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In-silico DESIGN, SYNTHESIS, AND ANTIBACTERIAL EVALUATION OF NOVEL TRIPHENYL-SUBSTITUTED IMIDAZOLES

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ABSTRACT

In an effort towards the discovery of new chemical entities to combat antimicrobial resistance, a series of novel 2,4,5-triphenyl imidazole derivatives were designed to target lanosterol 14α demethylase to screen the molecules for their antimicrobial potential. The designed molecules were synthesized and characterized by MS, FTIR, and NMR. The compounds were screened for their antimicrobial properties at a concentration of 100 µg/ml against Escherichia coli, Bacillus subtilis, and Aspergillus Niger. Compounds A9, B9, C9, and D9 were found to be active against Escherichia coli at the tested concentration. Computational studies including XP docking, induced fit docking, and MMGBSA were performed to assess their ability to bind at the active site.

Keywords: Computational, Antibacterial, Imidazole, Penicillins, Docking, MMGBSA.

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INTRODUCTION

Antimicrobial resistance (AMR) is a universal health and development threat.[1] It requires crucial strategic action in for achieving sustainable Development Goals (SDGs). We would no longer be able to treat infections and manage these risks to public health if antibiotics and antifungals lost their effectiveness. In recent years, several natural and synthetic antimicrobials have been discovered and successfully used in clinical practice.[2] Many of them are becoming ineffective owing to their misuse or development of resistance. Synthetic antimicrobial agents have significant contributions to clinical therapy starting from sulphonamides, Quinolones, and recently developed new-generation penicillins and cephalosporins.[3] They possess one or more heterocyclic moieties that are responsible for antimicrobial action. Heterocyclics including imidazole scaffold has attracted attention due to their variety of pharmacological action.[4] Imidazole is a basic functional moiety in antifungals like clotrimazole, miconazole, and ketoconazole. They are also present in antiparasitic drugs such as tinidazole and ornidazole. With this background, it was thought appropriate to design novel analogs of triphenyl-substituted imidazoles utilizing various computational tools. The designed analogs were synthesized, characterized and antibacterial screening was performed.

EXPERIMENTAL

Computational Studies

All the in-silico studies including molecular docking, induced fit docking, RMSD calculation, MMGBSA, and ADMET prediction were performed using Maestro suite Version 11.4 of Schrödinger supported on an

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HP computer having Intel core i3 processor, 4GB RAM, and Intel Haswell graphics card with Linux Ubuntu 18.04.1 LTS operating system.

Molecular Docking Studies

Molecular docking studies were performed for the designed compounds at the active site of lanosterol 14α demethylase (PDB id: 5V5Z). The crystal structure of the target was downloaded from the protein data bank and pre-processed as per the standard protocols. The compound structures were drawn and energy was minimized. Receptor GRID generation was carried out and designed ligands were docked at the receptor GRID. The docking[5] process was validated by calculating RMSD (root mean square deviation) values. The output results including docking scores, ligand interactions, and 3D interactions were analyzed and recorded.

MMGBSA

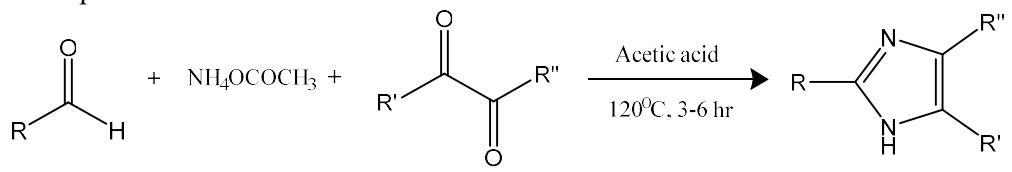
Prime MM-GBSA[6] is used to predict the free binding energy of the ligands in the protein-ligand complexes. Partial charges of the input ligand, implicit membrane model, or force field can be used for the calculation of ligand binding energies. Protein flexibility can also be considered by defining the protein flexibility region. Different sampling methods such as minimizing, minimizing side chains only, and minimizing polar hydrogens only can be used to describe the treatment of the defined flexible region. Prime MM-GBSA of the Prime module was used for calculating the ligand binding energies. The output file of Glide XP docking along with the VSGB solvation model and OPLS3e force field was utilized for binding energy calculations.

