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Evaluation of Antimicrobial Activity of Some Hybrids of Pyrimidine-Azole Derivatives along with Molecular Docking Study

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Abstract

Despite extensive research on antimicrobial drugs, efforts to find suitable alternatives to older drugs have not been very successful yet, due to microbial resistance. Heterocycles including azole and pyrimidine derivatives were used to design antimicrobial activity in this research, 12 novel pyrimidine-azole derivatives (*3a-3l*) that were previously synthesized were screened for their antibacterial and antifungal activities by using CLSI standard method. In this study, we used four species of bacteria, seven species of fungi, and five species of yeast. Molecular docking studies were also performed to investigate their binding mode and orientation toward lanosterol 14-α- demethylase (CYP51), as a plausible mechanism of azole antifungal compounds. The biological results showed that none of the compounds had antibacterial and antifungal effects compared to the control drugs. The molecular docking study showed that the compounds had a low binding affinity in the active site of the lanosterol 14-α- demethylase target, which confirmed the weak antifungal and antibacterial activities of these compounds.

Keywords: Antimicrobial, Molecular Docking, Azole-Pyrimidine hybrids.

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1. Introduction

The widespread use of antibiotic drugs led to increased resistance to treatment (1). Therefore, efforts are being made to design new and potent compounds with high efficacy. In the research, the design of the new antimicrobial compounds was developed with modifications to the scaffolds of the current drugs (2). Heterocycles including azole and pyrimidine derivatives were used to design the antimicrobial activity (3). Azole derivatives are the main backbone of most antifungal drugs (4, 5). Azoles antifungals are destructed fungal cell walls with binding to the iron atom of porphyrin of 14-alpha lanosterol demethylase enzyme through a nitrogen atom (6). Due to the indiscriminate prescription of azole drugs, resistance to them has increased. The resistance has been observed mostly in *C. albicans* and *A. fumigatus* species (7). Pyrimidine is the main scaffold of biological materials such as DNA, and RNA for which appropriate biological activities including anticonvulsant (8, 9), antibacterial (10, 11), antifungal(12), antiviral(13), and anticancer properties (14) have been reported. Extensive studies have been carried out on pyrimidine and azole hybrids, as antifungal and antibacterial agents (15). In a study, oxadiazole-pyrimidine hybrids showed high antibacterial and

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antifungal activity (16). Recently, molecules containing pyrimidine-based imidazole scaffolds were known as potent antimicrobial agents (17). Appna et al. evaluated some of the triazole-hydrazine pyrimidine derivatives for antifungal activity and presented promising results as antifungal agents (18).

Based on our study on the synthesis and biological evaluation of azole compounds (13, 14), in this study, we aimed to assess the antifungal and antibacterial properties of a variety of novel pyrimidine-azole derivatives that were previously synthesized. The effect of these compounds on the growth of yeasts such as *C. albicans*, *C. dublinii-ensis*, *C. glabrata*, *C. krusei*, *C.parapsilosis*, *C. tropicalis*, *C. neoformans* and *Filamentous Fungi* of *Aspergillus* (*A. flavus*, *A. fumigatus*, *A. clava-tus*), *Pseudoalscheria* (*P. boydii*), and *Exophiala dermatitidis* species and antibacterial effect on four bacterial species including *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were considered. The broth microdilution method was used for the evalu-ation of their antimicrobial activity. Subsequently, interaction with the lanosterol 14-α- demethylase enzyme (CYP51) was analyzed using molecular docking studies of the tested compounds.

2. Experimental section

2.1. Materials and methods

All reagents and solvents were obtained from Merck Company. Novel synthesized com-pounds (*3a-3l*) were obtained from Department of Medicinal Chemistry, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran (19, 20).

2.2. Antibacterial and antifungal assay

Micro-dilution method was used ac-cording to Clinical Laboratory Standard Institute (CLSI) for the determination of the minimum in-hibitory concentration (MIC) of the studied com-pounds (*3a–3l*) against the growth of bacteria and fungi. The Assay was performed at serial dilution on a range of 0.5-256 $\mu g.ml^{-1}$ for all compounds (*3a-3l*) against strains of gram-positive (*Staphy-lococcus aureus* and *Enterococcus faecalis*) and gram-negative (*Escherichia coli* and *Pseudomo-nas aeruginosa*) pathogenic bacteria. Ciprofloxa-cin was used as a standard drug.

