SYNTHESIS, MOLECULAR DOCKING AND ANTIMICROBIAL EVALUATION OF PYRAZINE SULFONAMIDES DERIVATIVES

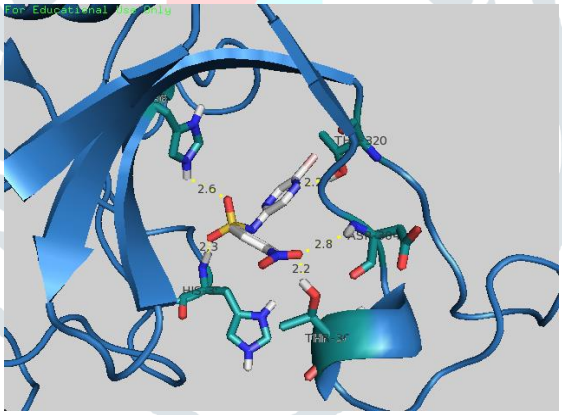
KHURSHEED AHMED[1], SAYYED NAZIA[1], SHETTY DEEPA[2], AIMAN KHAN[1], NARJIS SAYYED[1]

[1]Department of Chemistry, AbedaInamdar Senior College, Pune

[2]Department of Biotechnology, AbedaInamdar Senior College, Pune

ABSTRACT:

Pyrazine, in association with different scaffolds is one of the important classes of heterocyclic compounds those exhibit various biological activities. The basic scaffold used in clinically approved drugs is crucial in drug design whereas sulfamide ($R_2NSO_2NR_2$) functionality is highly acceptable in medicinal chemistry as it has the potential to form several electrostatic interactions with protein targets. While the sulfamide functionality is still fairly under-represented in medicinal chemistry, it is a valuable and versatile group that will gain increasing acceptance and favor in the future.In the present work, synthesis, structural characterization, molecular docking and antimicrobial evaluation ofpyrazine sulfonamide derivatives are reported. Based on the data **1b** successfully inhibits the transpeptidase through strong interactions and is showing strong inhibition against *Staphylococcus aureus***.**



1b docked in 5LB1 active pocket with the binding residues

Keywords:Sulfonamides, Pyrazine, Molecular docking.

1. INTRODUCTION:

Pyrazine sulfonamides are the class of chemical compound containingpyrazine ring and sulfonamide functionality. The drugs such as *Sulfalene*, *Sulfaquinoxaline*and*Sulfadiazene* are used for parasitic diseases which havepyrazine ring and sulfonamide functionality; therefore we envisagethat pyrazine sulfonamide derivatives will have potential to show antimicrobial activity.Pyrazineis one of the important class of heterocyclic compounds that can be isolated from natural sources or synthesized chemically. Many substituted pyrazines are produced naturally and are widely distributed in plants and animals including marine organisms. Pyrazines are also synthesized and degraded by several bacteria and fungi [1]. In insects, naturally occurring pyrazines serve as alarming pheromones, trial pheromones, repellents and site markers [2]. Pyrazinederivatives are important anthropogenic compounds, especially dihydropyrazines are essential for all forms of life due to their DNA strand-breakage

activity by their influence on apoptosis[3]. It is not surprising that many pyrazine derivatives possess numerous noteworthy pharmacological effects, including antimycobacterial, antibacterial, antifungal, antidiabetic, diuretic, anticancer, antiviral, hypnotic, and analgesic [4-8].

Pyrazine derivatives are frequently used as building blocks in drug discovery such as, varenicline (a partial agonist of certain subtypes of the nicotinic acetylcholine receptor)[9], bortezomib (proteasome inhibitor)[10],pyrazinamide (antitubercular agent) [11],Glipizide anti-diabetic [12],thionazine (insecticide and nematicide) [13], eszopiclone(nonbenzodiazepine hypnotic)[14], and Amiloride analogs which are blockers of epithelial sodium channel [15]. In addition n to this building block of pyrazinecarboxamide is an important part of many investigated pyrazine-based drugs.