Induced Fit Docking

Induced fit docking is a flexible docking technique that accounts for protein flexibility along with ligand flexibility using Glide and Prime modules.[7] An induced fit docking panel was used for the Induced fit docking of the ligands. The prepared ligands were docked into the binding site of the protein by using a flexible protein and flexible ligand. Induced fit docking was performed by employing standard sampling. A grid was generated using the co-crystallized ligand as the centroid. The constraints, Glide docking, and prime refinement options were set to default. Glide XP docking was performed and XP descriptors were written.

Synthesis of Title Compounds

1mmol of aldehyde, 1mmol of substituted benzil, 5mmol of ammonium acetate, and 10ml glacial acetic acid were taken in a 50ml round bottom flask. The contents of the RBF were refluxed at 120°C for 3-6 hours using an oil bath.[8] The completion of the reaction was monitored by TLC using Hexane: Ethyl acetate (3:1) as the mobile phase. After the completion of the reaction, the reaction mixture was allowed to cool to room temperature and poured into cold water. The precipitate formed was filtered and dried under a vacuum. The crude product obtained was recrystallized using methanol as solvent. The scheme for the synthesis of title compounds was presented in Scheme-1.



Substituted Aldehyde | Ammonium Acetate | Substituted benzil | 2,4,5-triarylimidazole

Derivatives

A9: R= 5-nitrofuryl
B8: R= 2-chloro-6-fluorophenyl
B9: R= 5-nitrofuryl
B11: R= 4-hydroxyphenyl
B15: R= 3-nitrophenyl
B17: R= 2-chlorophenyl
B18: R= 2-hydroxyphenyl
B19: R= 2-hydroxy-5-bromophenyl
C8: R= 2-chloro-6-fluorophenyl
C9: R= 5-nitrofuryl
C15: R= 3-nitrophenyl
C19: R= 2-hydroxy-5-bromophenyl
D8: R= 2-chloro-6-fluorophenyl
D9: R= 5-nitrofuryl
D11: R= 4-hydroxyphenyl
D15: R= 3-nitrophenyl
D18: R= 2-hydroxyphenyl
D19: R= 2-hydroxy-5-bromophenyl

A: R' = Phenyl
R" = Phenyl
B: R' = 4-bromophenyl
R" = 4-bromophenyl
C: R'= 4-methyl phenyl
R" = 4-methyl phenyl
D: R'= 2-chlorophenyl
R" = 3,4-dimethoxy phenyl

Scheme-1: Reported synthesis of triphenyl imidazole derivatives

Characterization

The synthesized compounds were characterized by Mass spectrometry, Fourier Transform Infrared spectroscopy, and Nuclear Magnetic Resonance spectroscopy.

Antimicrobial Assay

Mueller Hinton agar plates and Sabouraud dextrose agar plates were prepared by pouring approximately 100 ml of the media into sterile Petri plates. The bacterial suspension was inoculated onto the Mueller Hinton agar plates and fungal suspensions were inoculated onto the Mueller Hinton agar plates using a sterile swab by swabbing uniformly on the surface of the plate. Six wells of 6mm diameter were made in the inoculated agar plates using a sterile cork borer. 100µl of DMSO (negative control), standard drug (Ciprofloxacin for gram-negative bacteria, Amoxicillin for gram-positive bacteria, and Amphotericin B for fungi), and four different samples were added into the wells. The plates were refrigerated for 60 minutes to allow the diffusion of the sample. The plates were then incubated at 37°C for 18 hours for bacteria and at 30°C for 48 hours for fungi. After the incubation period, the zone of inhibition was measured.[9]

RESULTS AND DISCUSSION

Molecular Docking Studies and MMGBSA Free Energy Calculation

Molecular docking of the ligands was performed against lanosterol 14α demethylase with PDB Id 5V5Z. The protein was found to have a resolution of 2.9Å. The docking method was validated as the RMSD value between the co-crystallized ligand and the docked minimized co-crystallized ligand was found to be 1.919Å which should be ideally less than 2Å. The molecular docking results against lanosterol 14α demethylase are shown in Table-1 where the docking score and binding energies are reported.

