-tubercular, ant diabetic, antithrombotic, anti-inflammatory, etc. [12-17]. The cinnamic acid derivatives are mostly used in the cosmetics industry, polymer and

food as its structure contains acrylic acid, which is present in cis or trans configurations and its natural availability [18-20]. The presence of terminal acid functional groups makes it a fascinating fragment to link with other bioactive fragments. Some heterocyclic acrylic acids showed lipoxygenase and cyclooxygenase-1 inhibitors with potent antioxidant and anti-inflammatory activity [21-29].

Material and Methods:

Chemistry

The produced compounds were characterized using analytical methods such as 1H NMR, 13C NMR, and HRMS. The agar diffusion assay and the broth microdilution assay were employed to evaluate the in vitro antibacterial properties of the produced compounds. When tested against the identified bacteria, all of the compounds exhibited moderate to good antimicrobial properties. Unless otherwise noted, all chemicals were bought from commercial suppliers and utilized without additional purification. The primary compounds were acquired from Avra Labs and Sigma Aldrich. Thin layer chromatography (TLC) analysis using Merck pre-coated silica gel 60 F254 aluminum sheets, visible by UV light, was used to track the evolution of the reactions. Every reaction was conducted in an inert atmosphere. Melting points were uncorrected and recorded using the Casia-Siamia (VMP-AM) melting point equipment. TLC, NMR, and HRMS were used to evaluate the intermediates' purity. NMR, HRMS, and HPLC were used to verify the resulting compounds' purity, and all structures matched the suggested characterizations. A Varian NMR spectrometer operating at 400 MHz was used to record the 1H NMR spectra. The 13C was measured using a 100 MHz Varian NMR spectrometer [1-11]. The chemical shifts are provided as NMR spectra in δppm units. The abbreviations used include singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). WATERS Corporation manufactured the Micromass-QUATTRO-II, which was used to collect mass spectra.

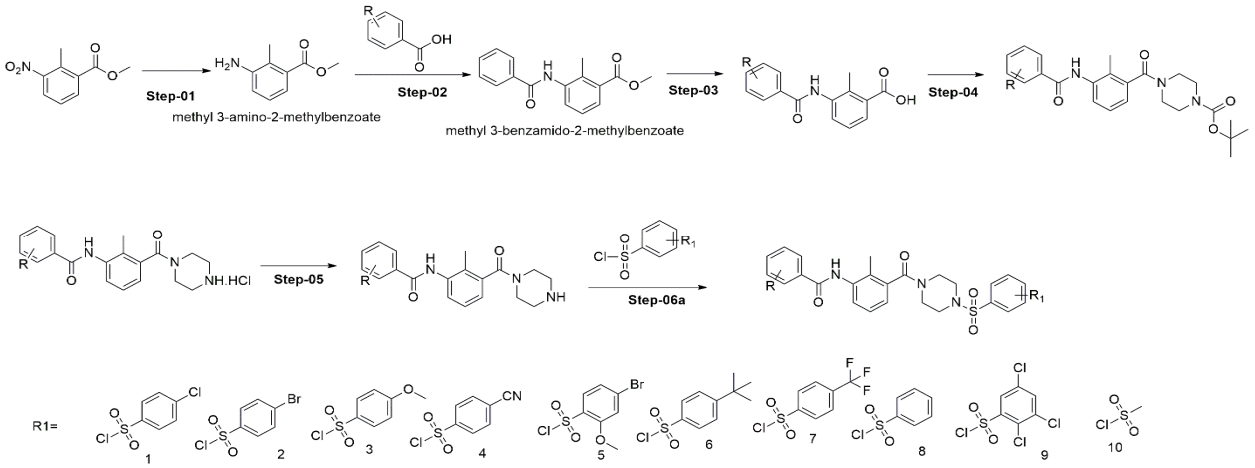
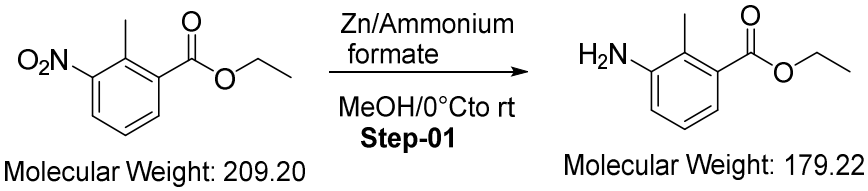


