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MOLECULAR DOCKING STUDIES OF SOME NOVEL PYRROLYL PYRAZOLE DERIVATIVES

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ABSTRACT

The antibacterial target, enoyl-acyl carrier protein (ACP) reductase, is a homotetrameric enzyme that catalyzes the last reductive step of fatty acid biosynthesis. In the present paper, Surflex docking has been carried out on a series (10 compounds) of enoyl ACP reductase inhibitors, using the SYBYL-X 2.0 package (Tripos Inc., St. Louis, USA). Surflex-docking studies revealed that the carbonyl group and pyrazole ring were significant for binding to the receptor, and it is also found that the pattern of binding of tested compounds is same as that of the 4TZK ligand, this in turn helped in understanding of specific activity of compounds.

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INTRODUCTION

Tuberculosis (TB), a contagious disease transmitted through the air, and caused by the bacterium *Mycobacterium tuberculosis*, is an important world-wide public health problem, which was declared a global health emergency in 1993 by the World Health Organization (WHO). According to statistics, one-third of the world's population is currently infected with the TB bacillus, each year, 8 million people world-wide develop active TB and about 1.7 million people die. *M. tuberculosis*, is an infection caused by slow-growing bacteria in parts of the body having high level of blood and oxygen is often found in lungs, called pulmonary TB. The disease also spreads to other parts of the body, called as extra-pulmonary TB that may be latent or active. In other case, treatment of the active TB is more complex due to multi-drug resistance (MDR-TB), extensive-drug resistance (XRD-TB) and HIV infection. Approximately about 50% of India's population is reported to be tuberculin test positive. Every year about 0.4 million deaths and one million new cases of TB are reported. This led to the declaration of TB as a global emergency by WHO in 1993. The regeneration of TB is closely linked to the emergence of HIV and total deficiency of the immune system [1].The situation has become more critical because of the presence of some complicating factors like, emergence of MDR-TB [2], HIV co-infection [3], lack of patient compliance with chemotherapy, and variable efficacy of Bacilli-Calmette Guerin (BCG) vaccine. Multi drug resistant tuberculosis is defined as disease due to *M. tuberculosis* that is resistant to Isoniazid and Rifampicin with or without resistance to other drugs. In the years to come TB is bound to be an important health problem, particularly in immuno-compromised host.Mycolic acid biosynthesis has been carried out [4] by numerous successive enzymatic cycles equivalent to Fatty Acid Synthase (FAS) systems viz., FAS I and II. Mycolic acid is a unique signature fatty acid, which is a core constituent of the mycobacterial cell wall present in fatty acid synthase system of *M. tuberculosis.* InhA, the enoyl acyl carrier protein reductase (ENR) from *M. tuberculosis* is the key enzyme for type II fatty acid synthesis (FAS II), which catalyses NADH-dependent reduction of 2-trans-enoyl-ACP (acyl carrier protein) to yield NAD+ and reduced enoyl thioester-ACP substrate, which in turn, helps the synthesis of mycolic acid.

Recently, pyrrole derivatives have emerged as chemotherapeutic agents potentially useful for inhibiting the activities of *M. tuberculosis* and other atypical mycobacteria, including *M. avium* complex, an opportunistic pathogen that greatly contributes to the death of AIDS patients, and it is also the most important simple heterocycles that is found in a broad range of natural products and drugs that are of growing relevance in materials science. Porretta and co-workers [5] have reported the antimycobacterial activity of pyrrole derivative, and Di santo et al.[6] more recently ascribed appreciable inhibiting action to pyrrolnitrin and some related nitropyrroles and also pyrrole has a broad spectrum of activity like, antitubercular [7,8], antibacterial, anti-inflammatory [9], antiviral [10], antifungal [11]. Previously we have described *in silico* studies on pyrrole derivatives as antitubercular agents [12-14]. In continuation of the work on pyrroles herein we report molecular docking studies of pyrrole incorporated prazole deivatives [15], as possible enoyl-ACP reductase inhibitors.