The antifungal activities of compounds (*3a–3l*) were assayed *in vitro* at a serial dilution of 0.5-256 $\mu g.ml^{-1}$ against the yeasts and filamentous fungi species. For the measurement of minimum in-hibitory concentrations (MIC) of the studied com-pounds, we used the broth micro-dilution method based on the CLSI (Clinical & Laboratory Stan-dards Institute) protocol. Firstly, stock suspensions of yeasts and fungi were prepared in $1\text{-}5\times10^6$ cells/mL, so they were diluted to 50 and 1000 times with RPMI for filamentous fungi and yeasts, respec-tively. To each well, an amount of 0.1 mL of the inoculums was added and incubated at 30 °C for 24-48 h. In this method, the un-inoculated medium and fluconazole were utilized as blank and control.

2.3. Molecular docking

The X-ray crystal structure of lanosterol 14-α- demethylase enzyme (CYP51) was down-loaded from the Protein Data Bank http://www.rcsb.org/pdb (PDB ID: 5TZ1) (21). Adding explic-it hydrogens and removing the co-crystal ligand and solvent molecules from the PDB file were per-formed for the preparation of protein molecules. For starting docking studies, the structures were drawn in ChemDraw Professional 16.0, optimized with Chem3D 16.0, and finally saved as pdbqt for-mat (22). The molecular docking was performed using Autodock Vina. The visualization of interac-tions and binding modes was done using Discov-ery Studio 2016 Client.

3. Results and discussion

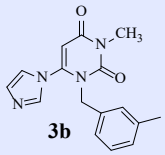
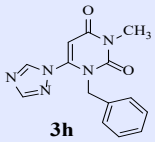
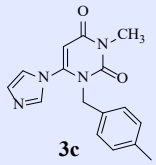
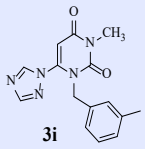
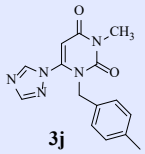
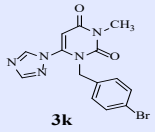
3.1. Chemistry

The physicochemical properties (Log P, MW, M.p.) were determined, as presented in Table 1.

3.2. Antimicrobial assay

Twelve newly synthesized pyrimidine-azole derivatives were evaluated for antimicrobial activity. In this study, drug sensitivity was deter-mined using the protocol CLSI (23). The antibac-terial activity for pyrimidine-azole derivatives was done against standard gram-positive (*S. aureus* and *E. faecalis*) and gram-negative (*E. coli* and *P.*

Table 1. The structures and physicochemical properties of the test compounds (*3a-3l*).

ID	Structure	Log P	M.P. (°C)	ID	Structure	Log P	M.P. (°C)
3a		0.92	-	3g		2.42	160-165
3b		1.41	120-125	3h		1.27	90-95
3c		1.41	100-105	3i		1.76	125-135
3d		1.75	110-115	3j		1.76	125-130
3e		1.59	138-140	3k		2.1	120-125
3f		2.08	120-123	3l		0.15	100-105

aeruginosa) bacteria. The results showed that none of the studied compounds had antibacterial activity in the concentration range of 0.5-256 µg/ml. Also, the results of antifungal and anti-yeast showed that all of the compounds had no inhibitory effect on microorganisms in the concentration range of 0.5-

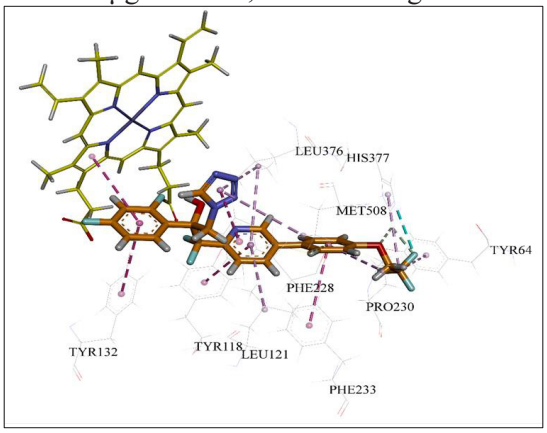


Figure 1. 3D structure and binding pose of co-crystal (VT1) in the active site of 14-α lanosterol demethylase enzyme (5TZ1).

