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Antimicrobial screening and molecular docking studies of some novel triazoloquinazolinone derivatives

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ABSTRACT

Triazoloquinazolinoneand their derivatives have been studied extensively for various biological activities such as antimicrobial, anti-inflammatory, antitumor, antioxidant and anti-HIV activity. All the synthesized compounds have been screened for their antibacterial activity againstS.aureus, B.subtilis, S.typhi, E.coli, V.cholerae and K.pneumonia and antifungal activity againstA.flavus, A.niger,C.albicans, Mucor,Candida 6 and Rhizopus.Evaluation of antimicrobial activity shows that several compounds exhibits good activity when compared with the reference drug candidates and thus could be promising new lead molecules. The molecular docking studies have widened the scope of developing a new class of antimicrobial agents.

Key words: Triazole, antibacterial, antifungal activity, molecular docking

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INTRODUCTION

The emergence and spread of antimicrobial resistance have become one of the most serious public health concerns across the world. The search for new antimicrobial compounds is a challenging task as bacteria are continuously developing resistance to antimicrobial compounds; however, infections due to such bacterial strains are infrequent although potentially fatal [1-3]. The ever growing resistance to antibiotics leads to continuous screening for new biologicallyeffective compounds of either natural or synthetic origin. Quinazoline derivatives are extensivelyused in pharmaceutical industry, medicine and in agriculture for their wide scope of biologicalactivity [4]. Quinazolinoneanalogs have been reported for various biological activities such asanti-inflammatory [5], antimicrobial [6], antioxidant [7],anticancer [8]and antihypertensive activities [9]. In the recent years, being focused on green chemistry using environmentally benign reagents and conditions is one of the most fascinating developments in the synthesis of widely used organic compounds. Multi-component reactions (MCRs) play an important role in combinatorial chemistry because of the ability to synthesize target compounds with greater efficiency and atom economy by generating structural complexity in a single step from three or more reactants. Hence, triazoloquinazolinone derivatives have an important role in the pharmaceutical industry as active pharmaceuticals. The prevalence of triazoloquinazolinone derivatives in biologically active molecules has stimulated the need for efficient ways to make these heterocyclic leads. In view of these observations, it was thought worthwhile to synthesize triazoloquinazolinone derivatives(**4-12**).

MATERIALS AND METHODS

In vitro antibacterial and antifungal activity

The minimum inhibitory concentration (MIC) in µg/mL was determined by the serial dilution method [10]. The respective test compounds (**4-12**) were dissolved in DMSO to obtain 1 mg/mL stock solution. Seeded broth (broth containing microbial spores) was prepared in nutrient broth (NB) from 24-h-old bacterial cultures on nutrient agar (HiMedia, Mumbai) at 37 ± 1°C, while fungal spores from 1- to 7-day-old Sabouraud agar (HiMedia, Mumbai) slant

cultures were suspended in Sabouraud dextrose broth (SDB). The number of colony forming units (cfu) of the seeded broth were determined by the plating technique, and adjusted in the range of 10^4–10^5cfu/mL. The final inoculum size was 10^5cfu/mL for the antibacterial assay and 1.1–1.5 × 10^2cfu/mL for the antifungal assay. Testing was performed at pH 7.4 ± 0.2 for bacteria (NB) and at pH 5.6 for fungi (SDB). Exactly 0.4 mL of the solution of the test compound was added to 1.6 mL of seeded broth to form the first dilution. One milliliter of this was diluted witha further 1 mL of seeded broth to give the second dilution, and so on, till six such dilutions were obtained. A set of assay tubes containing only seeded broth were kept as control. The tubes were incubated in BOD (biochemical oxygen demand) incubators at 37 ± 1°C for bacteria and 28 ± 1°C for fungi. The MICs were recorded by visual observation after 24 h (for bacteria) and 72–96 h (for fungi) of incubation. Ciprofloxacin wasused as the standard drug for bacterial studies and Fluconazole as the standard drug for fungal studies.