Experimental

Molecular docking

Molecular modeling was carried out using Sybyl-X, version 2.0 [16], running on a Intel® Core TM i3-2130 CPU@ 3.40GHz processor using Windows 7 professional workstation. Surflex-Dock algorithm of sybyl was used to dock designed compounds. The crystal structure of *Mycobacterium tuberculosis* enoyl reductase (InhA) complexed with 1-cyclohexyl-*N*-(3,5-dichlorophenyl)-5-oxopyrrolidine-3-carboxamide were downloaded from the Protein Data Bank (PDB entry code 4TZK, PDB extracted from the Brookhaven Protein Database http://www.rcsb.org/pdb) and used for initial docking studies. Co-crystalized ligand and water molecules were removed from the structure, H-atoms were added and side chains were fixed during protein preparation. The structure was then subjected to an energy refinement procedure. Gasteigere-Huckel charges [17] were calculated for the ligand, while Amber 7FF02 were used for the protein. The model was then subjected to energy minimization following the gradient termination of the Powell method for 3000 iterations using Tripose force field with non-bonding cutoff set at 9.0 and the dielectric constant set at 4.0. The binding of the pyrrolyl derivatives was also estimated using a variety of scoring functions that have been compiled into the single consensus score (CScore). The CScore module (Total Score) available in Sybyl includes the G_Score, PMF_Score, D_Score and ChemScore scoring functions.

RESULTS AND DISSCUSION

Molecular Docking studies

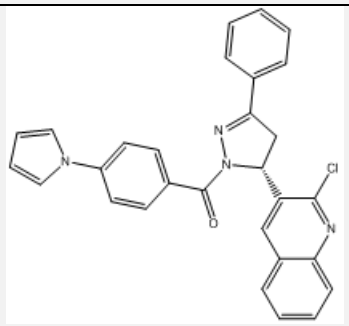
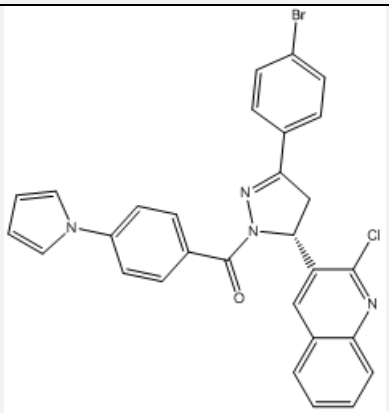
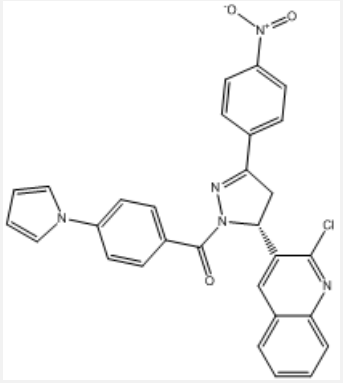
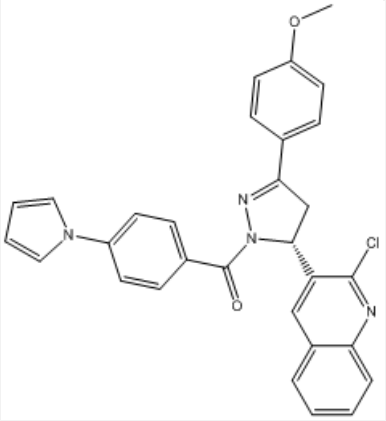
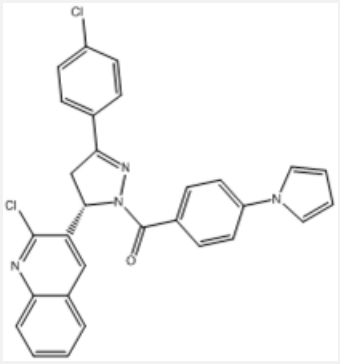
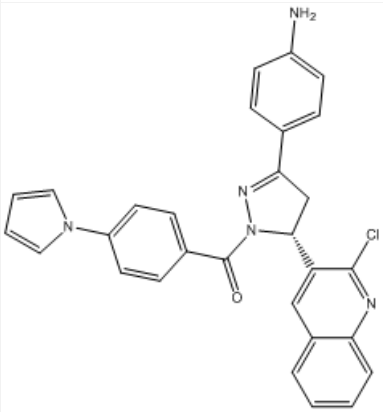
To investigate the detailed intermolecular interactions between the ligand and the target protein, a program Surflex-Dock was used. Three-dimensional structure information on the target protein was taken from the PDB entry 4TZK. Processing of the protein included the deletion of the ligand and the solvent molecules as well as the addition of hydrogen atoms. All the 10 inhibitors were docked into the active site of ENR as shown in figures **1A** and **1B**. The predicted binding energies of the compounds are listed in Table 2. As depicted in the figures **2A** and **2B** compound **5g** makes six hydrogen bonding interactions at the active site of the enzyme (PDB ID: 4TZK), among those two interactions were of oxygen atoms of nitro group with hydrogen atoms of TYR158 and NAD500 (O----H-TYR158, 1.95 Å; 1.99 Å; O----H-NAD500, 2.12 Å), nitrogen atom of nitro group makes interactions with hydrogen atoms of TYR158 and NAD500 (-N------H-NAD500, 2.06 Å; O----H-TYR158 2.29 Å) and oxygen atom of carbonyl group makes an interaction with the hydrogen atom MET98 (O----H-MET98, 2.15 Å). As depicted in fig. **3(A-B)**, compound **5c** makes three hydrogen bonding interactions at the active site of the enzyme (PDB ID: 4TZK), among those an interaction was of nitrogen atom present at 2nd position of pyrazole ring with hydrogen atom of NAD500 (-N------H-NAD500, 2.12 Å), oxygen atom of carbonyl group makes an interaction with the hydrogen atom NAD500 (O----H- NAD500, 2.13 Å) and remaining another hydrogen bonding interaction raised from the oxygen atom of nitro group with hydrogen atom of NAD500 (O----H- NAD500, 2.25 Å).

