International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Issue 1 | JAN-MAR| 2018 | 644-651
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

ANTIMICROBIAL SCREENING AND MOLECULAR DOCKING STUDIES OF IMIDAZO [1, 2-*b*] PYRIDAZINES AS POSSIBLE DIHYDROPTRERoATE SYNTHETASE(DHPS) INHIBITORS

Naresh K[1] and Sruthi K[2]

[1]Department of Pharmaceutical Chemistry, G. Pulla Reddy College of Pharmacy, Osmania University, Hyderabad, India.

[2] Department of Pharmaceutical Chemistry R.B.V.R.R Women's College of Pharmacy, Osmania University, Hyderabad, India.

***Corresponding Author Email: naresh4nani@gmail.com**

ABSTRACT

In the present investigation antimicrobial screening of imidazo[1,2-b] pyridazines was studied as continuation to our previous work. Twenty six derivatives covering benzamide and acetamide series of imidazo[1,2-b] pyridazines were screened against four bacterial and two fungal strains. Minimum inhibitory concentrations were determined for some of the potent compounds found in the screening. Molecular docking studies also performed on the target protein DHPS to evaluate the DHPS binding and inhibition ability.

KEY WORDS

imidazo[1,2-b] pyridazines, Dihydropteroate synthetase (DHPS), Antimicrobial, Sulfonamide, antibacterial.

INTRODUCTION

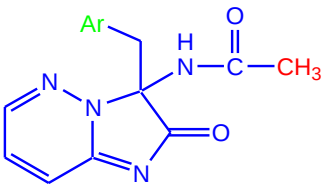
Dihydropteroate Synthetase (DHPS) belongs to the category of synthase enzyme family. It plays a crucial role in producing dihydropteroate which is essential for folic acid synthesis in bacteria. As the enzyme is not expressed in eukaryotes it promises a significant target for the development of antibacterial drugs Eg: Sulfonamides. These sulphonamides have been reported for the treatment of various Gram-positive and Gram-negative bacterial infections in combination with inhibitors of dihydrofolatereductase (DHFR). For example, co-trimoxazole is a commonly-used sulfamethoxazole-trimethoprim combination for many bacterial infections[1].

Even though the mutations in the dihydropteroate synthase have severely compromised the usefulness of these drugs, DHPS inhibitors still offer a promising clinical utility in the current scenario, as the design of novel inhibitors with a distinct mechanism is practically proven to be difficult resulted in high failure rates. DHPS mutations have been frequently characterized in many clinical isolates, relegating sulfonamide-based therapies to second- or third-line options. By the careful envisage on the DHPS catalytic mechanism and the mechanistic means of sulfa drug resistance, research is focussed on the development of new set of inhibitors that target the DHPS-active site of the enzyme[2].

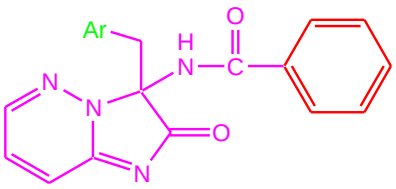
The enzyme has two substrates of a pteridine derivative and PABA. The pterin-binding pocket has been visualized in all the available crystal structures of DHPS and is found to be highly conserved. The pocket is located within the TIM barrel, directly below two flexible loops (loop1 and loop2) which are visualised to contain important requisites of the active site and is bounded by several key conserved residues that recognize the pterin-pyrophosphate substrate. The inhibitors of the constrained pterin binding pocket would be predicted to have a better spectrum of activity against both Gram-positive and Gram-negative

bacteria, and also be less able to tolerate resistance mutations[3-11].

The above observations prompted us to carry out the antimicrobial screening of our previously reported imidazo[1,2-b] pyridazine derivatives[12-13] to identify potential antimicrobial compounds. Molecular docking studies also performed to investigate the inhibition ability of the compounds on DHPS presuming that the imidazo[1,2-b] pyridazine ring bio-isoster of its substrates pteridine.