Computational Methods

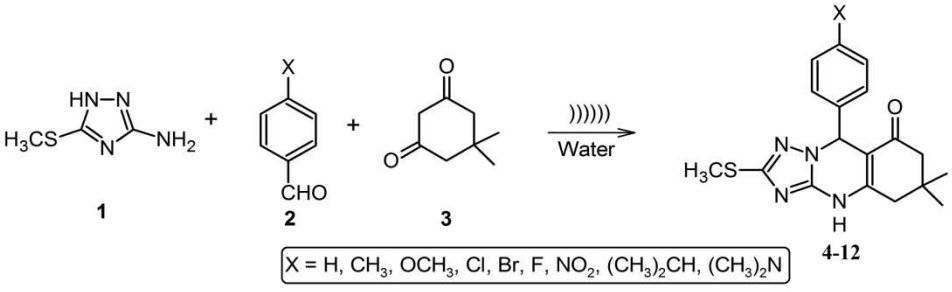
Docking calculations were carried out using DockingServer(**www.dockingserver.com**) (*Bikadi, Hazai, 2009*) [11]. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on corresponding protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (*Morris, Goodsell et al., 1998*) [12]. Affinity (grid) maps, 0.375 Å spacing were generated using the Autogrid program (*Morris, Goodsell et al., 1998*) [13]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively.

Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method (*Solis and Wets, 1981*)[14]. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

RESULTS AND DISCUSSION

Chemistry

The conventional approach for the synthesis of triazoloquinazolinone derivatives as follows: Dimedone reacts with 5-(methylthio)-1*H*-1,2,4-triazol-3-amine and corresponding aryl aldehyde to produce triazoloquinazolinone derivatives (**4-12**) and this efficient method using water as a solvent under ultrasound technique is represented in **Scheme 1**. The reaction mixture was located in ultrasonic bath for 40–50 min and the progress of reaction was monitored by thin layer chromatography (TLC).



Scheme 1

Antibacterial activity

The *in vitro* antibacterial activity of the title compounds **4-12** was determined by serial dilution method.All the synthesized Compounds, **4-12**were assessed to elicit their antibacterial activity *in vitro*against *Staphylococcus aureus*, *Bacillussubtilis*, *Salmonella typhi*,*Vibrio cholerae*, *Escherichia coli* and *Klebsiellapneumonia*. The antibacterial potency of the synthesized compounds was compared with Ciprofloxacin using their minimum inhibitory concentration (MIC) by serial dilution method; the values are summarized in **Table 1**. Close surveys of the MIC values indicate that all the compounds exhibited a varied range(12.5–200 µg/mL) of antibacterial activityagainst all the tested bacterial strains. The MIC values of compounds**7, 9**and**10**showed maximum inhibition activity (12.5 µg/mL) against*S. typhi*. Amongthe various substituted compounds, compound **4** against *V. cholerae*and *S. aureus*, compound **5** against *B. subtilis*,compound **7** against *K. pneumonia*, compound **8**against *V. cholerae*, compound **10** against*K. pneumonia*, compound **11** aganist*B. subtilis*and compound **12** against *E. coli* did not show any activity even at maximum concentration (200 µg/mL).Electron withdrawing substituents like chloro,fluoro and nitro substituted compounds **7,9** and **10**exerted excellent antibacterial activities.Fluorination

increases the lipophilicity due to strong electron withdrawing capability of fluorine.Moreover, fluorine substitution was commonly used in contemporarymedicinal chemistry to improve metabolic stability, bioavailability and protein ligand interactions.

Table -1 *In vitro* antibacterial activities of 4-12 against clinically isolated bacterial strains

Compound	Minimum inhibitory concentration (MIC) in µg/mL					
	S. aureus	*B. subtilis*	*S. typhi*	*V. cholerae*	*E.coli*	*K. pneumonia*
4	-	50	100	-	100	100
5	50	100	50	100	50	50
6	100	50	100	50	25	100
7	12.5	12.5	12.5	25	100	-
8	100	100	100	-	50	100
9	25	12.5	12.5	25	12.5	50
10	50	12.5	12.5	100	50	-
11	100	-	50	100	100	100
12	100	100	100	100	-	100
Ciprofloxacin	12.5	12.5	12.5	25	12.5	25

'—' no inhibition even at a higher concentration of 200 µg/mL

Antifungal activity

In order to extend the antimicrobial evaluation, the antifungal screening was also done, which revealed that the synthesized compounds (**4-12**) showed good inhibition against various tested fungal strains viz., *Aspergillusflavus*, *Aspergillusniger*, *Candida albicans*, *Mucor*, *Candida 6* and*Rhizopus*. Here, Fluconazole was used as standard drug.The results indicate that among the tested compounds, compound **9**showed maximum inhibition activity (6.25 µg/mL) against *C. albicans.* Among the various substituted compounds, compound **4**and **8** against*Mucor*,compound **5** against *A. flavus*,compound 7 against *Candida 6*,compound **6** and**8** against *Rhizopus*, compound **11** against *A. niger*, compound **5, 10** and **12** against *A.flavus*did not show any activity even at maximum concentration (200 µg/mL).However, the introduction of halogen functionality at *para* position of phenyl groups in compound **7, 9** and **10** registered moderate inhibition potency against all the tested fungal organisms with MIC ranging from 6.25 - 100 µg/mL. The Fluoro substituted compound **9** shows maximumantifungalpotency against*C. albicans.* A modification of *para* proton (compound **4**) by chloro, fluoro and nitro group i.e., compounds **7**, **9**and **10** shows moderate activity against the entire tested fungal strains but registered high inhibition against *C.albicans*(6.25-25µg/mL). Results of antifungal studies have been presented in **Table 2**.