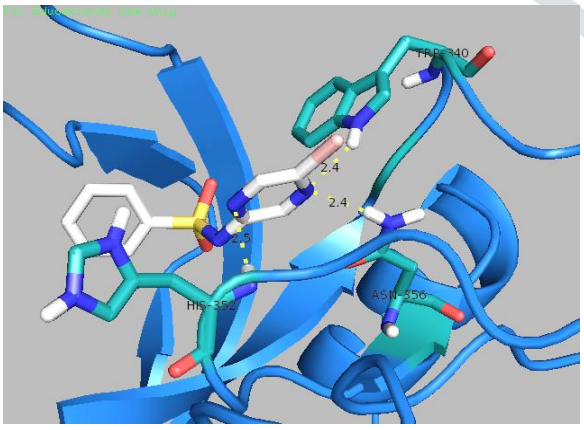
Sulfonamides (sulfa drugs) were the first drugs largely employed and systematically used as preventive and chemotherapeutic agents against various diseases. Over 30 drugs containing this functionality are in clinical use, including antihypertensive agent bosentan, antibacterial, translation initiation inhibitors. More recently, sulfonamides are used as an anticancer agent, as the antiviral HIV protease inhibitor and in Alzheimer's disease. Sulfonamide pharmacophore is an important structural core in medicinal chemistry. In the literature, sulfonamides with different pharmacological profiles, such as antimicrobial, [16] diuretic,[17]hypoglycaemic,[18]antithyroid,[19]antitumoral,[20] and antiviral [21] activities, are described. In addition, the antiparasiticefficacy of several benzenesulfonamides has been reported[22].

2. MOLECULAR DOCKING STUDIES

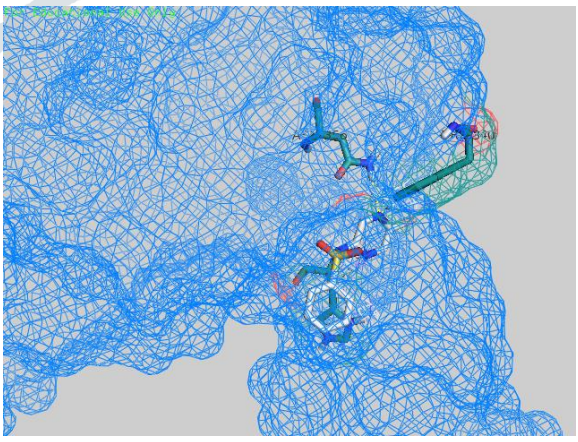
Cell wall is a known drug target in case of many bacterial infections. The faults in cell wall lead to the death of bacteria. Perfect cell wall is formed with the help of cross linking of peptidoglycanscarried out by L,D-transpeptidase. It this cross linking enzyme can be inhibited by certain molecules, it will lead to imperfect cross linking leading to the death of bacterial cell.

In order to explore the possible target of synthesized derivatives, molecular docking studies were carried out on the crystal structure of *Mycobacterium tuberculosis* L,D-transpeptidase-2 (LdtMt2) BC-module. The PDB file (5LB1) was downloaded from Protein Databank. The file was cleaned and polar hydrogen was added to the structure. The pdb structure was converted to pdbqt structure by adding kollmann charges.

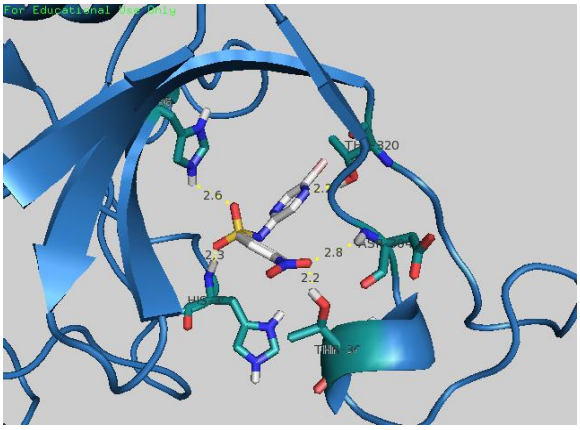
The pdbstructures of synthetic derivatives were prepared in discovery studio. The pdb structures were cleaned and saved as pdbqt structure. Grid box, coordinates and dimensions were finalized for final docking procedure as per the reported active pocket of the target protein. AutodockVina 4.2 was used for conducting docking studies. The images were created with the help of Python Molecular Viewer.