The binding interaction of 4TZK_ligand with enzyme active sites shows two bonding interactions and the docked view of the same has been depicted in Fig. **4(A-B)**.

All the compounds showed consensus score in the range 5.45-1.43, indicating the summary of all forces of interaction between ligands and the InhA. Also we saw that the studied compounds have showed same type of interactions with amino acid residues (TYR158 and NAD500) as that of reference 4TZK_ligand. These scores indicate that molecules preferentially bind to InhA in comparison to the reference 4TZK ligand (Table 2).

The Lipinski's 'rule of 5' was calculated for the compounds **1-10**. The poor absorption or permeation is most likely when, there are more than 5 H bond donors, the molecular weight is above 500, the cLogP is above 5 and there are more than 10 H-bond acceptors [18]. We have calculated theoretical cLogP, molecular weight (MW) and number of hydrogen bond donors and acceptors using sybyl-X.2.0. Observing the results in Table 3 it can be said that compounds **1-10** satisfied the physicochemical parameters range established by the Lipinski's rule.

Table 1: Structures and inhibitory activity of pyyrole derivatives (1-10) used for docking study.

Comp	Structure	MIC	Comp.	Structure	MIC
5a		1.6	**5b**		6.25
5c		1.6	**5d**		12.5
5e		1.6	**5f**		1.6