256 mg/ml (24). Generally, in concentrations less than 256 µg/ml, the compounds were not able to inhibit the growth of the studied bacterial and fungal strains.

3.3. Molecular docking studies

The molecular docking study was performed to confirm the obtained biological results. The tested compounds were docked on lanosterol 14-α- demethylase enzyme with PDB ID: 5TZ1. The co-crystal ligand of 5TZ1 is a tetrazole-based antifungal drug candidate (VT1). The co-crystal docked on the 5TZ1 and the binding energy was obtained at -12.5 kcal.mol^{-1}. The 3D crystal structure of the enzyme is shown in Figure 1. The key residues in the active site were His377 Tyr 118, Tyr 132, Phe 228, Phe 233, and Hem 601. Among the residues, His 377 participated in hydrogen bonding interaction, and other residues formed π interactions.

The RMSD value of re-docking of VT1 is 1.07 Å that indicated the validation of docking. The re-docking conformation of docked pose and co-crystal of VT1 is shown in Figure 2.

The docking results of twelve molecules on 14-alpha demethylase protein are shown in Ta-

Table 2. The binding energy and involved residues in the active site for test compounds (3a-3l).

ID	Involved Residues in HB	Involved Residues in π interaction	Involved Residues in Hydrophobic	Binding Energy (Kcal/mol)
3a	Tyr132 Ile304	Tyr118, Hem601 Leu376, Hem601	Ile131, Hem601	-9.1
3b	-	Tyr118, Leu376 Tyr118, Hem601	Ile131, Hem601, Leu376	-9.3
3c	Tyr132 Ile304	Tyr118, Tyr132 Hem601, Leu376 Tyr118, Phe233 Phe126	Ile131, Hem601 Leu121	-9.6
3d	Tyr132 Ile304	Tyr118, Tyr132 Hem601, Leu376 Tyr118, Phe126 Phe233	Ile131 Leu121	-9.3
3e	Ile304 Tyr132	Tyr118, Hem601 Leu376, Phe228 Hem601	Ile131, Ile304 Hem601, Met508	-9.6
3f	Gly307	Leu376, Tyr118 Phe233, Hem601 Leu376	Gly307, Phe126 Leu121, Ile131	-9.1
3g	Tyr132 Ile304	Tyr118, Tyr132 Hem601, Leu376 Tyr118, Phe126 Phe228, Phe233	Ile131, Ile304 Hem601, Leu121	-9.8
3h	Tyr132, Ile304	Leu376, Hem601 Tyr118	Ile131, Hem601	-9.1
3i	Tyr132	Tyr118, Leu376 Tyr118, Hem601	Ile131, Hem601 Leu376	-9.3
3j	Tyr132 Ile304	Tyr118, Tyr132 Hem601, Leu376, Tyr118, Phe126, Phe233	Ile131, Hem601 Leu121	-9.7
3k	Tyr132 Ile304	Tyr118, Tyr132 Hem601, Leu376 Tyr118, Phe126 Phe233	Ile131, Hem601 Leu121	-9.5
3l		Hem601, Tyr132 Hem601	Ile131, Hem601 Tyr118	-9.0

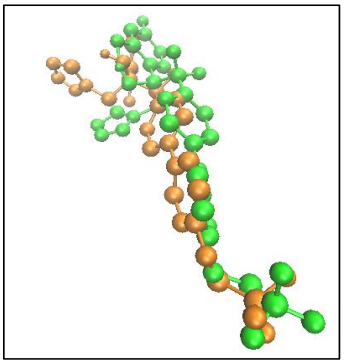


Figure 2. The superimposition docked pose and co-crystal of VT1 in the active site of lanosterol 14-α-demethylase enzyme with PDB ID: 5TZ1.