Figure 01: Synthetic scheme for 4-(tert-butyl)-N-(3-(4-((4-phenyl)sulfonyl)piperazine-1-carbonyl)-2-methylphenyl)benzamide

The synthesis of 4-(tert-butyl)-N-(3-(4-((4-chlorophenyl) sulfonyl) piperazine-1-carbonyl)-2-methylphenyl) benzamide

Step-01: Synthesis of ethyl 3-amino-2-methylbenzoate



Calculations:

Sr.No	Chemical Name	Mole.wt	Wt	Mole Eq.	Moles
1	Step-01 Product	209.20	1	1	0.00298
2	Reagent-1 Zinc	65.38	0.585	3	0.00896
3	Reagent-2 Ammonium formate	63.04	0.564	3	0.00896
4	Solvent (MeOH)		20.00 mL		

Procedure:

1] Reagent 1 (0.585 g, 3 eq) was added to a stirred solution of Step-01 product (1.00 g, 1 eq) in solvent (20.00 mL) at 0°C.

2] Reagent 2 (0.564 g, 3 eq) is then gently added pinch-wise at 0°C. (The creation of exotherms)

3] For two hours, the reaction mixture was agitated at room temperature. TLC tracked the reaction's development. In the solvent, 50% solvent. Iodine, UV, and ninhydrine.

Work-up:

4] To get crude material, the reaction mixture was filtered through a celite bed and vacuum-concentrated once the starting material reaction was finished.

5] The resulting crude material was chilled for 30 minutes at 0°C after being dissolved in 100.00 mL of DCM.

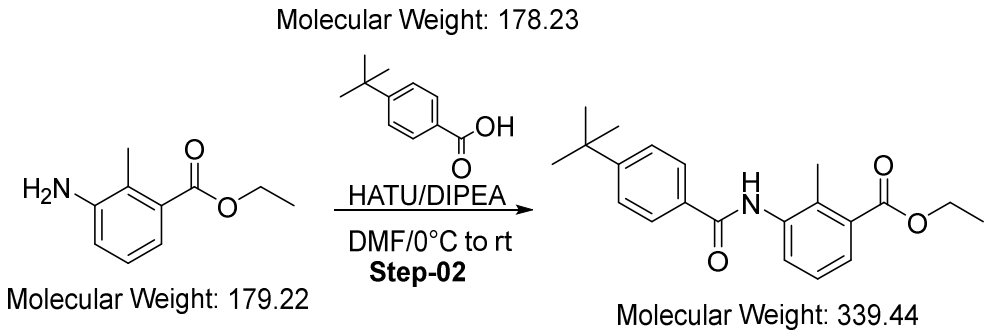
6] There is precipitation of ammonium formate, then decan DCM, dried over sodium sulphate, concentrate under vacuum to get 1.20 g crude material.

Purification:

7] By employing 40% silica 100-200 mesh column chromatography, the obtained crude substance was refined. To obtain 0.8 g of the desired result, ethyl acetate is used as an eluent in hexane.

8] Nature of product: Brown solid.

Step-02: Synthesis of ethyl 3-(4-(tert-butyl) benzamido)-2-methylbenzoate



Calculations:

Sr.No	Chemical Name	Mole.wt	Wt	Mole Eq.	Moles
1	Step-04 Product Amine	179.22	0.5	1	0.00166
2	Acid	178.23	0.340	1.1	0.00182
3	Reagent 1 HATU	379.25	0.944	1.5	0.00249
3	Reagent 2 DIPEA d-0.742	129.25	0.643g	3	0.00498
4	Solvent DMF		10.00 mL		

Procedure:

1] Reagents 1 (0.944 g, 1.5 eq) and 2 (0.866 mL, 2 eq) were added to a stirred solution of step-04 product (0.5 g, 1 eq) in solvent (5.00 mL) at 0°C.