Table -2 *In vitro* antifungal activities of 4-12 against clinically isolated fungal strains

Compound	Minimum inhibitory concentration (MIC) in µg/mL					
	A. flavus	*A. niger*	*C. albicans*	*Mucor*	*Candida 6*	*Rhizopus*
4	50	50	50	-	50	100
5	-	100	25	100	100	100
6	50	50	50	100	50	-
7	50	50	12.5	50	-	100
8	100	100	50	-	50	-
9	50	25	6.25	25	25	50
10	-	100	12.5	50	50	50
11	50	-	25	100	100	-
12	-	100	50	100	-	-
Fluconazole	12.5	25	6.25	25	25	50

'—' no inhibition even at a higher concentration of 200 µg/Ml

Molecular docking studies

Molecular docking studies were conducted in order to validate the obtained pharmacological data and to provide understandable evidence for the observed antimicrobial activity of all synthesized compounds. Molecular docking study is a well-established technique to determine the interaction of two molecules and find the best orientation of ligand would form a complex with overall minimum energy. All the synthesized compounds **(4-12)** were docked *Structure of TREX1 in complex with a nucleotide*of *S.typhi*at ten different orientations.The structure of the protein mentioned above [PDB:**3B6O**] was retrieved from the Protein Data Bank [www.rcsb.org(DOI:10.2210/pdb3b6o/pdb)] and further modified for docking calculations. The ligand molecules were drawn and analysed using Chem Draw Ultra 8.0. **3D**, coordinates were prepared using dock server. Based on the *in vitro* antimicrobial studies, it is worthwhile to do *in silico*studies; it supports the *in vitro* activity.

In silico studies revealed all the synthesized molecules showed good binding energy toward the target protein ranging from -8.36to -6.91 kcal/mol. The docking results revealed that compound**10** showed minimum binding energyof -8.09 kcal/mol, which is due to dipole-dipole and hydrogen bond interaction with amino acids of targeted

protein.It was observed that the most active compound of the series, i.e., compound**7**was predicted to be most active *in silico* too. The other compounds like **7**and **9** having significant antibacterial activity are also found to have good docking scores as shown in **Table 3**.The acting force of this binding mode is mainly depends on hydrogen bonding, electrostatic forces, van-der Waals forces and hydrophobic interaction due to non-polar residue interaction and water structure effect alteration. Docked ligand molecule **4** with the secondary structure of*TREX1 in complex with a nucleotide* of *S. typhi*in solid and ribbon model is depicted in **Figure 1**. The surface cavity with target molecule **4** at the active pocket of the protein structure is depicted in **Figure 2**. 2D plot of hydrogen bond forming amino acids with target ligand, HB plot of interacted residues in protein and molecular interactions of *S. typhi* with compound **4**is depicted in **Figure 3, 4&5** respectively.

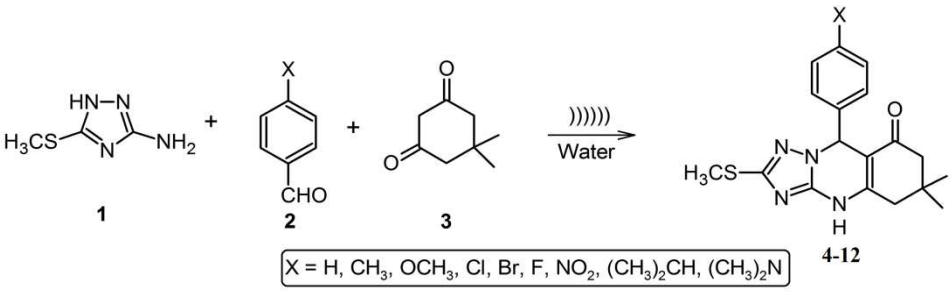
Table 3 Molecular docking results of the target molecules with*TREX1 in complexwith a nucleotide*from *Salmonella typhi* (PDB ID:1B6O)