ble 2. As indicated the Table 2, the binding energy for all compounds was in the range of -9 to -9.8 k cal.mol^{-1}; it was found that none of the compounds had the binding affinity to the active site of the protein. Figure 3 and 4. is shown the interaction and binding pose of the studied structures. The results indicated that all compounds had the same binding pose in the active site and a small part of the compounds was located in the binding pocket. Compound 6g with a binding energy of -9.8 kcal. mol^{-1} was the best compound that established the hydrogen bonding with Tyr132 and Ile304 and the

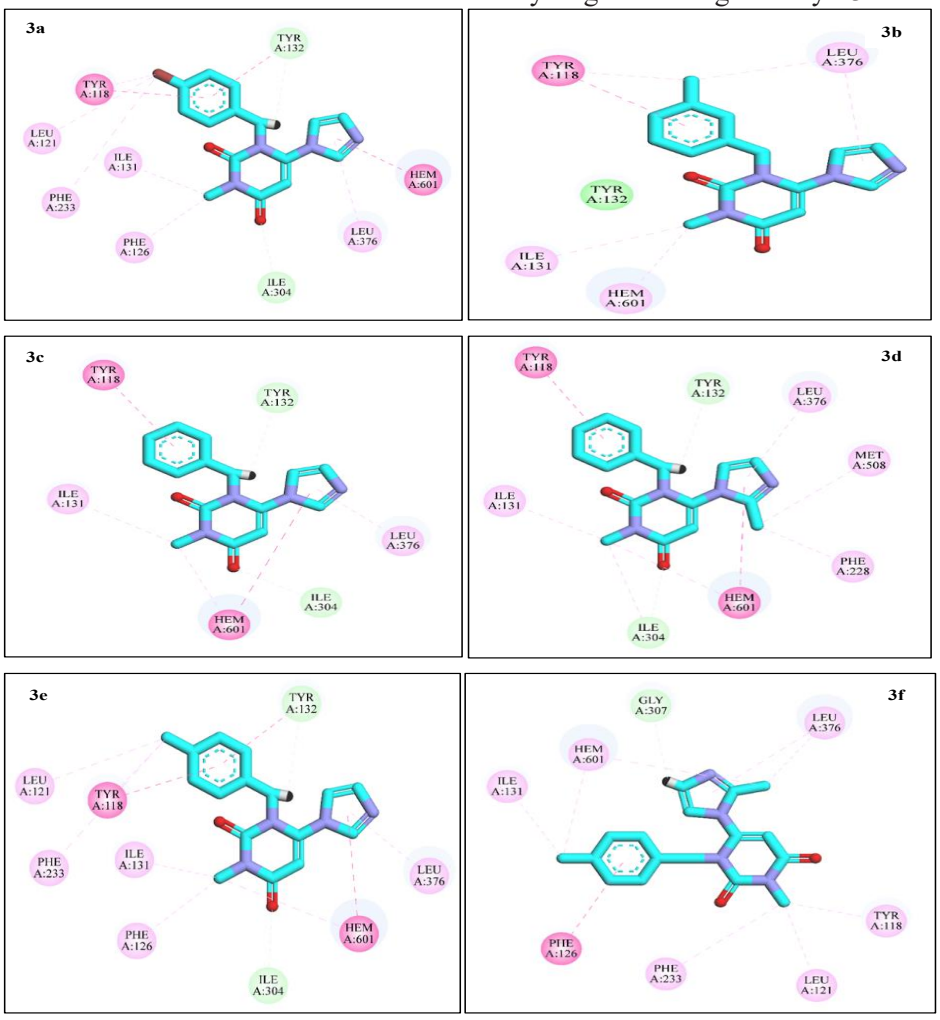


Figure 3. 2D Structures and interactions of the test compounds (3a-3f).