2] For five minutes, the reaction mixture was agitated at RT. The addition of amine (0.34 g, 1.1 eq) came next.

3] For 12 hours, the reaction mixture was agitated at room temperature.

4] TLC tracked the reaction's development. [70 percent solvent in solvent, iodine, and UV]

Work-up:

5] Precipitation occurred when the starting material reaction mixture was finished and put onto 50 milliliters of ice-cold water.

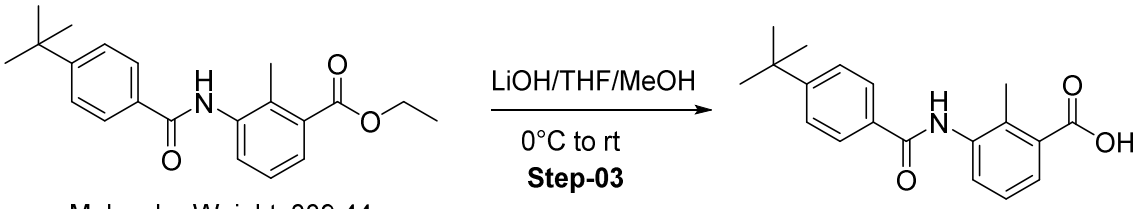
6] To achieve 0.8 g of crude material, the resulting ppt was filtered and cleaned with cold water.

Purification:

7] To obtain a final product of 0.7g, the resulting crude material was triturated using hexane.

8] Product nature: solid off-white.

Step-03: Synthesis of 3-(4-(tert-butyl)benzamido)-2-methylbenzoic acid



Molecular Weight: 339.44

Molecular Weight: 311.38

Calculations:

Sr.No	Chemical Name	Mole.wt	Wt	Mole Eq.	Moles
1	Step-02 Product	339.44	0.7	1	0.0021
2	LiOH	23.95	0.102	2	0.0042
3	THF		7.00		
4	MeOH		2.00		
5	H2O		5.00		

Procedure:

1] LiOH (0.102 g, 2 eq) and H2O (5.00 mL) were added to a stirred solution of Step-03 product (0.7 g, 1 eq) in THF (7.00 mL) and MeOH (2.00 mL). After that, the reaction mixture was agitated for 12 hours at room temperature.

2] TLC tracked the reaction's development. [50 percent solvent in solvent, iodine, and UV].

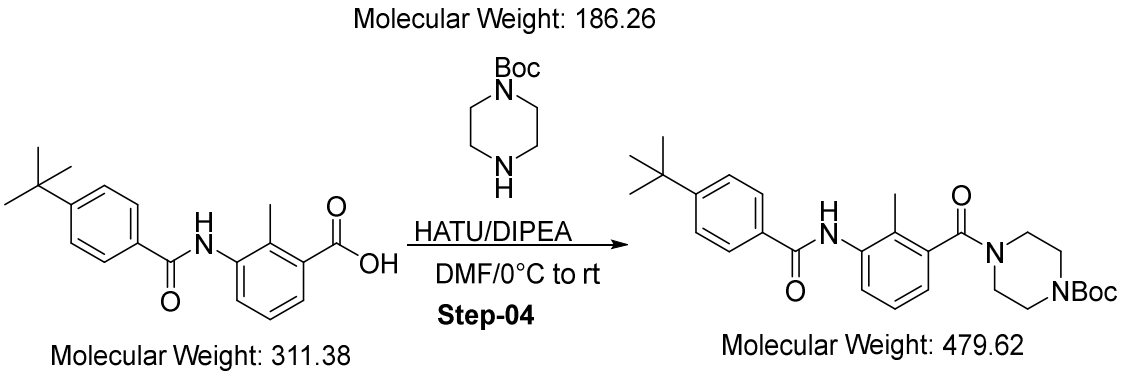
Work-up:3] Following the end of the initial material reaction, the mixture was vacuum-concentrated to obtain Crude material which was dissolved in H2O (25.00 mL) and then acidify to PH 2 to 3 using 2 N. HCl (5.00 mL) to get precipitation, which was filtered and dried under vacuum to afford 0.6 g off white solid

Purification:

4] To obtain 0.5 g of the final product, the obtained crude material was triturated using 30.00 mL of hexane.