Compound	Binding Energy (kcal/mol)	Docking Energy (kcal/mol)	Inhibition Constant (µM)	Intermolec. Energy (kcal/mol)
4	-7.98	-7.46	1.42	-9.24
5	-8.06	-6.99	1.23	-9.07
6	-7.36	-6.73	4.00	-8.84
7	-7.85	-7.02	1.77	-9.00
8	-7.94	-7.46	1.52	-9.48
9	-7.96	-7.16	1.45	-9.12
10	-7.92	-7.42	1.56	-9.48
11	-7.71	-7.20	1.60	-9. 02
12	-6.97	-6.86	1.98	-8.67

The *in vitro* antifungal MIC values are correlated well with binding energies obtained throughmoleculardocking with *DihydrofolateReductase*(PDB:**1AI9**) of *C.albicans*[www.rcsb.org(DOI: 10.2210/pdb1ai9/pdb)]. Docked ligand molecule **10** with the secondary protein structure of *DihydrofolateReductase*in solid and ribbon model is depicted in **Figure 6**. The minimum fungal inhibition potency against*C. albicans* of compounds **7**, **9** and **10** showed excellent docking energies. Their bindingenergies are -8.00, -7.43 and -7.66 kcal/mol respectively[**Table 4]**. From the comparative analysis, the above compounds**7**, **9** and **10** shows good *in vitro*antifungal activity which is further supported by their *in silico*analysis. The above mentioned compounds utilize their amino head group to interact with the crucial amino acid residues such as Thr 147through hydrogen bonds.The surface cavity with target molecule **10** at the active pocket of the protein structure is depicted in **Figure 7**. 2D plot of hydrogen bond forming amino acids with target ligand, HB plot of interacted residues in protein and molecular interactions of *C. albicans* with compound **10**is depicted in **Figure 8, 9 & 10** respectively.Therefore, it is pleasing to state that the docking studies have widened the scope of developing a new class of antimicrobial agents.

Table 4 Molecular docking results of the target molecules with *DihydrofolateReductase*from *Canidaalbicans* (PDB ID: 1AI9)

Compound	Binding Energy (kcal/mol)	Docking Energy (kcal/mol)	Inhibition Constant (µM)	Intermolec. Energy (kcal/mol)
4	-7.53	-8.08	3.01	-8.14
5	-7.57	-7.87	2.84	-8.07
6	-7.26	-7.81	4.78	-8.05
7	-8.00	-8.50	1.36	-8.63
8	-7.56	-7.92	2.88	-8.16
9	-7.43	-7.78	3.60	-8. 02
10	-7.60	-8.31	2.44	-8.47
11	-7.48	-8.18	3.29	-8.38
12	-7. 18	-8.29	4.25	-8.29



Scheme 1

Scheme 1.Scheme for the synthesis of triazoloquinazolinone derivatives (4-12)

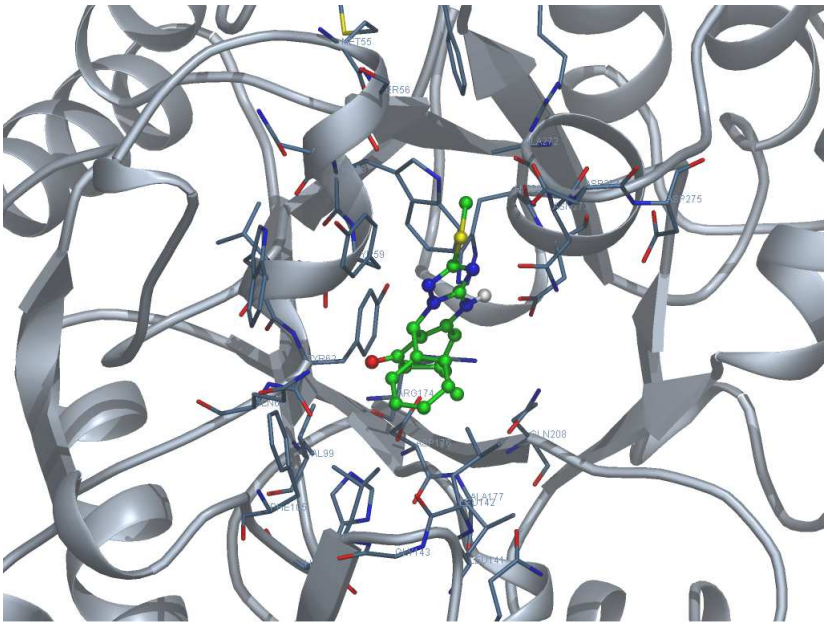


Figure 1

Figure 1.Docked ligand molecule 4 with the secondary structure of *alpha-amylase* with*TREX1 in complex with a nucleotide*from *Salmonella typhi* (PDB ID:1B6O) in solid and ribbon model