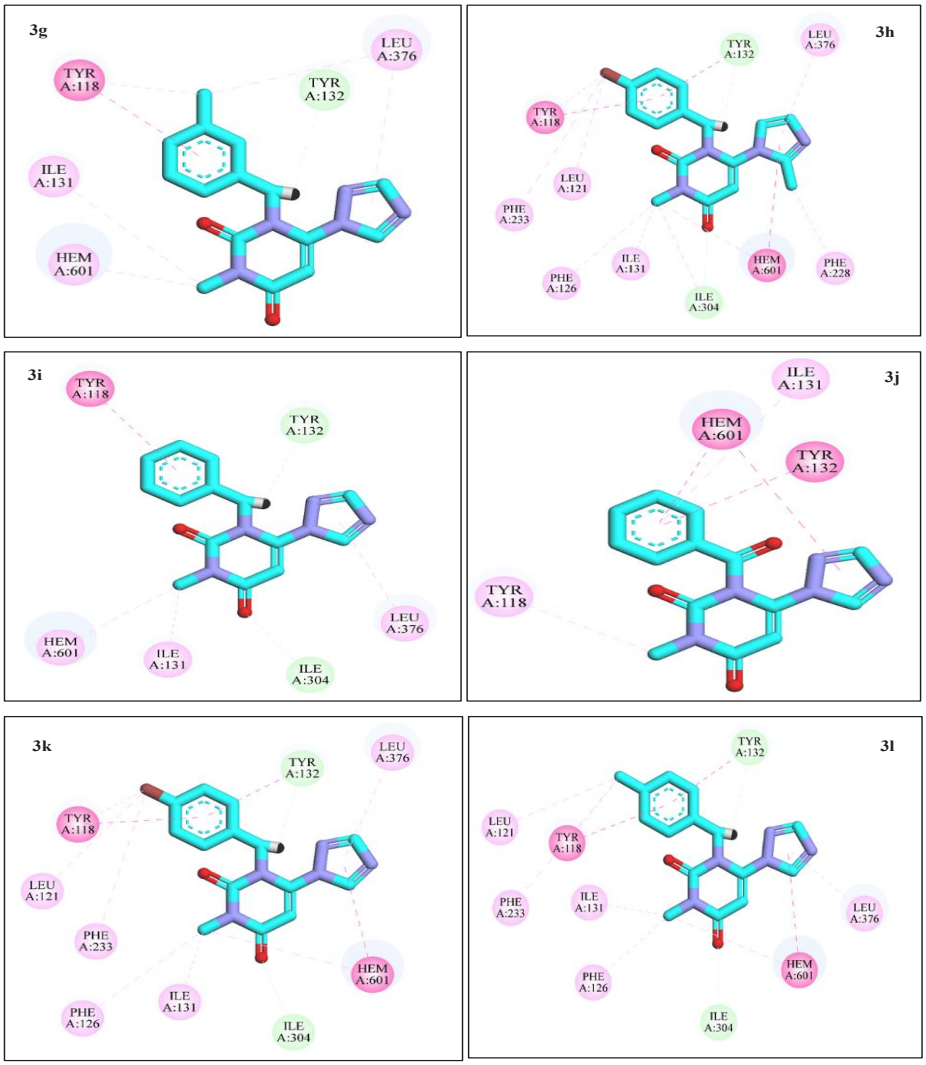


Figure 4. 2D Structures and interactions of the test compounds (3g-3l).

π contacts with Tyr118, Tyr132, Hem601, Leu376, Tyr118 and Phe126 residues. The rest of the compounds had almost the same interactions in the active site; among the key residues, most of the compounds interacted with Tyr 132 as hydrogen bonding and Tyr 118 as π interaction. Generally, the docking results proved that all compounds had a weak binding with the active site of 14-α lanosterols demethylase, which is consistent with the results of antimicrobial activities.