Table-1: Docking and MMGBSA Results of Designed Ligands Against Lanosterol 14α Demethylase

Ligand	Docking score	MMGBSA binding energy (Kcal/mol)
B17	-8.588	-46.68
D19	-8.534	-42.52
C15	-8.132	-40.87
B18	-8.109	-47.58
D8	-7.993	-24.24
B9	-7.982	-49.68
D18	-7.941	-49.05
C9	-7.866	-41.92
D15	-7.837	-28.97
B19	-7.744	-41.87
D11	-7.697	-53.35
B11	-7.595	-49.28
B15	-7.553	-46.54
C8	-7.499	-36.78
C19	-7.455	-37.83
B8	-7.321	-41.80
A9	-7.048	-47.49
D9	-6.880	-45.64
Itraconazole	-6.490	-68.29

Table-2: Ligand Interactions of Docked Ligands with Lanosterol 14α Demethylase

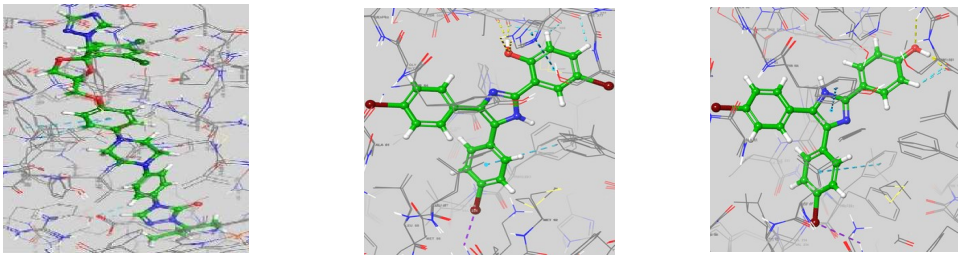
Ligand	Docking score	Hydrogen Bond	Halogen bond	Pi-Pi stacking	Pi cation
B17	-8.588	TYR 505	LYS 90	PHE 233	

D19	-8.534	HIE 377, SER 507		PHE 233	
C15	-8.132	TYR 505		PHE 233	
B18	-8.109	HIE 377, SER 507	LYS 90	PHE 233	
D8	-7.993		HIE 377	PHE 380	
B9	-7.982	TYR 505	SER 378, LYS 90	TRP 383	
D18	-7.941	SER 507		PHE 233	
C9	-7.866	TYR 505, GLN 66			
D15	-7.837	CYS 530		TYR 132, TYR 118, PHE 233, PHE 280, HEM 601	
B19	-7.744	Met 508	PHE 380	PHE 233	
D11	-7.697	HIE 377, MET 508		PHE 233, HIE 377	
B11	-7.595	TYR 505, GLN 66	LYS 90		
B15	-7.553			PHE 233, HIE 377	PHE 380
C8	-7.499			PHE 233	
C19	-7.455	MET 508		PHE 233	
B8	-7.321		LYS 90	PHE 233	
A9	-7.048			PHE 233, HIE 377	PHE 380
D9	-6.880	TYR 505, GLN 66			
Itraconazole	-6.490			TYR 118	HEM 601

Ligands B17 and D19 possessed appreciable docking scores of -8.588 and -8.534 respectively at the ligand binding site of lanosterol 14α demethylase protein 5V5Z. The standard drug itraconazole showed a docking score of -6.490. Compounds D11 and B11 showed strong binding with the receptor due to their minimum binding energies of -53.35, -49.68, and -49.28 Kcal/mol respectively. A summary of the ligand interactions of the docked ligands against lanosterol 14α demethylase is shown in Table-2. The 3D interactions of the standard drug and most active molecules were presented in Fig.-1.

Induced Fit Docking Against Lanosterol 14α Demethylase

The induced fit docking results of the ligands against lanosterol 14α demethylase are shown in Table-3. The IFD scores of many designed ligands were better than that of the standard drug Itraconazole.



(a) (b) (c)

Fig.-1: 3D Ligand Interaction Diagram of (a) Itraconazole (b) B19 (c) B11 with lanosterol 14α demethylase (PDB Id: 5V5Z)

The standard drug showed pi-pi stacking with residue PHE233 and PHE380. Similar interactions were observed in compounds B19, B11, C15, D18, D9, B17, B15, B18, B9, B8, D15, C8, and C9 (Fig.-10).

Many of the designed ligands showed interaction similar to standard drugs thus establishing the potential of the ligands as lanosterol 14α demethylase inhibitors.