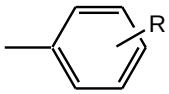
5a-n



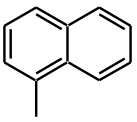
6a-n

5a-h and 6a-h

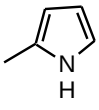
Ar =



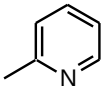
5i and 6i



5j and 6j

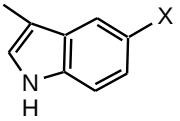


5k and 6k



5land 6l

5m /6m (X = H)
5n/6n (X = Br)



EXPERIMENTAL

Materials used for the antimicrobial activity were procured from Hi-media and SD fine chemicals. The standard drug samples were procured from CDTL, Hyderabad.

***In vitro* anti microbial activity**

All the synthesized compounds were evaluated for the antimicrobial activity using disc diffusion method against four bacterial strains among two gram positive and two gram negative and two fungal strains as per the procedure described in our earlier work[14].

Antibacterial screening

All the test compounds were prepared in DMSO at a 1000 µg/ml and standard drug solution was prepared at 100µg/ml. All the experiments were carried out under aseptic condition, sterile petriplates containing sterilized agar medium were prepared and standard bacterial solution were transferred on to the agar plate aseptically[15]. Sterile filter paper disc previously soaked in the appropriate test standard solution were placed at previously marked places on the inoculated agar plates. Then all the plate was kept a side for fifteen minutes to allow the diffusion of solution and then incubated for 24hrs at 37±2^{0}C. After incubation the zone of inhibition around the filter paper disc was measured using antibiotic zone reader. All the above experiments were carried out in triplicate and calculated the mean and standard deviation. The zone of inhibition of the test compounds were compared with the standard ciprofloxacin.

Antifungal screening

Anti fungal activity was carried out to assess the anti fungal activity of the synthesized compounds against *Candida albicans* and *Asperigillus falvus*. Compounds were tested at 1000 µg/ml concentration prepared in DMSO. Positive and negative controls were maintained for the entire experiments. Filter paper disc method was employed as per the procedure described above using appropriate culture media. All the petriplates were incubated for 24 hrs at 27±2^{0}C then the zone of inhibition was measured in mm and Mean, and SD values were determined. Clotriamazole was taken as standard drug.

Determination of MIC -Micro dilution method:

Minimum inhibition concentration for some of the test compounds and standard drugs were determined by broth micro dilution method against four bacterial strains *E. coli* (NCIM 2068) *P.aeruginosa (*NCIM 2862*), S.aureus (*NCIM 2079*), B.subtilis (*NCIM 2921*)* and two fungal strains *Candida albicans* and *Aspergillus flavus*. Standardized inoculum containing bacterial organism was diluted with Mueller-Hinton broth to get 5X10^5 cfu/ml in every plate and fungal inoculum was diluted to 5.0X10^2 c.fu/ml in RPMI 1640 media. Two-fold dilutions were prepared for test and standard compounds by diluting with DMSO. Sterile micro dilution plates were marked appropriately and loaded with the bacterial and

fungal inoculums separately and the test and standard solutions were introduced into the plates Positive control plates were maintained without adding drug solution and negative control was maintained by adding sterilized Mueller-Hinton broth or RPMI 1640 media to check the sterility. To each of the above microplates containing 100 µL of drug solution, 100 µL of standardized bacterial and fungal suspension was added and incubated for 48 hours at 37±2°C. The minimum inhibitory concentration (MIC) was determined.