5] The product is a solid off-white color.

Step-04: Synthesis of tert-butyl 4-(3-(4-(tert-butyl)benzamido)-2-methylbenzoyl)piperazine-1-carboxylate



Calculations:

Sr.No	Chemical Name	Mole.wt	Wt	Mole Eq.	Moles
1	Step-03 Product Acid	311.38	0.5	1	0.00166
2	Amine	186.25	0.340	1.1	0.00182
3	Reagent 1 HATU	379.25	0.944	1.5	0.00249
3	Reagent 2 DIPEA d-0.742	129.25	0.643g	3	0.00498
4	Solvent DMF		10.00 mL		

Procedure: 1]Reagents 1 (0.944 g, 1.5 eq) and 2 (0.866 mL, 2 eq) were added to a stirred solution of step-04 product (0.5 g, 1 eq) in solvent (5.00 mL) at 0°C.

2] For five minutes, the reaction mixture was agitated at RT. The addition of amine (0.34 g, 1.1 eq) came next.

3] For 12 hours, the reaction mixture was agitated at room temperature.

4] TLC tracked the reaction's development. [70 percent solvent in solvent, iodine, and UV]

Work-up:5] Precipitation occurred when the starting material reaction mixture was finished and put onto 50 milliliters of ice-cold water.

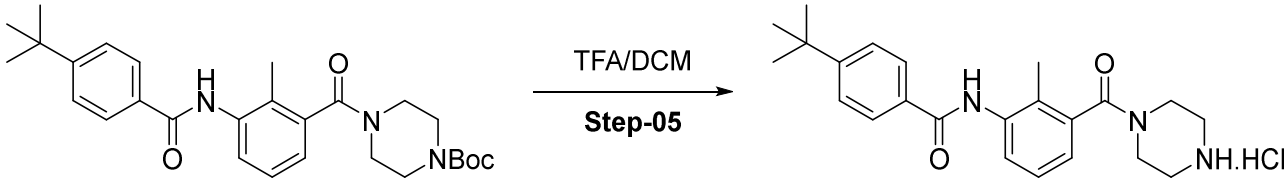
6] To achieve 0.8 g of crude material, the resulting ppt was filtered and cleaned with cold water.

Purification:

7] To obtain a final product of 0.7g, the resulting crude material was triturated using hexane.

8] Product nature: solid off-white.

Step-05: Synthesis of 4-(tert-butyl)-N-(2-methyl-3-(piperazine-1-carbonyl) phenyl) benzamide hydrochloride



Molecular Weight: 479.62 Molecular Weight: 415.96

Calculations:

Sr.No	Chemical Name	Mole.wt	Wt (g)	Mole Eq.	Moles
1	Step-05 Product	468.98	0.7	1	0.0015
2	4 M HCl in 1,4-Dioxane:		10.00 mL		

Procedure:

1]1 M 1,4-Dioxane: HCl (10.00 mL) was used to dissolve the Step-05 product (0.7 g, 1.1eq).

2] For 12 hours, the reaction mixture was agitated at room temperature.

3] TLC tracked the reaction's development. [60 percent solvent in solvent, iodine, and UV]

Work-up:

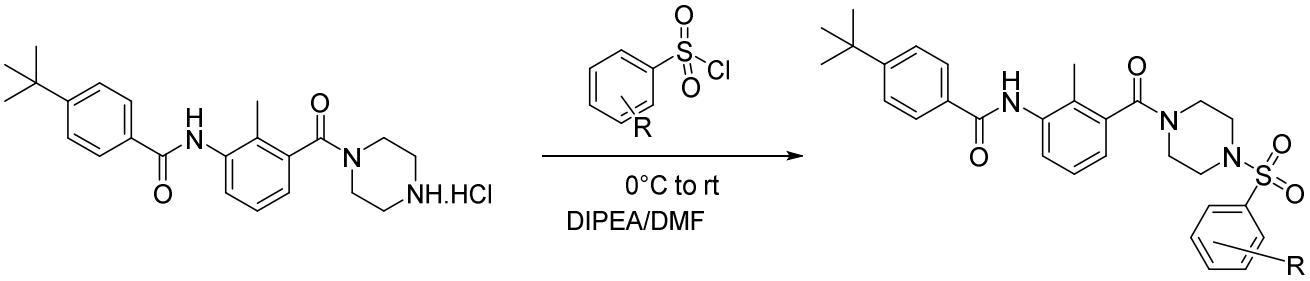
4] The reaction mixture was concentrated under vacuum to dryness once the starting material reaction was finished, yielding 0.71 g of white solid.