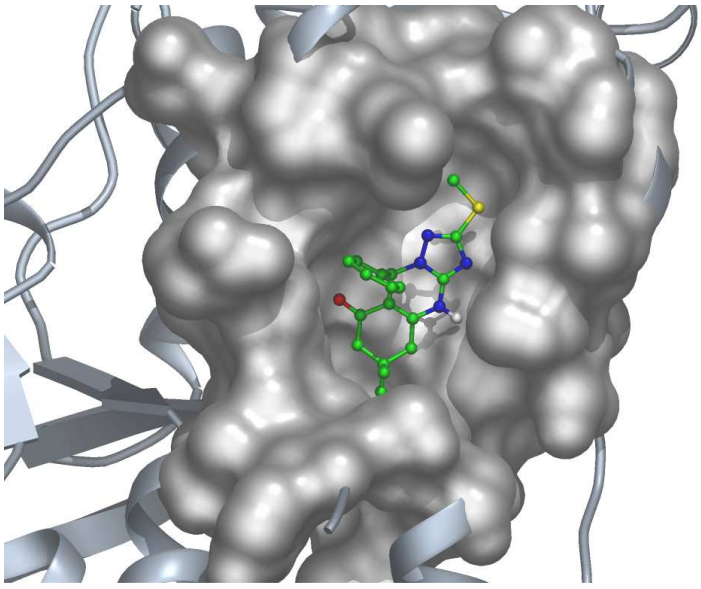


Figure 2

Figure 2.The surface cavity with target molecule 4 at the active pocket of the protein

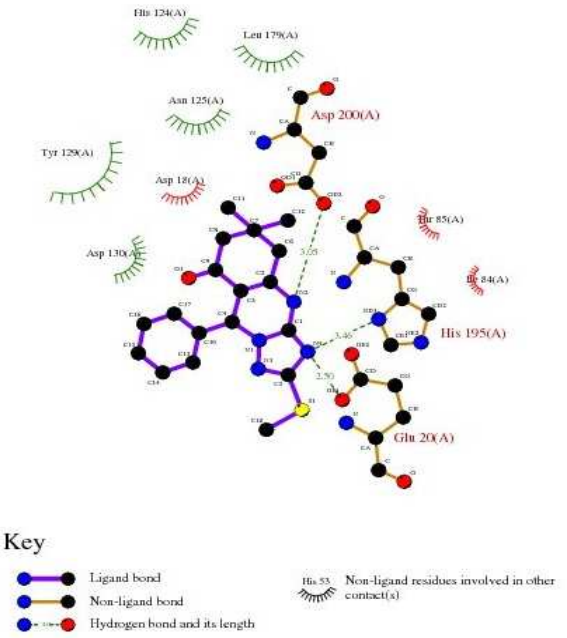


Figure 3

Figure 3. 2D plot of hydrogen bond forming amino acids with target ligand for compound 4