Table 1: Anti-microbial activity of N-1 substituted 2,3-dihydro Imidazole[1,2-b] Pyridazine Acetamide derivatives

Compound	Zone of inhibition(mm) Mean ± SD[$]					
	P. a*	***E. c****	***S. a****	***B. s****	***C. a**** [#]	***A. f**** [#]
5a	9±0.1	10±0.2	14±0.1	16±0.1	9±1.2	-
5b	-	-	16±0.2	15±0.2	11±1.2	-
5c	-	-	14±0.6	12±0.6	11±0.1	-
5d	11±0.2	10±0.2	17±0.4	18±0.2	14±0.1	12±0.2
5e	9±0.5	9±0.1	20±0.2	20±0.3	12±0.2	12±0.2
5f	10±0.2	11±0.5	20±0.2	22±0.1	12±0.3	16±0.5
5g	12±0.14	11±0.2	22±0.1	23±0.5	16±0.1	14±0.4
5h	14±0.6	14±0.2	20±0.1	19±0.1	14±0.4	14±0.2
5i	12±0.2	16±0.4	18±0.3	17±0.2	10±0.5	-
5j	12±0.5	10±0.5	17±0.5	18±0.1	10±0.4	10±0.3
5k	-	-	16±0.2	17±0.5	11±0.6	-
5l	-	-	18±0.1	18±0.2	12±0.2	12±0.1
5m	14±0.2	16±0.3	22±0.1	21±0.2	14±0.1	12±0.4
5n	14±0.8	15±0.5	22±0.2	23±0.1	14±0.5	15±0.4
[£]Standard	24±0.1	23±0.1	24±0.2	25±0.1	18±0.1	17±0.1

[$] All experiments were carried out in triplicate and calculated mean and standard deviation

****Bacterial strains P.a – Pseudomonas aeruginosa, E.c- Escherichia coli, S.a - Staphylococcus aureus, B. s- Bacillus subtilis, [#] Fungal strains C.a- Candida albicans, A. f- Aspergillus flavus.***

[£] Standard – Ciprofloxacin for anti bacterial activity and Clotrimazole for Anti-fungal activity

Table 2: anti microbial activity of of N-1 substituted 2,3-dihydro Imidazo[1,2-b] Pyridazine Benzamide derivatives

Compound	Zone of inhibition(mm) Mean ± SD[$]					
	P. a*	***E. c****	***S. a****	***B. s****	***C. a**** [#]	***A. f**** [#]
6a	-	9±0.2	10±0.2	11±0.2	11±0.2	-
6b	-	9±0.2	10±0.5	12±0.1	10±0.2	-
6c	9±0.2	8±0.3	10±0.3	12±0.5	11±0.2	12±0.2
6d	12±0.1	11±0.4	12±0.1	19±0.1	16±0.3	10±0.1
6e	12±0.1	12±0.1	15±0.1	17±0.2	12±0.1	10±0.3
6f	12±0.2	10±0.2	16±0.2	17±1.1	14±0.5	14±0.1
6g	10±0.4	10±0.5	22±0.2	22±1.2	14±0.1	14±0.5
6h	18±0.1	17±0.1	20±0.6	21±0.5	14±0.5	16±0.4
6i	-	-	16±0.4	16±0.2	12±0.5	-
6j	-	-	10±0.2	12±0.2	10±0.47	-
6k	-	9±0.2	10±0.2	14±0.1	11±0.6	09±0.1
6l	-	-	12±0.1	14±0.3	-	09±0.2
6m	14±0.2	12±0.1	17±0.3	16±0.3	14±0.3	14±0.1
6n	12±0.1	12±0.2	20±0.3	17±0.1	14±0.4	14±0.6
[£] Standard	24±0.1	23±0.2	24±0.1	25±0.1	18±0.2	17±0.1

[$] All experiments were carried out in triplicate and calculated mean and standard deviation

****Bacterial strains P.a – Pseudomonas aeruginosa, E.c- Escherichia coli, S.a - Staphylococcus aureus, B. s- Bacillus subtilis, [#] Fungal strains C.a- Candida albicans, A. f- Aspergillus flavus.***

[£] Standard – Ciprofloxacin for anti bacterial activity and Clotrimazole for Anti-fungal activity

RESULTS AND DISCUSSION

Antibacterial activity

Anti bacterial activity of synthesized compounds revealed that compounds 5e, 5f, 5g, 5h, 5m and 5n showed excellent activity against G^{+ve} strains with a ZOI ranging from 20-23 mm, comparable with the standard drug ciprofloxacin. Compounds 5i, 5j and 5l exhibited potent activity (ZOI 17-19 mm) and the remaining compounds showed moderate to weak anti bacterial activity. Nevertheless, all the compounds of the series 5a-5n were inactive against G-ve bacterial strains.