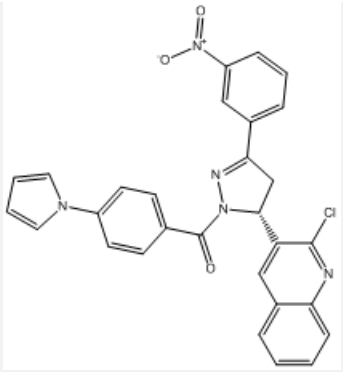
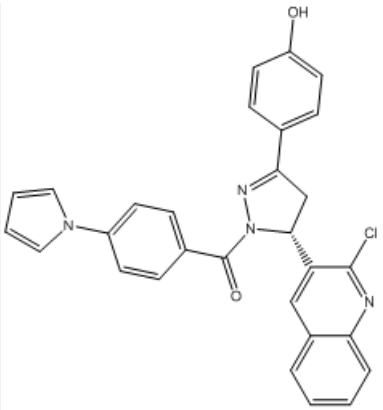
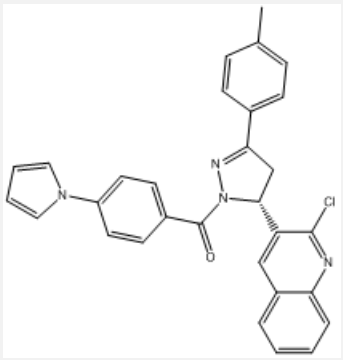
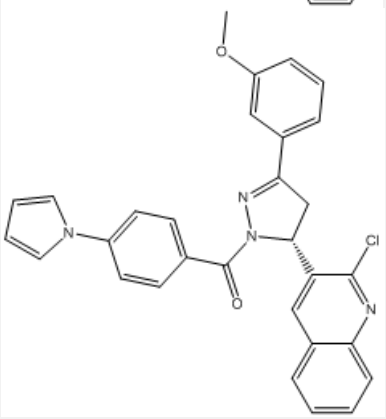
5g		1.6	**5h**		1.6
5i		1.6	**5j**		3.12

Table 2. Surflex Docking score (kcal/mol) of the pyrrole derivatives.

Compounds	C Score[a]	Crash Score[b]	Polar Score[c]	D Score[d]	PMF Score[e]	G Score[f]	Chem Score[g]
Ligand	7.19	-2.06	1.36	-159.93	-54.08	-285.50	-39.55
5j	5.45	-1.72	0.97	-138.791	-51.148	-265.661	-40.301
5d	5.07	-1.55	0.00	-131.923	-50.907	-246.460	-34.646
5a	4.55	-3.44	0.01	-139.072	-30.224	-267.526	-40.663
5h	4.41	-2.45	0.42	-140.749	-55.945	-279.925	-40.349
5g	4.14	-2.03	2.98	-117.042	-38.537	-225.442	-36.647
5b	3.86	-3.95	0.96	-155.614	-49.850	-317.846	-41.255
5e	2.96	-2.81	0.01	-134.730	-54.075	-280.774	-40.142
5c	2.93	-3.89	1.49	-128.975	-68.636	-288.032	-41.354
5i	2.34	-7.79	0.00	-203.385	-24.165	-358.373	-53.782
5f	1.43	-2.16	0.02	-131.424	-61.405	-253.842	-37.484

[a] CScore (Consensus Score) integrates a number of popular scoring functions for ranking the affinity of ligands bound to the active site of a receptor and reports the output of total score.
[b] Crash-score revealing the inappropriate penetration into the binding site. Crash scores close to 0 are favorable. Negative numbers indicate penetration.
[c] Polar indicating the contribution of the polar interactions to the total score. The polar score may be useful for excluding docking results that make no hydrogen bonds.
[d] D-score for charge and van der Waals interactions between the protein and the ligand.
[e] PMF-score indicating the Helmholtz free energies of interactions for protein-ligand atom pairs (Potential of Mean Force, PMF).
[f] G-score showing hydrogen bonding, complex (ligand-protein), and internal (ligand-ligand) energies.
[g] Chem-score points for H-bonding, lipophilic contact, and rotational entropy, along with an intercept term.

Table 3. QSAR parameters: cLogP, molecular weight (MW), number of H bond donors and acceptors value for compounds 1-10.