Table-3: Induced Fit Docking of Ligands Against Lanosterol 14α Demethylase

Ligand	IFD score	Interactions			
		Hydrogen Bond	Halogen bond	Pi-Pi stacking	Pi cation
B19	-1020.43	HIE 377, SER 507	LYS 90	PHE 233, HIE 377	
D11	-1018.34	SER 506, TYR 505, SER 378		HIE 377	
D19	-1018.07	HIE 377, SER 378, MET 508		TYR 118, HIE 377	
C19	-1017.90	TYR 132, GLY 303		PHE 228	
B11	-1017.47	HIE 377, SER 378	LYS 90	TYR 64, PHE 233	
C15	-1017.36			PHE 233, HIE 377	PHE 380, HIE 377
D18	-1017.09	LYS 90, HIE 377, SER 507		TYR 64, PHE 233	
C9	-1016.56	TYR 505		TYR 64	
D9	-1015.88	LYS 90		PHE 233, HIE 377	PHE 380, HIE 377
B17	-1015.86		HIE 377, SER 378	PHE 233, PHE 380	
B15	-1015.85			PHE 233, HIE 377	
D8	-1015.69	MET 508	SER 378	TYR 118, PHE 228	
Itraconazole	-1015.53			PHE 233, PHE 380	
B18	-1015.41	HIE 377, SER 507	LYS 90	TYR 64, PHE 233, HIE 377	
B9	-1015.36	TYR 505	HIE 377, SER 378	PHE 380, HIE 377	
B8	-1014.84		LYS 90, HIE 377, SER 378	PHE 233, HIE 377	
D15	-1014.75	MET 508		TYR 118, PHE 228, PHE 380	TYR 505
C8	-1014.72			PHE 233, HIE 377, ILE 379	
A9	-1014.01	LYS 90, TYR 401		PHE 233, PHE 380, TYR 401	HIE 120, PHE 233

Synthesis

The designed compounds were synthesized and their physicochemical properties were analyzed. A list of the synthesized compound and their physicochemical properties is given in Fig.-2 and Table-4 respectively. The synthesized test compounds were characterized by IR, MASS, and NMR spectroscopic methods. The structure assignment was achieved with the help of both ^{1}H and ^{13}C NMR techniques. Molecular weight was confirmed by LCMS mass spectroscopy. Important functional groups were assigned using IR spectroscopy. Figure-3 and 4 represent the mass and ^{1}H NMR of the representative compound. The

characteristic peaks in ^{1}H NMR spectra were mainly in the aromatic region (7-8 ppm) and corresponding peaks in the aliphatic region (2-4 ppm).