Purification:

5] To obtain 0.5g of the finished product, the acquired crude material was triturated using 25.00 mL of hexane.

6] Product nature: solid off-white.

Step-06b: Synthesis of 4-(tert-butyl)-N-(2-methyl-3-(piperazine-1-carbonyl) phenyl) benzamide Substituted sulfonamide derivatives



Calculations:

Sr.No	Chemical Name	Mole.wt	Wt	Mole Eq.	Moles
1	Step-06 Product	405.32	0.1	1	0.000246
2	Sulfonyl chloride	211.06	0.0572	1.1	0.000271
3	Reagent 1 DIPEA d-0.742	129.25	0.093g (0.125mL)	3	0.00072
4	Solvent DMF		2.00 mL		

Procedure:

1] Reagent 1 (0.125 mL, 3 eq) was added to a stirred solution of step-06 product (0.1 g, 1 eq) in solvent (2.00 mL) wad.

2] The reaction mixture was stirred at room temperature for 5 minutes. Subsequently, sulfonyl chloride (0.057 g, 1.1 eq) was added.

3] The reaction mixture was stirred at room temperature for 12 hours.

4] TLC was used to track the reaction's progress. [80% solvent in solvent. UV and Iodine]

Work-up:

5] The reaction mixture was poured into ice-cold water (50.00 mL) after the starting material was complete, and precipitation occurred.

6] The obtained ppt was filtered and washed with cold water to yield 0.8 g crude material.

Purification:

7] A final product of 0.71 g was achieved by triturating the acquired crude material with hexane.

8] Product nature: solid off-white.

Results and Discussion:

Molecular Modeling

We describe how the affinities and stabilities of these compounds in the pockets of their target proteins can be predicted using the atomistic interactions between receptors and possible therapeutic candidates. All three methods demonstrated the effectiveness of the derivatives.

1. **Docking**

We examined the derivatives' primary interactions with the target receptor using molecular docking. The findings demonstrated that Maestro-Glide's parameters accurately simulated these chemicals' binding mechanism.

The strong compounds SM14 and SM16 exhibited the same binding orientation as the standard in the amide's first series. In compounds with side chains, the heterocyclic atoms form hydrogen bonds with a receptor's amino acid. The molecule interacts well with the essential amino acid. More effective than other compounds, the SM14 chemical groups create the strongest covalent connection with the protein's primary active site. Although they had a lower binding affinity than SM15, the SM16 chemical groups also exhibited the strongest bond contact with the protein's primary active site. Other substances interact hydrophobically and establish hydrogen bonds with essential amino acids.

Table 01: Docking scores with receptor interactions

Code	**Docking Scores-** (kcal/mol)		**Hydrogen Bonding Interactions**	**Hydrophobic Interaction**
	5BNR	**2VF5**		
SM11	-5.234	-6.232	Arg36, Asn210, Ala143	Pro45, Leu80
SM12	-6.889	-6.887	Arg36, Asn210	Tyr51, Pro52

SM13	-6.099	-5.098	Arg36, Gln1633,	Pro145, Leu144
SM14	-7.123	-8.112	Asn210, Ala143	Phe50, Phe50, Pro52
SM15	-7.087	-6.443	Arg36, Asn210, Gln1633	Pro145, Leu144
SM16	-7.223	-5.783	Gln1633, Asn210	Tyr42, Phe52, Pro50
SM17	-6.221	-6.099	Asn210, Ala143	Pro120, Leu140, Pro52
SM18	-5.342	-6.090	Arg36, Gln1633,	Tyr51, Pro52
SM19	-6.900	-4.343	Ala143	Trp68, Pro45
SM20	-4.882	-4.222	Asn210, Arg36,	Tyr78, Pro50
STD	-7.902	-7.788	Asn210, Ala143	Trp146, Pro145, Leu144