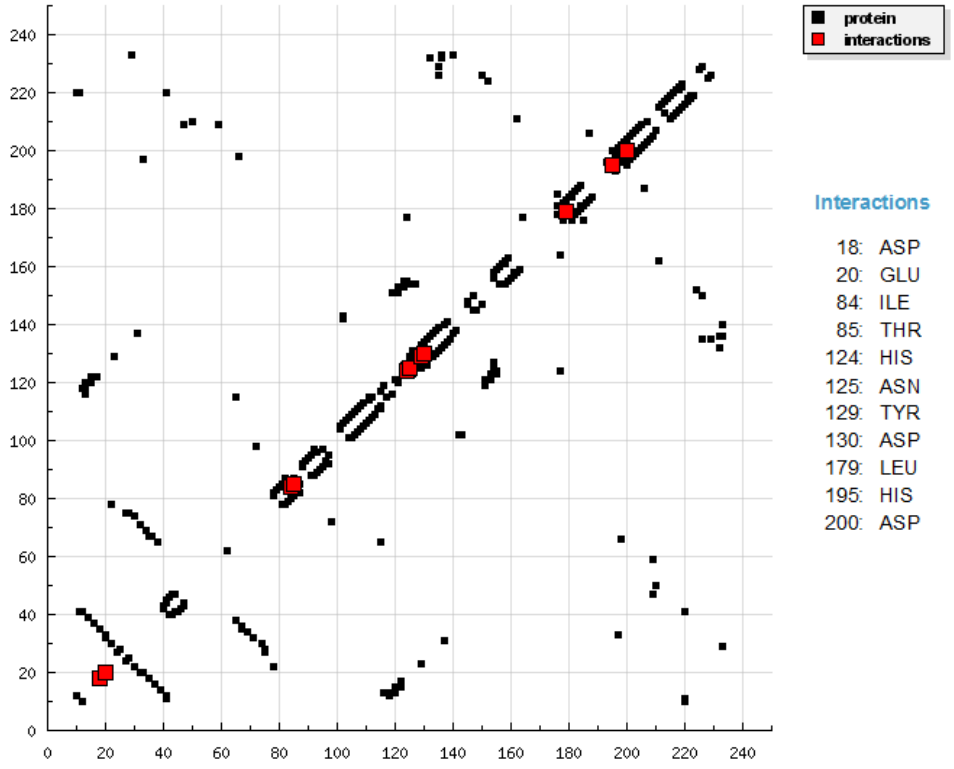


Figure 4

Figure 4.HB plot of interacted residues in protein with compound 4

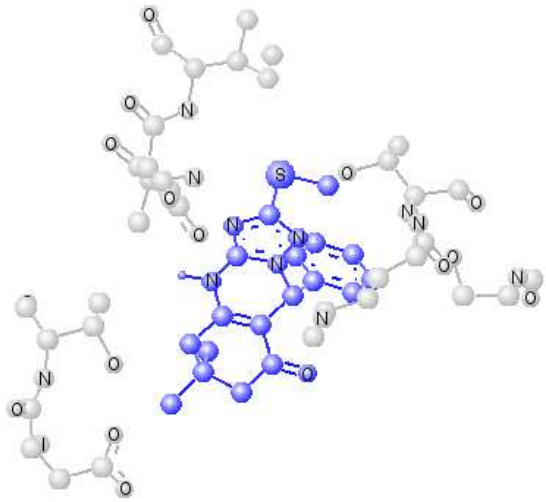


Figure 5

Figure 5. Molecular interactions in protein with compound 4

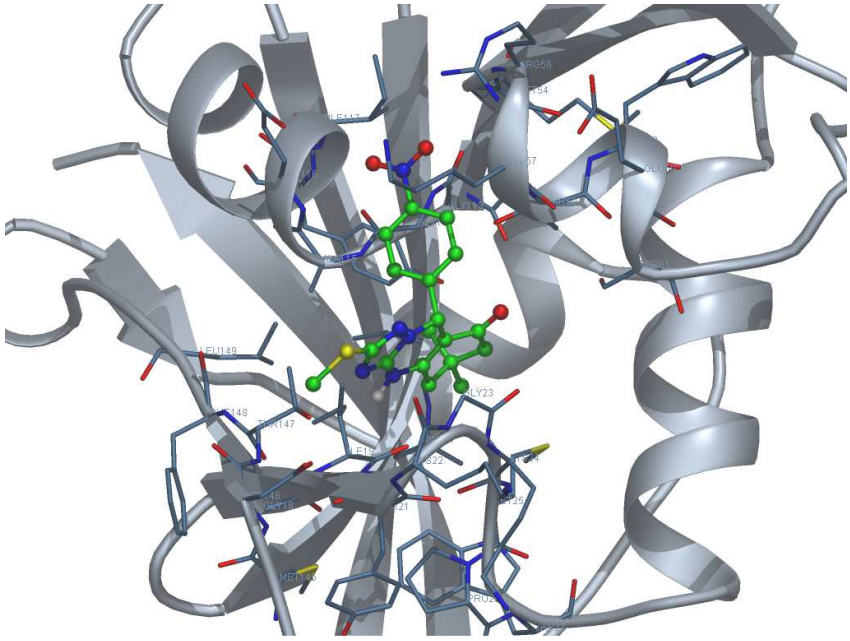


Figure 6

Figure 6.Docked ligand molecule 10 with the secondary structure of *DihydrofolateReductase*from *Canida albicans* (PDB ID: 1AI9) in solid and ribbon model