4. Conclusion

A series of pyrimidine-azole hybrid derivatives, which had been previously synthesized (19-20) were assayed as antifungal and antibacterial activities by using CLSI standard method. The biological evaluation of all compounds showed that none of the compounds had an inhibitory effect in the concentration range of 0-256 µg/ml. The molecular docking analysis was carried out for all of the derivatives against lanosterol 14-α- demethylase protein (5TZ1). The docking results indicated that all of the compounds exhibited a low binding affinity compared to the internal ligand of the 5TZ1 protein which confirmed the obtained biological effects.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Masiá Canuto M, Gutiérrez Rodero F. Antifungal drug resistance to azoles and polyenes. *Lancet Infect Dis*. 2002 Sep;2(9):550-63. doi: 10.1016/s1473-3099(02)00371-7. PMID: 12206971.
2. Arastehfar A, Gabaldón T, Garcia-Rubio R, Jenks JD, Hoenigl M, Salzer HJF, et al. Drug-Resistant Fungi: An Emerging Challenge Threatening Our Limited Antifungal Armamentarium. *Antibiotics (Basel)*. 2020 Dec 8;9(12):877. doi: 10.3390/antibiotics9120877. PMID: 33302565; PMCID: PMC7764418.
3. Sui YF, Li D, Wang J, Bheemanaboina RRY, Ansari MF, Gan LL, et al. Design and biological evaluation of a novel type of potential multi-targeting antimicrobial sulfanilamide hybrids in combination of pyrimidine and azoles. *Bioorg Med Chem Lett*. 2020 Mar 15;30(6):126982. doi: 10.1016/j.bmcl.2020.126982. Epub 2020 Jan 20. PMID: 32001137.
4. Eze CC, Ezeokonkwo AM, Ugwu ID, Eze UF, Onyeyilim EL, Attah IS, et al. Azole-Pyrimidine Hybrid Anticancer Agents: A Review of Molecular Structure, Structure Activity Relationship, and Molecular Docking. *Anticancer Agents Med Chem*. 2022 Aug 4;22(16):2822-2851. doi: 10.2174/1871520622666220318090147. PMID: 35306990.
5. Bheemanaboina RRY, Wang J, Hu YY, Meng JP, Guan Z, Zhou CH. A facile reaction to access novel structural sulfonyl-hybridized imidazolyl ethanols as potential DNA-targeting antibacterial agents. *Bioorg Med Chem Lett*. 2021 Sep 1;47:128198. doi: 10.1016/j.bmcl.2021.128198. Epub 2021 Jun 10. PMID: 34119615.
6. Shafiei M, Peyton L, Hashemzadeh M, Foroumadi A. History of the development of antifungal azoles: A review on structures, SAR, and mechanism of action. *Bioorg Chem.* 2020 Nov;104:104240. doi: 10.1016/j.bioorg.2020.104240. Epub 2020 Aug 28. PMID: 32906036.
7. Beck KR, Telisman L, van Koppen CJ, Thompson GR 3rd, Odermatt A. Molecular mechanisms of posaconazole- and itraconazole-induced pseudohyperaldosteronism and assessment of other systemically used azole antifungals. *J Steroid Biochem Mol Biol.* 2020 May;199:105605. doi: 10.1016/j.jsbmb.2020.105605. Epub 2020 Jan 23. PMID: 31982514.
8. Amr Ael-G, Sayed HH, Abdulla MM. Synthesis and reactions of some new substituted pyridine and pyrimidine derivatives as analgesic, anticonvulsant and antiparkinsonian agents. *Arch Pharm (Weinheim)*. 2005 Sep;338(9):433-40. doi: 10.1002/ardp.200500982. PMID: 16134091.
9. Alam O, Mullick P, Verma SP, Gilani SJ, Khan SA, Siddiqui N, et al. Synthesis, anticonvulsant and toxicity screening of newer pyrimidine semicarbazone derivatives. *Eur J Med Chem*. 2010 Jun;45(6):2467-72. doi: 10.1016/j.ejmech.2010.02.031. Epub 2010 Feb 18. PMID: 20211511.
10. Cieplik J, Stolarczyk M, Pluta J, Gubrynowicz O, Bryndal I, Lis T, et al. Synthesis and antibacterial properties of pyrimidine derivatives. *Acta Pol Pharm*. 2011 Jan-Feb;68(1):57-65. PMID: 21485702.
11. Zhuang J, Ma S. Recent Development of Pyrimidine-Containing Antimicrobial Agents. *ChemMedChem*. 2020 Oct 19;15(20):1875-1886. doi: 10.1002/cmdc.202000378. Epub 2020 Sep 11. PMID: 32797654.
12. Wu W, Lan W, Wu C, Fei Q. Synthesis and Antifungal Activity of Pyrimidine Derivatives Containing an Amide Moiety. *Front Chem*. 2021 Jul 12;9:695628. doi: 10.3389/fchem.2021.695628. PMID: 34322475; PMCID: PMC8311460.
13. Khan S, Kale M, Siddiqui F, Nema N. Novel pyrimidine-benzimidazole hybrids with antibacterial and antifungal properties and potential inhibition of SARS-CoV-2 main protease and spike glycoprotein. *Digit Chin Med*. 2021;4(2):102-19.
14. Basha J, Goudgaon NM. A comprehensive review on pyrimidine analogs-versatile scaffold with medicinal and biological potential. *J Mol Struct.* 2021;1246:131168.
15. Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. *Microorganisms*. 2021 Sep 27;9(10):2041. doi: 10.3390/microorganisms9102041. PMID: 34683362; PMCID: PMC8541629.
16. Desai NC, Vaghani HV, Jethawa AM, Khedkar VM. In silico molecular docking studies of oxadiazole and pyrimidine bearing heterocyclic compounds as potential antimicrobial agents. *Arch Pharm (Weinheim).* 2021 Oct;354(10):e2100134.