Among the synthesized Imidazopyridazine benzamides 6a-6n, compounds 6g, 6h, 6n exhibited highest activity than the remaining compounds on G^{+ve} strains. Whereas, 6d, 6e, 6f and 6m showed potent inhibition of the growth with ZOI ranging from 16-19 mm. However, except compound 6h (17-18 mm) all the other compounds of the series were inactive on the growth of G^{-ve} bacterial strains.

From the results of the antibacterial activity of all the series of compounds using disc diffusion method, potent compounds were selected for the determination of MIC using agar micro dilution method.

Antifungal activity

The antifungal activity of the title compounds revealed that among the compounds of imidazo[1,2-*b*] pyridazines, the acetamide series compounds 5f, 5g and 5n exhibited good anti fungal activity and the remaining compounds were weakly active against the fungal strains. In the imidazo[1,2-*b*] pyridazines benzamide derivatives compounds 6d and 6h showed remarkable inhibition on the growth of fungi, where as compound 6f, 6g, 6m and 6n were moderately active. The remaining compounds of the series were inactive on the tested fungi.

Table 3: MIC values of N-1 substituted 2,3-dihydro Imidazo[1,2-b] Pyridazine and acetamide(5a-5n) and Benzamide derivatives(6a-6n)

Entry	Compound	Minimum Inhibitory Concentration(µg/ml)					
		EC*	PA*	SA*	BS*	CA#	AF#
1	5d	64	128	1	1	NT	NT
2	5e	128	128	2	2	NT	NT
3	5f	>256	256	1	0.5	32	1
4	5g	128	128	0.5	0.5	4	2
5	5h	>256	>256	2	2	32	8
6	5i	>256	>256	8	16	NT	NT
7	5J	128	>256	16	32	NT	NT
8	5m	128	128	16	16	16	16
9	5n	64	64	32	16	4	1
11	6d	128	64	32	16	NT	NT
12	6e	128	128	64	64	NT	NT
13	6f	128	256	32	64	32	32
14	6g	128	64	32	16	2	0.250
15	6h	128	32	16	8	2	1
16	6m	>256	126	8	16	16	32
17	6n	>256	126	4	8	8	8
18	Cipro£	0.250	0.250.	0.125	0.125	NT	NT
19	Clotrimazole£	NT	NT	NT	NT	0.06	0.125

Bacterial strains P.a – Pseudomonas aeruginosa, E.c- Escherichia coli, S.a - Staphylococcus aureus, B. s- Bacillus subtilis, # Fungal strains C.a- Candida albicans, A. f- Aspergillus flavus.

£ Standard – Ciprofloxacin for anti bacterial activity and Clotrimazole for Anti fungal activity

Minimum inhibitory concentration -Micro dilution method

Micro dilution method was employed to determine the MIC of selected 17 compounds from all the series against the same set of bacterial strains used for the disc diffusion method as per the method described in the experimental section. Ciprofloxacin and clotrimazole were taken as standard drugs for the comparison of MIC of test compounds.