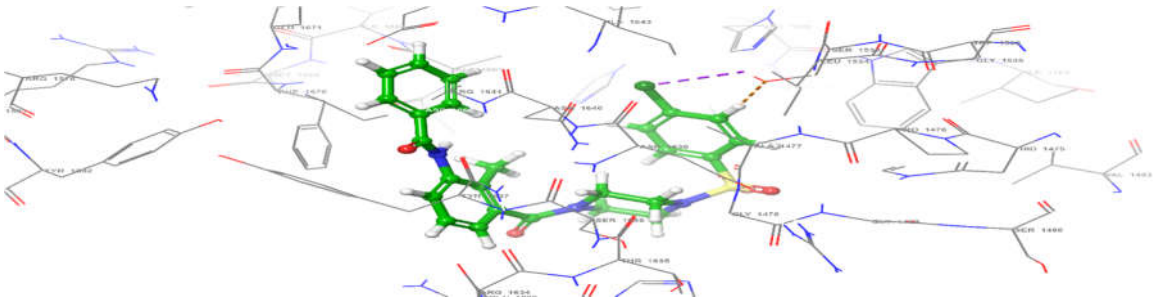


Figure: 02- The 3D Pose of the SM12 compounds

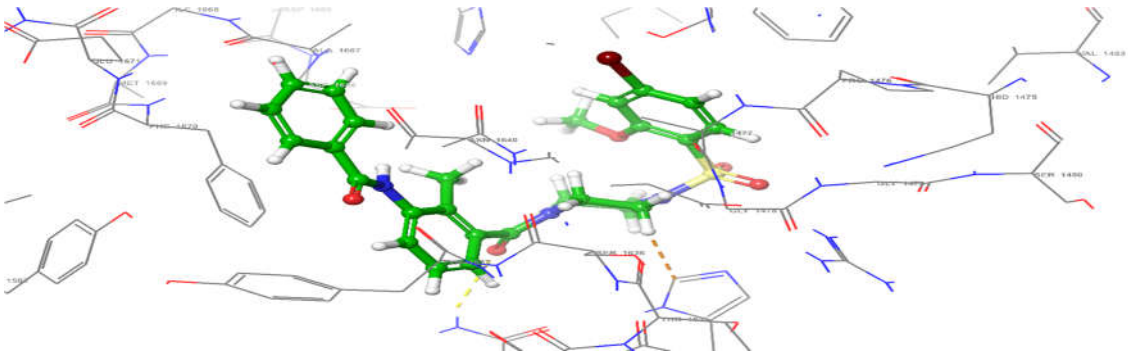


Figure 03- The 3D Pose of the SM14 compounds

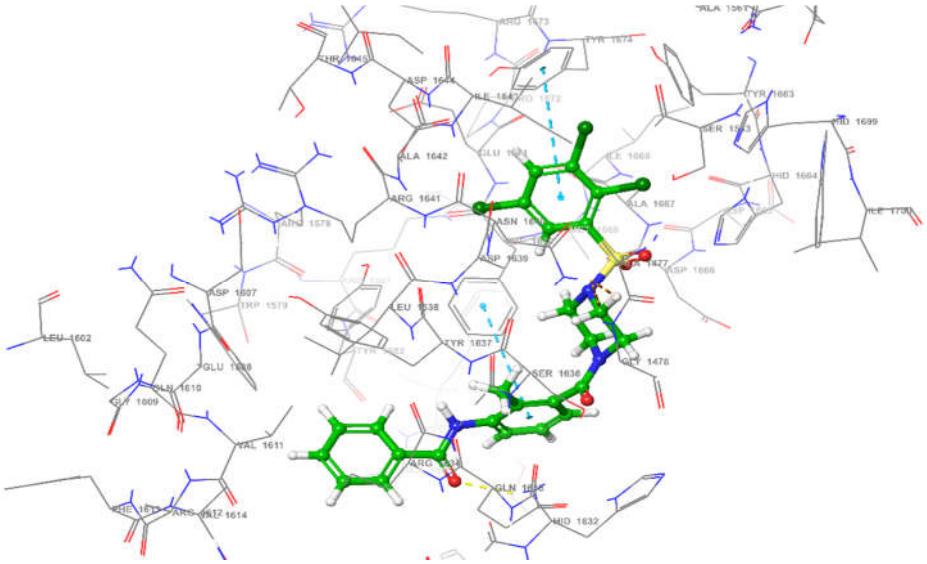


Figure 04- The 3D Pose of the SM17 compounds

Biological activity evaluation

The study synthesized and investigated the antibacterial and antifungal properties of ten novel sulfonyl piperazine derivatives (SM-11 and SM-20) in vitro. Using a 96-well microtiter plate and the micro-broth dilution method, the MIC of these compounds was quantitatively determined. Standard reference medications for antibacterial and antifungal screening were ampicillin and nystatin, respectively. The microbiological panel included fungal strains (A. niger NCIM 501 and C. albicans NCIM 3471), Gram-negative bacteria (E. coli NCIM 2065 and P. vulgaris NCIM 2813), and Gram-positive bacteria (S. aureus NCIM 2079 and B. subtilis NCIM 2063). Based on the MIC values, the antimicrobial profiles of the synthesized compounds were diverse, which is suggested to be caused by the different substituents and structural modifications of the synthesized piperazines. Compound SM14 was the most active sulfonyl-substituted derivative in general terms displaying MIC values of 92–134 µg/mL with lower MIC values of 118 µg/mL and 134 µg/mL against *A. niger* and *C. albicans* respectively. These values (all ≤125 µg/mL) are comparable to Ampicillin and Nystatin, demonstrating that broad-spectrum could be considered. Compound SM16 was the most active antibacterial compound against *B. subtilis* and *E. coli* 94–128 µg/mL of MIC values. This indicates an excellent bacteriostatic effect. On the other hand, compound SM12 displayed good activity against Gram-positive as well as Gram-negative bacteria, which points to a mode of action that would not be turned off by bacterial cell wall composition.