Compounds	cLOG P	Donor	Acceptor	MW
5a	6.43	0	5	476.95
5b	7.29	0	5	555.85
5c	3.14	0	8	521.95
5d	6.35	0	6	506.98
5e	7.14	0	5	511.40
5f	5.20	1	6	491.97
5g	3.14	0	8	521.95
5h	5.76	1	6	492.95
5i	6.93	0	5	490.98
5j	6.35	0	6	506.98

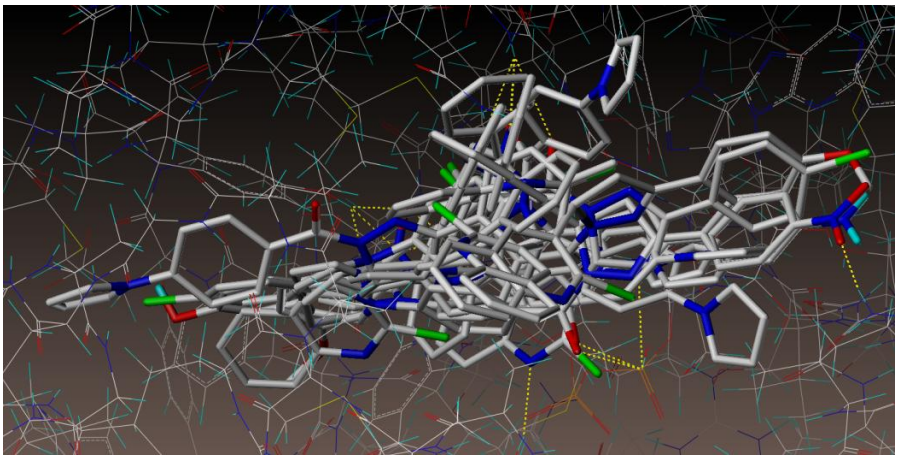


Figure 1. All 10 compounds aligned in the docking site of the enzyme (PDB ID: 4TZK).

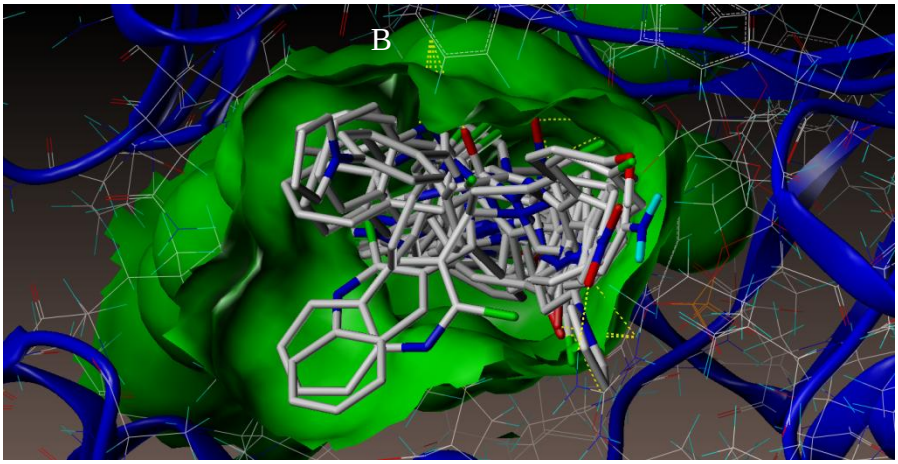


Figure 2. Interaction of ligand at the binding site of the enzyme (PDB ID: 4TZK).