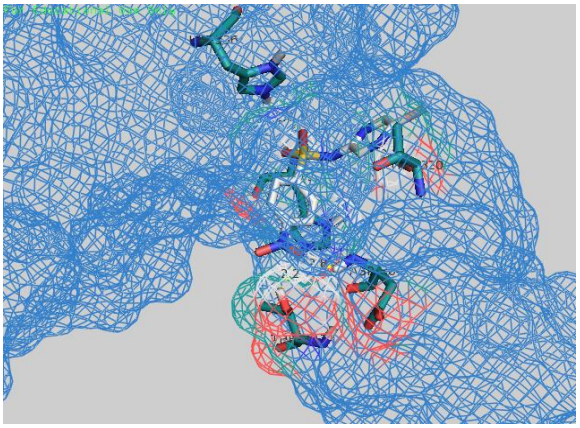
a



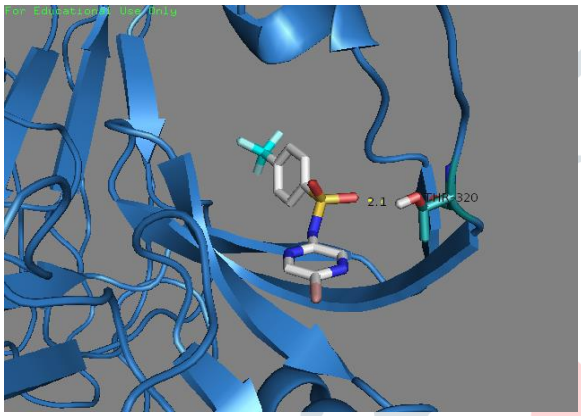
b



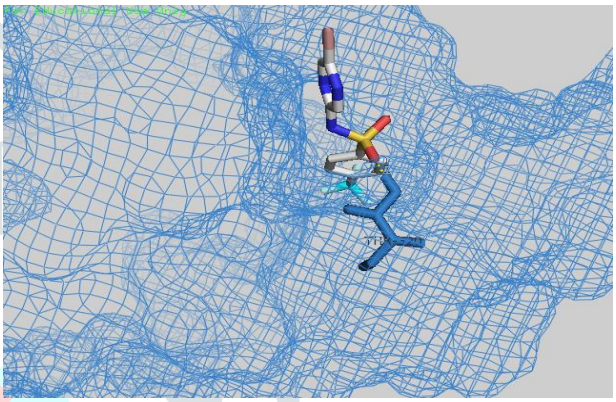
c



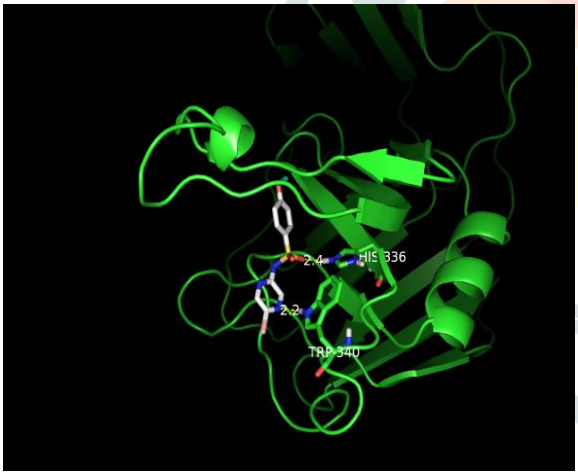
d



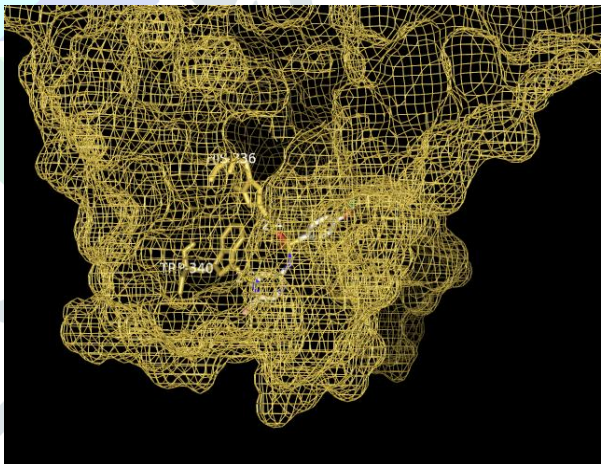
e



f



g



h

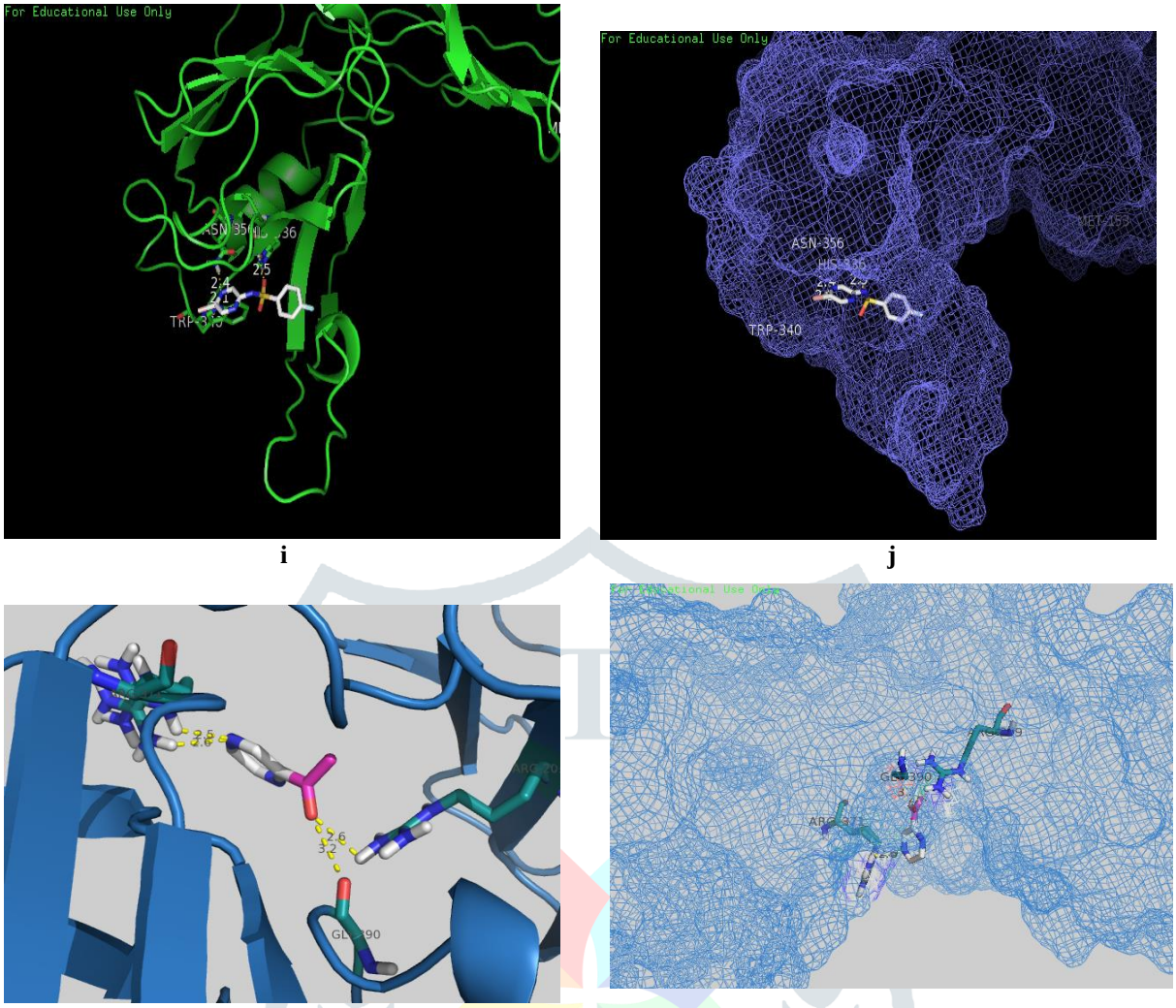


Fig.1 synthesized compound docked with 5LB1 active pocket. (a) 1a docked in 5LB1 ;(b)1a docked in 5LB1 Wire Mesh View; (c)1b docked in 5LB1;(d) 1b docked in 5LB1 Wire Mesh View;(e) 1c docked in 5LB1;(f) 1c docked in 5LB1 Wire Mesh View; (g) 1d docked in 5LB1;(h) 1d docked in 5LB1 Wire Mesh View;(i) 1e docked in 5LB1;(j) 1e docked in 5LB1 Wire Mesh View;(k) PMZ docked in 5LB1;(l) PMZ docked in 5LB1 Wire Mesh View.