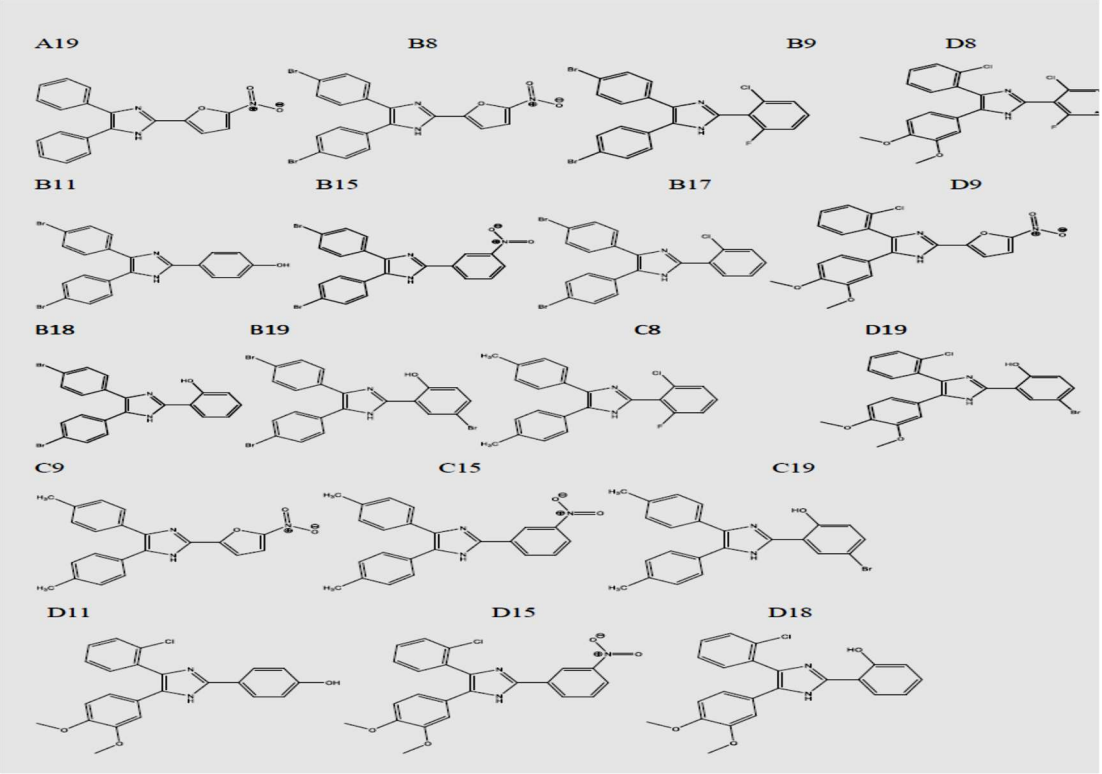


Fig.-2: Structure of Synthesized Test Compounds

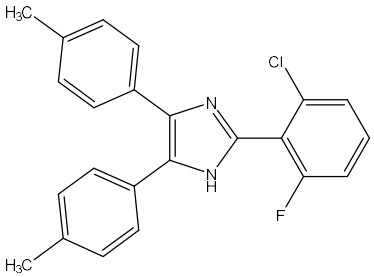
Table-4: Physicochemical Properties of the Synthesized Compounds Characterization

Compound	Molecular formula	Molecular weight (g)	Percentage yield (%)	Rf value	Melting point (°C)
A9	$C_{19}H_{13}N_3O_3$	331.33	98.5	0.53	264-266
B8	$C_{21}H_{12}Br_2ClFN_2$	506.60	74.3	0.68	204-206
B9	$C_{19}H_{11}Br_2N_3O_3$	489.12	95.5	0.15	270-272
B11	$C_{21}H_{14}Br_2N_2O$	470.16	75.1	0.05	222-224
B15	$C_{21}H_{13}Br_2N_3O_2$	499.16	78.9	0.81	278-280
B17	$C_{21}H_{13}Br_2ClN_2$	488.61	82.4	0.68	208-210
B18	$C_{21}H_{14}Br_2N_2O$	470.16	85.5	0.67	238-240
B19	$C_{21}H_{13}Br_3N_2O$	549.06	86.9	0.83	216-218
C8	$C_{23}H_{18}ClFN_2$	376.86	71.3	0.56	118-120
C9	$C_{21}H_{17}N_3O_3$	359.39	94.8	0.11	126-128
C15	$C_{23}H_{19}N_3O_2$	369.42	52.1	0.64	296-298
C19	$C_{23}H_{19}BrN_2O$	419.32	73.0	0.78	170-172
D8	$C_{23}H_{17}Cl_2FN_2O_2$	443.30	38.8	0.08	88-90
D9	$C_{21}H_{16}ClN_3O_5$	425.83	80.8	0.05	112-114
D11	$C_{23}H_{19}ClN_2O_3$	406.87	60.3	0.07	104-106
D15	$C_{23}H_{18}ClN_3O_4$	435.86	83.9	0.43	238-240
D18	$C_{23}H_{19}ClN_2O_3$	406.87	43.3	0.18	198-200
D19	$C_{23}H_{18}BrClN_2O_3$	485.76	62.7	0.32	84-86

Antibacterial Activity

The antimicrobial activity of the synthesized compounds was tested against Escherichia coli, Bacillus subtilis, and Aspergillus Niger at a concentration of 100µg/ml by agar well diffusion method.[10] Out of the

tested compounds, Compound A9, B9, C9, and D9 were found to be active against Gram negative[11] bacteria E. coli but were not active against B. subtilis and A. Niger. All the other compounds were inactive at the tested concentration against all three organisms.[12] The structure-activity relationship suggests that derivatives possessing nitro groups on the C ring were active against E. coli. Structure activity-guided insights suggest the strong relationship between powerful electron-withdrawing groups with the gram-negative activity spectrum. The activity values were presented in Table-5 and the zone of inhibition is presented in Fig.-5.



Molecular weight = 376.86g

Compound C8 Peaks**:** [M+1] peak was found at 377.24

Fig.-3: Mass Spectrum of the Representative Compound C8

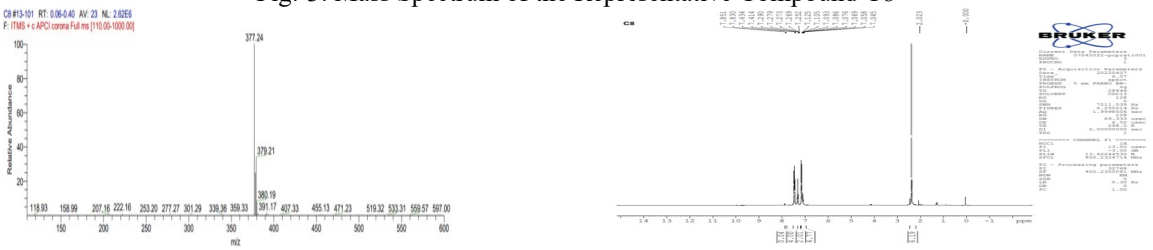


Fig.-4: ^{1}H NMR Spectrum of the Representative Compound C8

Table-5: Zone of Inhibition of Active Compounds Tested Against E. coli

Compound	Zone of inhibition (mm)
A9	22
B9	11
C9	17
D9	17.3
Ciprofloxacin (50µg/ml)	31.67