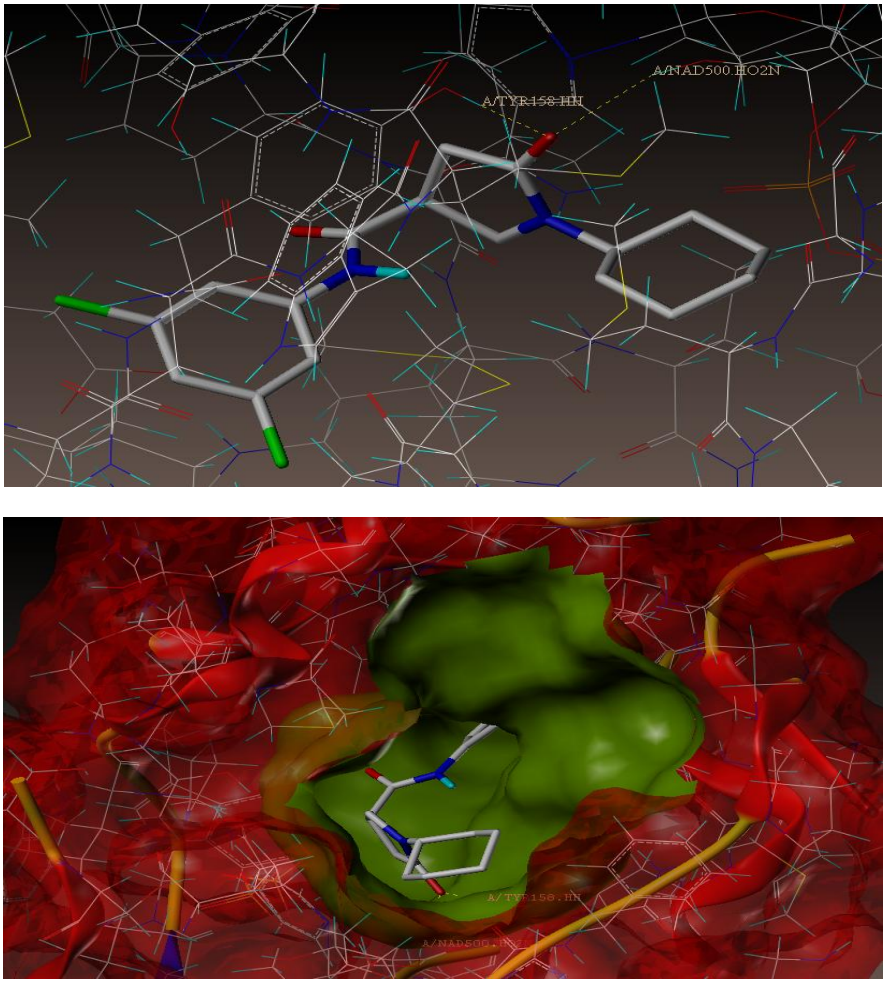


Figure 3. Interaction of compound 5g at the binding site of the enzyme (PDB ID: 4TZK).