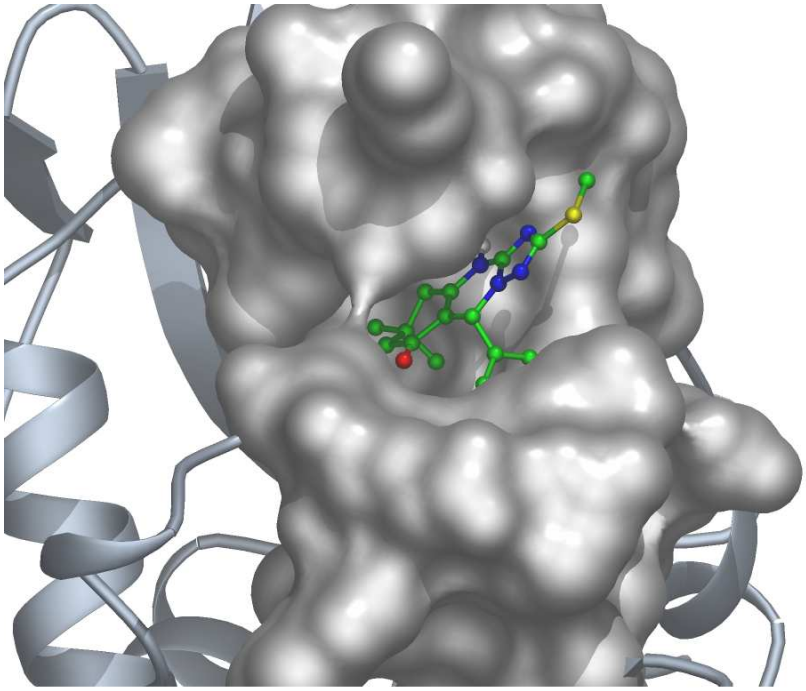


Figure 7

Figure 7.The surface cavity with target molecule 10 at the active pocket of the protein

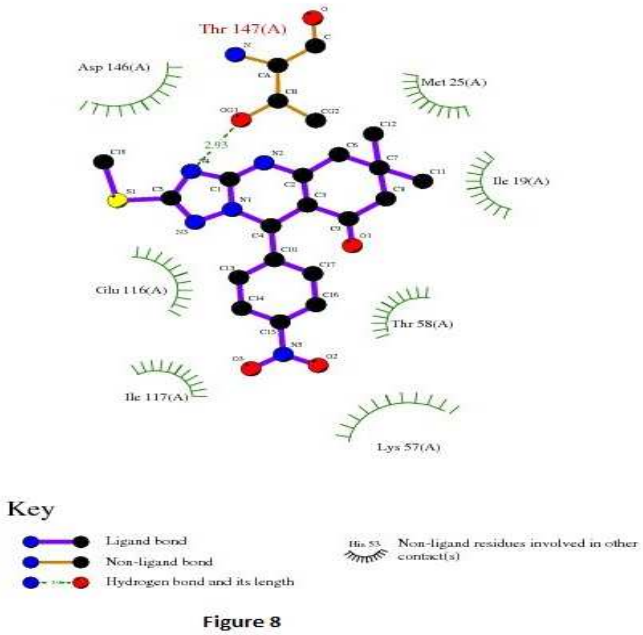


Figure 8

Figure 8. 2D plot of hydrogen bond forming amino acids with target ligand for compound 10