doi: 10.1002/ardp.202100134. Epub 2021 Jun 24. PMID: 34169569.
17. Desai D, Shende P. Drug-free cyclodextrin-based nanosponges for antimicrobial activity. *J Pharm Innov*. 2021;16:258-68.
18. He GX, Xue LW. Synthesis, Structures, and Antibacterial Activities of Hydrazone Compounds Derived from 4-Dimethylaminobenzohydrazide. *Acta Chim Slov.* 2021 Sep;68(3):567-574. PMID: 34897529.
19. Barzegar F. Design, synthesis and docking studies of pyrimidine-azole hybrid derivatives (I) as anti-fungal and anti-bacterial agent [Thesis]. Shiraz: Shiraz University of Medical Sciences, Faculty of Pharmacy; 1400.
20. Madadelahi ardekani Sedighe, Design, synthesis and docking studies of pyrimidine-azole hybrid derivatives (II) as anti-fungal and anti-bacterial agent [Thesis]. Shiraz: Shiraz University of Medical Sciences, Faculty of Pharmacy; 1401.
21. Onar HÇ, Kara EM, Ceyhan D, Erçağ E. Molecular Docking Studies and Microbial Activities of Mono-, Di-and Tri-Substituted Simple Coumarins. European Journal of Advanced Chemistry Research. 2023;4(1):22-30.
22. Zare F, Solhjoo A, Sadeghpour H, Sakhteman A, Dehshahri A. Structure-based virtual screening, molecular docking, molecular dynamics simulation and MM/PBSA calculations towards identification of steroidal and non-steroidal selective glucocorticoid receptor modulators. *J Biomol Struct Dyn.* 2023 Sep-Oct;41(16):7640-7650. doi: 10.1080/07391102.2022.2123392. Epub 2022 Sep 22. PMID: 36134594.
23. Siva S, Li C, Cui H, Meenatchi V, Lin L. Encapsulation of essential oil components with methyl-β-cyclodextrin using ultrasonication: Solubility, characterization, DPPH and antibacterial assay. *Ultrason Sonochem.* 2020 Jun;64:104997. doi: 10.1016/j.ultsonch.2020.104997. Epub 2020 Feb 7. PMID: 32058914.
24. Aljamali D, Mahmood N, Jawad SF. Preparation, spectral characterization, thermal study, and antifungal assay of (Formazane-Mefenamic acid)-Derivatives. *Egypt J Chem.* 2022;65(2):449-57.