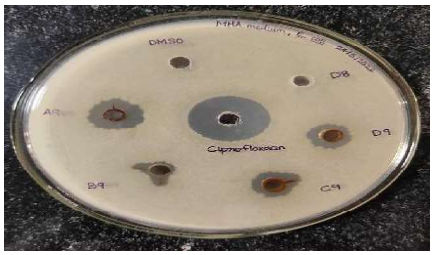


Fig.-5: Antimicrobial Assay of Active Compounds Against *E. coli*

CONCLUSION

A series of novel 2,4,5- triaryl imidazole derivatives were designed, synthesized, and characterized by various spectroscopic techniques. The designed compounds were further investigated by in silico studies against lanosterol 14α demethylase. They were subjected to in vitro antimicrobial assay. Four compounds A9, B9, C9, and D9 showed promising antibacterial activity against *Escherichia coli* at the tested concentration of 100 µg/ml. The results obtained in this study support the potential of these molecules as antibacterial agents. Antimicrobial screening could be carried out at different concentration ranges to negate the false negative results obtained in this study. In future studies, an attempt will be made to perform the

antibacterial activity of the compounds against the clinical isolates to further substantiate their antibacterial potential.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the faculty authors can be verified from their ORCID ids, given below:

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REFERENCES

1. C. I. R. Chandler, *Palgrave Communications*, **5**, Article 53(2020), https://doi.org/10.1057/s41599-019-0263-4
2. D. F. Pancu, A. Scurtu, I. G. Macasoi, D. Marti, M. Mioc, C. Soica, D. Coricovac, D. Horhat, M. Poenaru, C. Dehelean, *Antibiotics*, **10(4)**, 401(2021), https://doi.org/10.3390/antibiotics10040401
3. A. B. Shaik, R. P. Yejella, S. Shaik, *International Journal of Medicinal Chemistry*, **2017**, Article ID 6873924, (2017), https://doi.org/10.1155/2017/6873924
4. G. S. Andrei, B. F. Andrei, P. R. Roxana, *Mini Reviews in Medicinal Chemistry*, **21(11)**, 1380(2021)
5. R. A. Friesner, J. L. Banks, R. B. Murphy, T. A. Halgren, J. J. Klicic, D. T. Mainz, M. Repasky, E. H. Knoll, D. E. Shaw, M. Shelley, J. K. Perry, P. Francis, P. S. Shenkin, *Journal of Medicinal Chemistry*, **47**, 1739(2004), https://doi.org/10.1021/jm0306430
6. M. P. Jacobson, D. L. Pincus, C. S. Rapp, T. J. F. Day, B. Honig, D. E. Shaw, R. A. Friesner, *Proteins*, **55(2)**, 351(2004), https://doi.org/10.1002/prot.10613
7. W. Sherman, H. S. Beard, R. Farid, *Chemical Biology & Drug Design*, **67(1)**, 83(2006), https://doi.org/10.1111/j.1747-0285.2005.00327.x
8. B. Somashekara, B. Thippeswamy, G. R. Vijayakumar, *Journal of Chemical Sciences*, **131(62)**, 1(2019), https://doi.org/10.1007/S12039-019-1639-0
9. B. Athanassiadis, P. V. Abbott, N. George, L. J. Walsh, *Australian Dental Journal*, **54(2)**, 141(2009)
10. S. Renjusha, P. H. Vaisakh, *Rasayan Journal of Chemistry*, **14(3)**, 1653(2021), https://doi.org/10.31788/RJC.2021.1436332
11. G. Sundararajan, R. G. D. Rajaraman, K. Krishnasamy, *Rasayan Journal of Chemistry*, **14(3)**,1659(2021), http://doi.org/10.31788/RJC.2021.1436428
12. U. B. Chougale, H. V. Chavan, S. M. Deshmukh, P. R. Kharade, S. R. Dhongade, *Rasayan Journal of Chemistry*, **13(3)**, 1506(2020), http://dx.doi.org/10.31788/RJC.2020.1335716

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