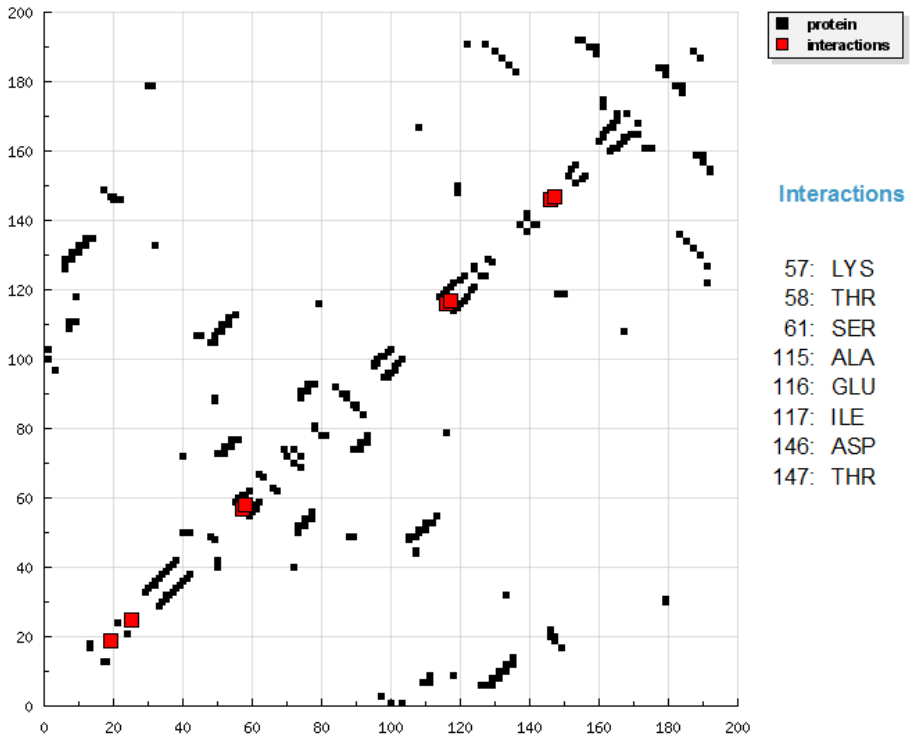


Figure 9

Figure 9.HB plot of interacted residues in protein with compound 10

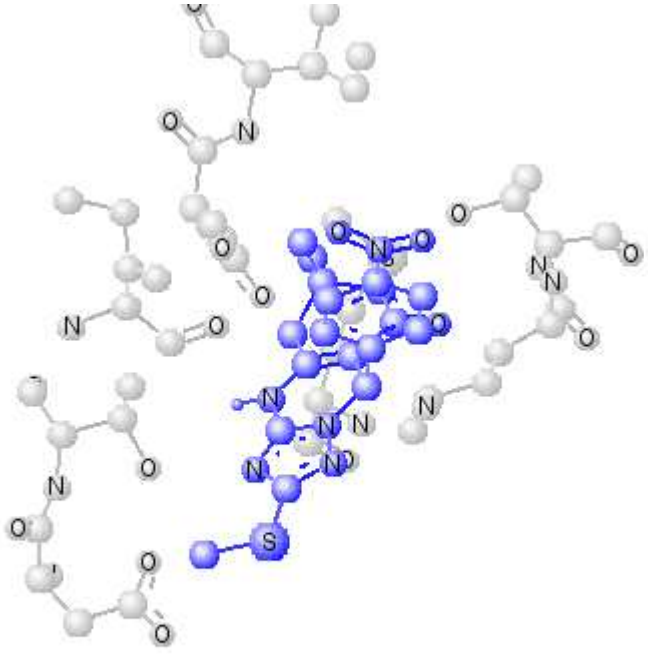


Figure 10

Figure 10.Molecular interactions in protein with compound 10

CONCLUSION

The antimicrobial activity results indicated that some of the tested compounds showed the most promising antibacterial and antifungal activities. These observations may promote a further development of our research in this field. Further development of this group of compounds may lead to compounds with better pharmacological profile than standard drugs and serve as templates for the construction of better drugs to combat bacterial and fungal infection. After studying the docking poses and binding modes of the docked compounds, the necessity of hydrogen bond formation for enhancing the activity of this class of compounds can be highly advocated.

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REFERENCES

[1] C. Foucault,P. Brouqui, *FEMS Immunology and MedicalMicrobiology*., **2007,** 49, 173.
[2] H.C. Neu, *Science*.,**1992** . 257, 1064.
[3] R. Wise, T. Hart, O. Cars et al.*BritishMedical Journal*., **1998**, 317, 609.
[4] S. Jantova, S. Stankovsky, K.Spirkova, *Biologia, Bratislava*., 59, **2004,**741.
[5] B.Maggio, G. Daidone, D. Raffa, S. Plescia, L. Mantione, V. M. C. Cutuli, N. G. Mangano, A.Caruso, (2001).*Eur. J. Med. Chem.*,**2001,**36**,** 737.
[6] G.Grover, S.G. Kini, *Eur. J. Med. Chem.*,**2006**, *41*, 256.
[7] S.M. Roopan, T. Maiyalagan, F.N. Khan, *Canadian Journal of Chemistry.*,**2008,** 86, 1019.
[8] P. Mani Chandrika, T. Yakaiah, A. Raghu Ram Rao,B. Narsaiah, N. Chakra Reddy,V. Sridhar,J. VenkateshwaraRao, *Eur. J. Med. Chem.*,**2008,**43, 846.
[9]V. Alagarsamy, U.S. Pathak,*Bioorg& Med. Chem.*,**2005**, 15**,** 1877.
[10] M.H. Dhar,M. M. Dhar,B.N. Dhawan,B.N. Mehrotra,C. Ray, Part I. *Indian J ExpBiol*, 6, 32–47.
[11] Z. Bikadi, E. Hazai, *J. Cheminf*., **2009,**1, 15.
[12] T.A. Halgren,*Journal of Computational Chemistry*.,**1998**, 17, 490.
[13] G.M. Morris, D.S Goodsell*et al*.,*Journal of Computational Chemistry*,**1998**, 19, 1639.
[14] F.J. Solis, R.J.B. Wets, *Mathematics of Operations Research*.,**1981,**6, 19.