The result of the MIC micro dilution method showed that except compounds, 5d, 5n, 6d, 6g, 6h all the remaining compounds tested were found to have MIC

values greater than 64µg/ml on all G^{-ve} strains which revealed moderate inhibition of the growth. However, the MIC values of most of the tested compounds on the G^{+ve} strains was apparently lower, among all the tested series acetamide series imidazo[1,2-*b*] pyridazines were found to have low MIC values than the benzamide series. The MIC of compound 5g bearing 4-OCH_3 against G^{+ve} bacteria was found be lowest (0.5 µg/ml) than the other compounds. Whereas the MIC of compounds 5d and 5f was 1 µg/ml. However, in imidazo[1,2-*b*] pyridazines benzamide derivatives except compounds 6h, 6m and 6n all the compounds tested showed greater MIC values were > 62 µg/ml on tested gram positive bacterial starins.

The MIC of the selected compounds further supports the antifungal activity of the compounds. Compound 6g bearing 4-OCH_3 was found to have remarkable inhibition with an MIC 0.250 µg/ml (*A. Flavus*), The MIC of Compounds 5f, 5n, and 6h was 1 µg/ml on *A.flavus*. The MIC of compounds 6g and 6h on *C. Albicans* was 2 µg/ml. However, the MIC of the remaining tested compounds was greater than 4 µg/ml and considered them as weak anti fungal agents

Molecular docking studies:

Molecular docking studies were carried out to evaluate the *in vitro* anti microbial potency of synthesized imidazo[1,2-*b*] pyridazine derivatives against the antimicrobial target dihydropteroate synthetase (DHPS), Crystal structure of DHPS (PDB ID: **1eye**) was downloaded from the protein data bank and used for the docking[16-17]. The docking studies were carried out by using Schrodinger- Glide with Maestro GUI. The ligand molecules were prepared in 2D sketcher and energy minimized using OPLS force field to prepare 3D structures using Ligprep tool. The structure of the target protein PDB was refined, water molecules were removed, and energy minimized using Protein Preparation wizard. The active site of the target was defined for the docking by preparing a receptor grid by selecting the co-crystal ligand of the receptor. Finally docking was performed using Glide for the prepared ligprep file and receptor grid file employing standard precision docking methodology. After docking run the glide score of the docked ligands and binding interactions of the docked ligands were considered (Table 5) for identification of potential compounds by comparing with that of standard drug Dapsone.

Standrad drug Dapsone interacts with the DHPS through a hydrogen with Arg253 the sulfone oxygen acts a hydrogen bond acceptor for this Hydrogen bond and the amino group of dapsone also donate a hydrogen and participate in one more H-bond interaction with the Phe 19.

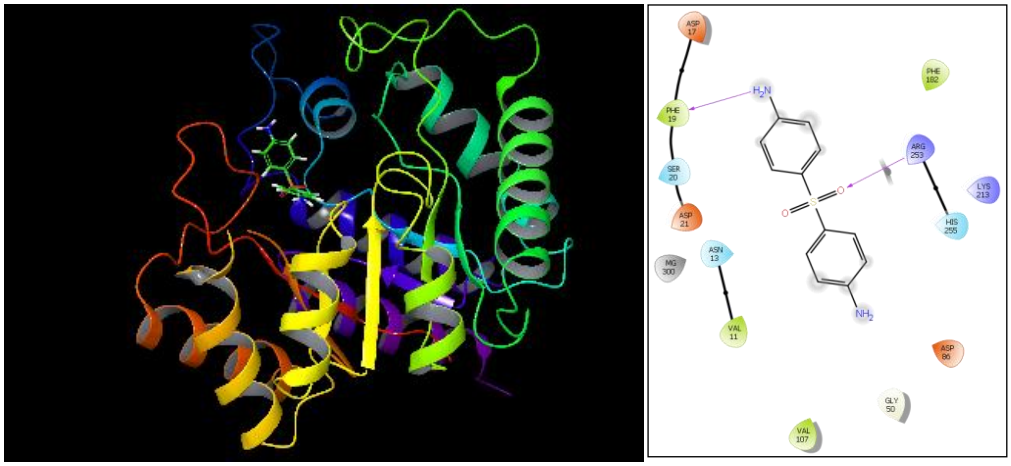


Figure 1: Interactions of Dapsone with DHPS (3EYE)