Table 1: binding energy prediction by docking studies

Sr. No.	Code	B.E. Kcal/mole	Bond Length Angstrom	Amino Acid Residues
1	1a	-6.9	2.4 2.5 2.4	TRP-340 HIS-352 ASN-356
2	1b	-7.2	2.8 2.2 2.2 2.6 2.3	ASP-304 THR-307 THR-320 HIS-336 HIS-352
3	1c	-6.9	2.1	THR-320
4	1d	-6.7	2.4 2.2	HIS-352 TRP-340

5	1e	-6.8	2.1 2.4 2.5	TRP-340 ASP-304 HIS-352
6	PZM	-4.2	2.6 2.5, 2.6 3.2	ARG-209 ARG-371 GLY-390

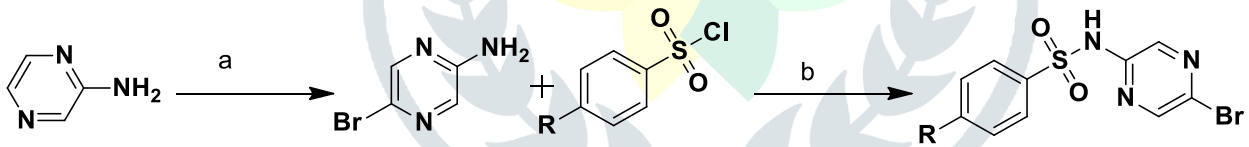
The docking studies show that all the synthesized derivatives are binding with the active pocket with greater binding affinities as compared to pyrazinamide (PZM) ,drug for treating tuberculosis. A molecule with smaller volume is able to slip in the active pockets very easily but this advantage of smaller volume translates into disadvantage in terms of interaction with the active pocket through its surface area. It easily slips into the pocket but with similar ease it can drift out of the pocket in absence of stronger binding interactions.

Therefore, the synthesized derivatives show higher binding affinity in comparison to pyrazinamide.

3. EXPERIMENTAL SECTION:

Solvents for synthesis were reagent grade and dried by standard procedures. The starting materials such as 2- amino pyrazine, *N*-Bromosuccinimide, Pyridine, and organic solvents etc. which were used for synthesis. All compounds were routinely checked by TLC on silica gel G plates using petroleum ether / ethyl acetate as solvent system and the developed plates were visualized by UV light and iodine vapours. The detailed synthesis has been shown in following reaction schemes.

5-Bromo 2- amino pyrazine (BP) was synthesized bybromination of 2- amino pyrazine using *N*- Bromosuccinimide& then it is further reacted with different Aromatic Sulphonyl chlorides in presence of base to yield different pyrazine sulfonamides. Structuralcharacterization of these sulfonamides is reported in this work.



1a: R= -H 1d: R= -OCH_3

1b: R= -NO_2 1e : R= -F

1c : R= -CF_3

Scheme: (a) *N*-bromosuccinamide, DCM, O°C (b) Pyridine O°C

Step I - General procedure for Synthesis 5-Bromo 2- amino pyrazine.

To a solution of 2- amino pyrazine (21.3mmol) in dry Dichloromethane (50ml) *N*- Bromo succinimide (21.03 mmol) was added at 0°C , the mixture was stirred at the room temperature for 24 hrs. Progress of the reaction was monitored by TLC. Reaction mixture was quenched by adding saturated solution of $NaHCO_3$ (30ml).Organic layer washed with water (30 ml), the combined aqueous layerwere extracted with DCM (100mlx 3) the combined organic extract were dried over Na_2SO_4 , filtered andconcentrated to furnish the 5-Bromo 2- amino pyrazine, which was recrystallized by ethanol (yield-70 %), M.P. 116°C.

Step II- Synthesis Pyrazine sulfonamide.

To an ice cooled solution of 2- amino 5- bromopyrazine (20 mmol) in pyridine (8 ml) the corresponding aromaticsulphonyl chloride (30 mmol) in pyridine (6 ml) was slowly added. The mixture was stirred at 0°C for 2 hr. Reaction was monitored by TLC.Reaction was quenched by adding water and the solid was collected by filtration& the solid product purified by silica gelcolumn chromatography.