SM13,SM17.SM18,SM19 and SM20 were the least active compounds in this series; they exhibited the least activity, reflecting relatively poor activity against all microorganisms.

Key Structure –activity relationship (SAR) insights derived from these results-

1) Chlorine-substituted phenyl ring (SM12) correlated with increased antibacterial and antifungal activity, which might be attributed to higher lipophilicity and better Interaction with microbial enzymes.

2) CN-substituted phenyl ring (SM14) correlated with increased antibacterial and antifungal activity, which might be attributed to higher lipophilicity and better Interaction with microbial enzymes.

3) The piperazine functionality in all compounds is pivotal as a pharmacophore and may favorably modulate receptor binding and pharmacokinetic profile.

Table 04. MIC (ug/ml) of the compound against microorganisms.

Sample ID	MIC(ug/ml)					
	Bacilli subtilis NCIM 2063	*Staphylococcus aureus NCIM 2079*	*Escherichia coli NCIM 2065*	*Proteus vulgaris NCIM 2813*	*Aspergillus Niger NCIM 501*	*Candida albicans NCIM*
SM11	120	116	138	120	140	160
SM12	95	94	102	104	120	106
SM13	130	136	146	130	132	130
SM14	92	92	100	96	118	134
SM15	102	98	126	106	122	106
SM16	94	104	120	102	128	112
SM17	125	118	134	132	160	130
SM18	130	120	138	128	126	148
SM19	134	126	158	122	130	108
SM20	124	116	175	110	130	102
Ampicillin	93	91	122	94	N.D	N.D
Nystatin	N.D	N.D	N.D	N.D	116	98

Note: N.D.-Not Done.

The synthesized compounds shown a moderate to highest activity against the bacterial and fungal strains. The potential compounds further can be studied for the in-vivo study.

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