Table 4: Docking scores of imidazo[1,2-*b*]pyridazin derivatives on DHPS enzyme(1EYE)

Title	Docking score	Glide gscore	Title	Docking score	Glide gscore
5a	-5.103	-5.103	6a	-4.151	-4.151
5b	-4.316	-4.316	6b	-3.17	-3.17
5c	-4.254	-4.254	6c	-3.395	-3.395
5d	-4.616	-4.616	6d	-4.697	-4.698
5e	-4.598	-4.599	6e	-5.494	-5.495
5f	-4.175	-4.175	6f	-3.78	-3.78
5g	-4.95	-4.95	6g	-4	-4
5h	-4.754	-4.757	6h	-4.857	-4.862
5i	-5.187	-5.187	6i	-4.075	-4.075
5j	-5.167	-5.167	6j	-3.598	-3.598
5k	-4.712	-4.722	6k	-4.881	-4.889
5l	-5.159	-5.159	6l	-3.568	-3.568
5m	-5.556	-5.556	6m	-4.43	-4.43
5n	-4.746	-4.746	6n	-3.966	-3.966
Dapsone	-5.26	-5.26	-	-	-

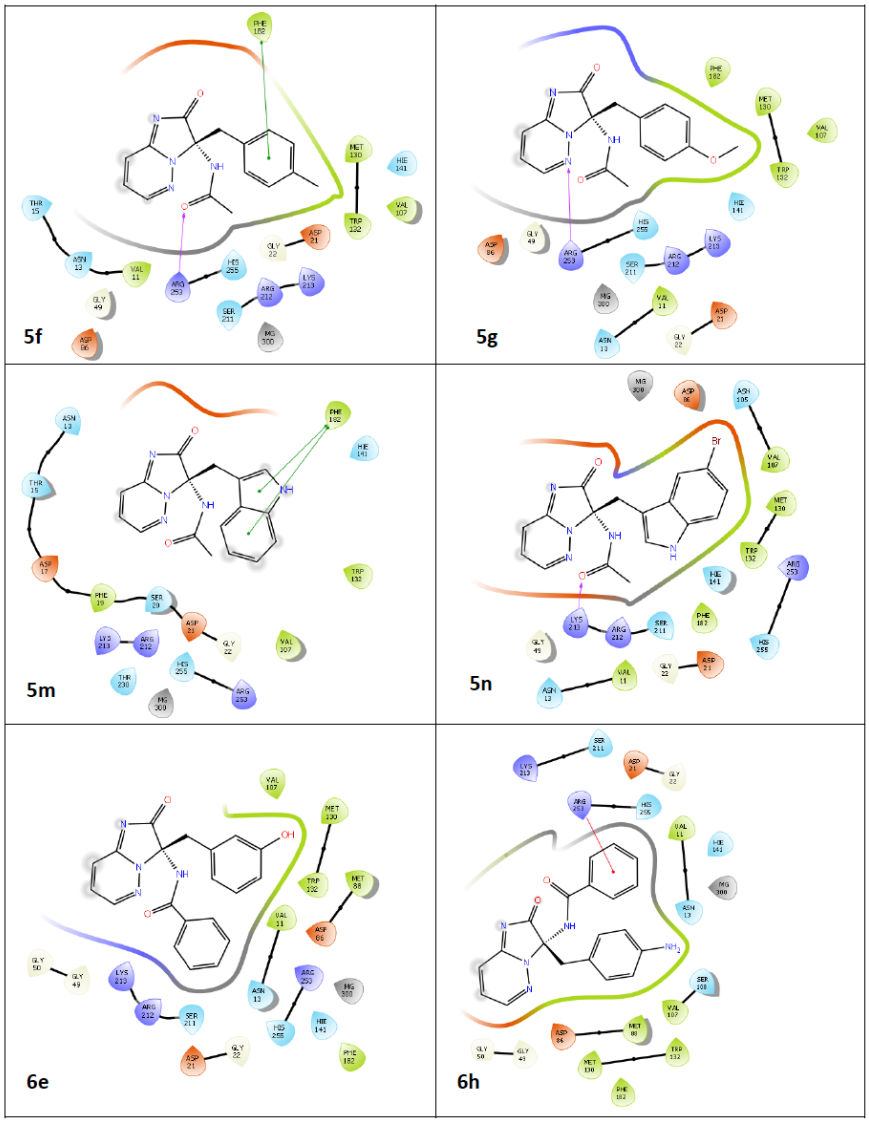


Figure 2: Ligand interaction diagrams (2D overlay) of *compounds* 5f, 5g, 5m, 5n, 6e and 6m with target enzyme dihydropteroate synthetase (DHPS) crystal structure (PDBID: 1EYE)

The binding of acetamides derivatives 5a-5g and their interactions has almost similar in the active site of DHPS. Compund 5m bound to the active site with highest binding affinity with a Gscore -5.556 the imidazole ring is flipped towards Phe182 and forms pi-pi interactions. Whereas compound 5f acetamido group forms a H bond with Arg253 and a hydrophobic pi-pi stake observed between benzyl ring and Phe 182. Moreover, compound 5g also bound in the similar fashion (figure 2). Compound 5n also made appreciable hydrophobic interactions with the Phe 182 and Trp 132. Compound 6e found to show highest binding affinity with a G score of -5.494 in the benzamides. The two-phenyl ring flipped away from each other and benzamido group has interacted with Lys213 and Arg 212 and the benzyl moiety made some polar intaerctions with Met 130. Wheras benzamido group of 6h made hydrophobic interactions with the Arg253 and His 256 and the p-amino benzyl group has oriented towards polar amino acids Asn13 and Ser 108. These observations suggest that compounds **imidazo[1,2-*b*] pyridazines** with benzyl substitution carrying a polar group at 4 position and compounds with an indole nucleus are the promising candidates for the DHPS inhibition as novel antibacterial agents.

CONCLUSION

From the present investigations it could be concluded that imidazo[1,2-*b*]pyridazin 2-one scaffold and their derivatives could be a novel candidates for the development of new antibacterial DHPS inhibitors. Further, through investigation would results in development novel antibacterial agents devoid of developing resistance by pathogenic bacteria.