4. STRUCTURAL CHARACTERIZATION:

1. N-(5-bromopyrazin-2-yl) benzenesulfonamide**(1a).** Yield 64%, pale brown colour: ^{1}HNMR (400 MHz, $CDCl_3$)δ: 11.6(s, 1H); 8.45 (s, 1H); 8.2 (s, 1H) 7.7-7.95 (m 5H): LCMS m/z (M^+ = 313, 100%) (M+2 = 315, 98%)
2. N-(5-bromopyrazin-2-yl)-4-nitrobenzenesulfonamide **(1b).** Yield 70 %, pale brown colour: ^{1}HNMR (400 MHz, $CDCl_3$)δ: 8.8(s, 1H); 8.5 (s, 1H);8.3 (s, 1H) 8.1-8.2 (m, 4H): LCMS m/z = (M-NO_2, 313, 100%) (M+2 = 315, 98%)
3. N-(5-bromopyrazin-2-yl)-4-(trifluoromethyl)benzenesulfonamide**(1c).** Yield 58 %, pale brown colour: ^{1}HNMR (400 MHz, $CDCl_3$)δ: 8.8(s, 1H); 8.3 (s, 1H); 8.25 (s, 1H) 7.8-7.9 (m, 4H): LCMS m/z = (M^+, 381, 100%) (M+2 = 383, 98%)
4. N-(5-bromopyrazin-2-yl)-4-methoxybenzenesulfonamide **(1d).** Yield 59 %, pale brown colour: ^{1}HNMR (500 MHz, $CDCl_3$)δ: 8.47(d, 1H); 8.27 (d, 1H);7.85 (d, 2H); 6.96 (d, 2H); LCMS m/z = (M^+, 344, 100%) (M+2 = 346, 98%)
5. N-(5-bromopyrazin-2-yl)-4-fluorobenzenesulfonamiden**(1e).** Yield 76 %, colourless: ^{1}HNMR (500 MHz, $CDCl_3$) δ: 8.48(d, 1H); 8.3 (d, 1H); 8.27 (d, 1H);7.85 (m, 2H); 7.20 (m, 2H); LCMS m/z = (M^+, 332, 100%) (M+2 = 334, 98%)

6. EVALUATION OF ANTIBACTERIAL ACTIVITY:

The antibacterial activity of the synthesized compounds was performed against Gram positive bacteria: *Staphylococcus aureus* and Gram negative bacteria: *Escherichia coli*. The antibacterial activity was performed by Agar disc-diffusion method. [23].

Table 2: Antimicrobial activity of compounds 1a-1e

Compound	Zone of inhibition (mM)			
	Gram Positive bacteria *Staphylococcus aureus*		Gram negative bacteria: *Escherichia coli*	
	2mM	10mM	2mM	10mM
1a	7	8	Not Detected	
1b	6	12		
1c	-	6		
1d	-	-		
1e	-	8		
BP	-	-		
Control (TMP-STX)	10			

7. RESULTS & DISCUSSION:

There are reports that have suggested that sulfonamides are useful for the treatment of some Staphylococcal infections. [24]. In the present investigation, few sulfonamides derivatives were evaluated for their antibacterial activity with the purpose of revealing possible leading compounds for the development of new antibacterial agents.

Trimethoprim/sulfamethoxazole (TMP/SMX) was used as a control in this study. It is an antibiotic which belongs to sulphonamide group. It consists of one part trimethoprim to five parts sulfamethoxazole. It is used for treatment of urinary tract

infections,Methiciilin resistant Staphylococcus aureus (MRSA) skin infections, travelers'diarrhea, respiratory tract infections, and cholera.

The antibacterial activity of synthesized compounds was evaluated at a concentration of 2mM and 10 mM.The data related to zone of inhibition against the bacterial cultures is shown in Table 2. The strongest inhibition against Gram positive bacteria *Staphylococcus aureus* was observed in case of compound 1b. On the contrary, the synthesized compounds did not show any inhibitory effect on Gram negative bacteria *E. coli*.The compound 1b showed better antibacterial activity when compared to the antibiotic TMP-STX which was used as a control in this study.

Fig.2 Plates showing Zone of inhibition for TMP-SXT,1a and 1b against *Staphylococcus aureus*

8. CONCLUSION

The results of antibacterial activity indicate that the compound**1b** are more effective against *Staphylococcus aureus* while none of the compound is effective against Gram negative bacteria *E. coli*.The result from docking studies also predictsthat compound **1b**has high binding energyand it will be able to successfully inhibit the transpeptidase through strong interactions

ACKNOWLEDGEMENT:

The author thank to the BCUD,SPPU for financial support.