REFERENCES

1. Rachel E Morgan, Gaëlle O Batot, Jennifer M Dement, Vincenzo A Rao, Thomas C Eadsforth and William N Hunter, Crystal structures of *Burkholderia cenocepacia* dihydropteroate synthase in the apo-form and complexed with the product 7,8-dihydropteroate, BMC Structural Biology2011, 11(21) 1-9.
2. *Iro Argyropoulou, Athina Geronikaki, Paola Vicini and Franca Zani,* Synthesis and biological evaluation of sulfonamide thiazole and benzothiazole derivatives as antimicrobial agents,Arkivoc 2009,6, 89 – 102,
3. Dalia Hammoudeh, Ying Zhao, Stephen W White, Richard Lee, Replacing sulfa drugs with novel DHPS inhibitors, *Future medicinal chemistry*, 2013, 5(11):1331-40
4. Aneesa, F., Ravindra, K., Bhagavanraju, M. (2015). Design, Synthesis, Antimicrobial and Anti-inflammatory Activity of N-Pyrazolyl Benzamide Derivatives, Medicinal chemistry, 5(12), 521-527.
5. Diwakar, R., Ramendra, K. S. (2011). Synthesis and antibacterial activity of benzamides and sulfonamide derived from 2-amino-5-bromo/nitropyridine against bacterial strains isolated from clinical patients, *Indian Journal of Chemistry*, 50(B), 931-936.
6. Carmen, L., Luminita, M., Mariana, C. C. (2011). Synthesis, Spectroscopic Properties and Antipathogenic Activity of New Thiourea Derivatives, *Molecules*,16, 7593-7607.
7. Ilkay, Y., Oren, Aki-Sener, E., Cengiz, E., Ozlem, T.A., Ismail, Y., Nurten, A. I. (2004) Synthesis and Microbiological Activity of Some Substituted N-(2−hydroxy-4-nitrophenyl) benzamides and Phenylacetamides as Possible Metabolites of Antimicrobial Active Benzoxazoles, Turkish Journal of Chemistry,28, 441-449.
8. Narayana, B., Raj, K.K.V., Ashalatha, B.V., Kumari, N.S., Sarojini, B.K. (2004) Synthesis of some New 5-(2-Suhstituted-l,3-Thiazol-5-yl)-2-HydroxyBenzamides and their 2-Alkoxy Derivatives as Possible Antifungal Agents, European Journal of Medicinal Chemistry, 39, 867-872.
9. Sener, E.A., Bingol, K.K., Arpacl, O.T., Yalcin, I., Altanlar, N. (2002). Synthesis and Microbiological Activity of some N-(2-Hydroxy-4-Substituted Phenylbenzamides, Phenylacetamides and Furamides as the Possible Metabolites of Antimicrobial Active Benzoxazoles I. *Farmaco,* 57 (45) 1-456.
10. Raffa, D., Daidone, G., Plescia, F., Schillaci, D., Maggio, B. (2002). Synthesis and Antifungal Activity of New N-(l-Phenyl-4-Carbetoxypyrazol-5-yl)-,N-(Indazole-3-yl)-andN-(Indazol--5-yL)-2-Iodobenzamides, *Farmaco,* 57, 183-187.
11. Sener, E.A., Bingol, K.K., Oren, I., Aipaci, O.T., Yalcin, I., Altanlar, N. (2000). Synthesis and Microbiological Activity of Some N-(o-Hydroxyphenyl) Benzamides and Phenylacetamides as the Possible Metabolites of Antimicrobial Active Benzoxazoles Part II. *Farmaco*,55, 469-476
12. Sruthi K, Sumakanth M, Mahendra Kumar CB, Naresh K, synthesis, *in silico* and *in vitro* anti-proliferative studies of some novel benzamido substituted imidazo[1,2-b] pyridazin-2-ones, *J.Fac.Phar.Anakara/Ankara.Ecz.Fak.Derg*, 2017, 41(1), 9-25.
13. Sruthi K, Sumakanth M, Mahendra Kumar CB, Naresh K, Design, Synthesis of N-(Substituted Imidazo [1,2-b] Pyridazine) Acetamides and Their Anti-Proliferative Studies on BRAFV600E Mutated A375 and Colo-205 Cell Lines, *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 2017, 12(3), 84-91.
14. J. Golus, R. Sawicki, J. Widelskiand G. Ginalska, The agar microdilution method - a new method for antimicrobial

susceptibility testing for essential oils and plant extracts, Journal of Applied Microbiology,2016, 121(5), 1291-1298.

15. Jayaveera KN, Rajeshwar Y, Naresh K, A study on antimicrobial and antioxidant activities of Schiff's bases derived from new thieno[2,3 - *d*]pyrimidines, *J Phar Chem*, 2013, 7, 12-16.
16. Friesner R.A, Murphy R.B, Repasky M.P, Frye L.L *et al*, Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes, J. Med. Chem, 2006, 49, 6177–6196.
17. Baca, A.M., Sirawaraporn, R., Turley, S., Sirawaraporn, W., Hol, W.G., Crystal structure of Mycobacterium tuberculosis 7,8-dihydropteroate synthase in complex with pterin monophosphate: new insight into the enzymatic mechanism and sulfa-drug action, J.Mol.Biol.2000, 302: 1193-1212.

***Corresponding Author:**
Naresh K*
Email: naresh4nani@gmail.com