9. REFERENCES:

1. Rajini RS, Aparna P, Sasikala C, Ramana CV. Microbial metabolism of pyrazines. *Crit Rev Microbiol***2011** 37:99-112
2. Woolfson A, Rothschild M. Speculating about pyrazines. *Proc Royal Soc***1990** 242:113-19
3. Yamaguchi T. The relationship between the chemical structures of dihydropyrazine derivatives and DNA strand-breakage activity. *Chem Pharm Bull(Tokyo)***2007** 55:532-6
4. R.Kalizan,et.al., PharmaceutischWeekblad, *Scientific Edition***1985** 7(4), 141-145.
5. E. Emary, *Journal Of The Chinese Chemical Society***2006** 53(2),391-401.
6. V. L. Rusinov,et.al. *Pharmaceutical Chemistry Journal*, **2005**39(12), 630-635.
7. A.Johnstonl, et.al.*Journal of Pharmacology and Experimental Therapeutics*, **1993** 264(2),604-608.
8. M. Shailaja, et.al. *Eur. J.Med.Chem*. **2010** 45(11), 5208-5216.
9. Coe JW, Brooks PR, Vetelino MG, et al. Varenicline: an alpha4beta2 nicotinic receptor partial agonist for smoking cessation. *J Med Chem***2005**48:3474-7
10. Adams J, Ma YT, Stein R, et al. Boronic ester and acid compounds. US5780454;**1996**
11. Welch JT, Mendez S, CynamonMH.Pyrazinamide for immunostimulation and the treatment of leishmaniases.US157396; **2012**

12. Reichman L.B.: *Chemotherapy***1996** 42, 2
13. Cynamon M.H., Klemens S.P. *J. Med. Chem.* **1992** 35, 1212.
14. Cotrel C, Jeanmart C, Messer MN. Pyrrolo(3,4-b)pyrazine derivatives. DE2300491; **1973**
15. Joule, J.A.; Mill, K. *Heterocyclic Chemistry*, 4th ed.; Blackwell publishing, UK 2002,221-22.
16. Argyropoulou, I.; Geronikaki, A.; Vicini, P.; Zani, F. Synthesis and Biological Evaluation of Sulfonamide Thiazole and Benzothiazole Derivatives As Antimicrobial Agents. *Arkivoc***2009**, 6, 89−102.
17. Vullo, D.; Innocenti, A.; Supuran, C. T. Diuretics with Carbonic Anhydrase Inhibitory Activity: Toward Novel Applications for Sulfonamide Drugs. In Drug Design of Zinc−Enzyme Inhibitors; Supuran, C. T., Winum, J.-Y., Eds.; Wiley & Sons, *Inc.: New York***2009** 155−170.
18. Selam, J. L. Pharmacokinetics of Hypoglycemic Sulfonamides: Ozidia, *a New Concept. Diabetes Metab.* **1997**, 23, 39−43.
19. Thornber, C. W. Isosterism and Molecular Modification in Drug Design. *Chem. Soc. Rev.***1979**, 8, 563−580.
20. Hu, L.; Li, Z.; Jiang, J.; Boykin, D. W. NovelDiaryl or Heterocyclic Sulfonamides As Antimitotic Agents. *Anti-Cancer Agents Med. Chem.* **2008**, 8, 739−745.
21. Supuran, C. T.; Innocenti, A.; Mastrolorenzo, A.; Scozzafava, A. Antiviral Sulfonamide Derivatives. *Mini-Rev. Med. Chem.* **2004**, 4, 189−200.
22. Norose, K.;Aosai, F.; Mun, H.-S.; Yano, A. Effects of Sulfamethoxazole on Murine Ocular Toxoplasmosis in Interferon-γ Knockout Mice. *Invest. Ophthalmol. Vis. Sci.***2006**, 47, 265−271.
23. Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing; Sixteen International supplements. CLSI document M100 S16. Vol 26-3; M7-A7, Vol.26-2; M2-A9, Vol. 26-1. Wayne PA. USA **2006**.
24. Altoparlak U, Kadanali A, Çelebi S: Slime factor positivity in coagulase negative staphylococci isolated from nasal samples of hemodialysis patients. *Int J ClinPract***2004**, 58:1112-1114.