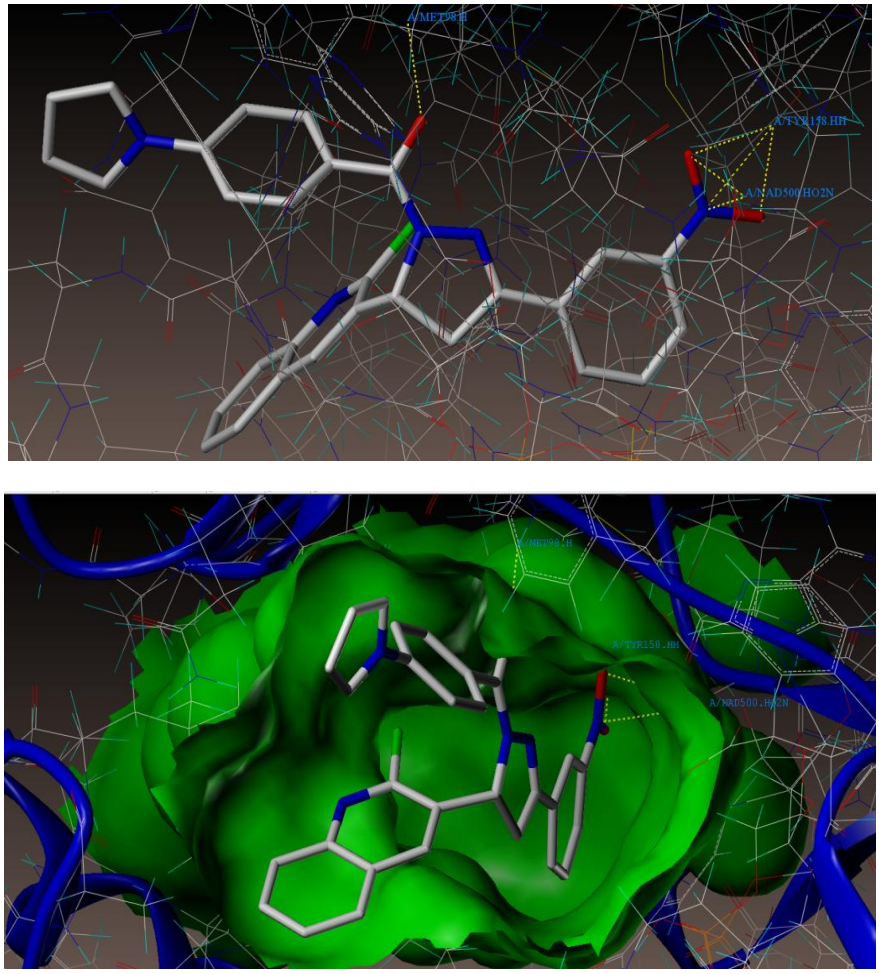
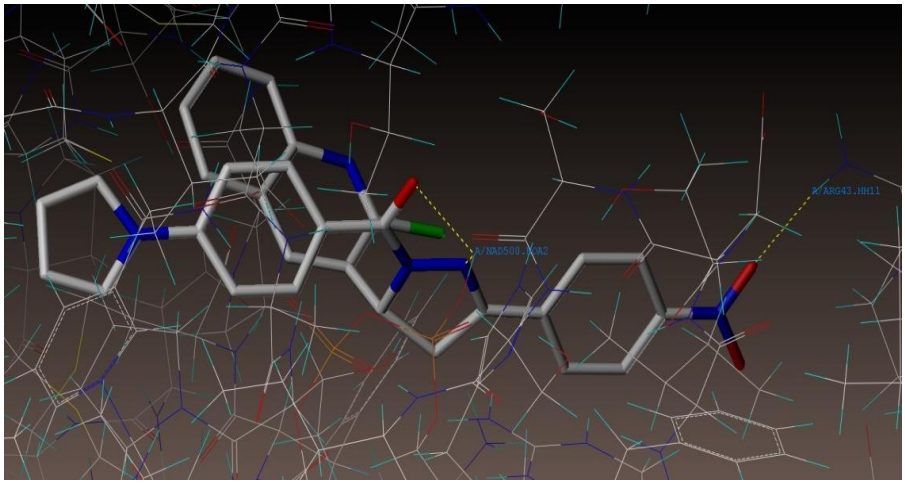
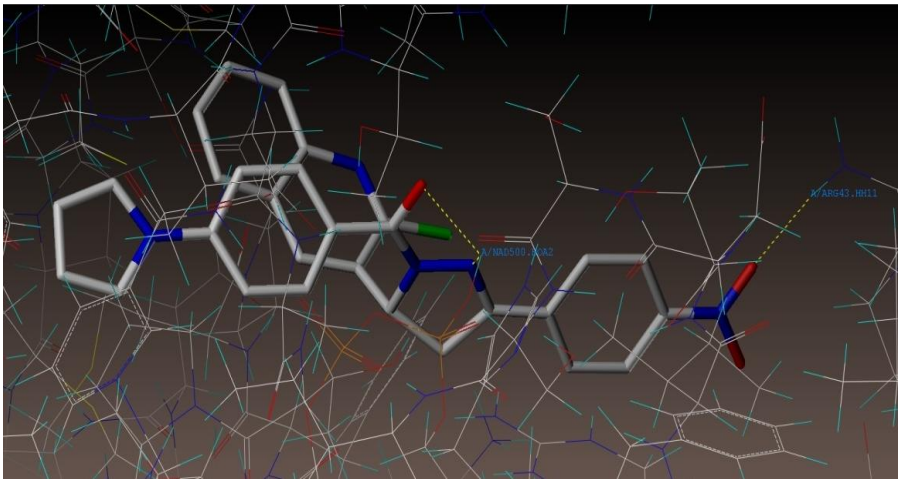


Figure 4. Interaction of compound 5c at the binding site of the enzyme (PDB ID: 4TZK).





CONCLUSION

Molecular docking study was carried out on a set of 10 pyrrolyl pyrazole derivatives and showed better intermolecular interaction with the target protein. All the compounds studied has showed better hydrogen bonding interaction with the amino acid at the active site of the enzyme used and the other studied parameters like, ligand-ligand, and Van-der walls interaction, Helmholtz free energy, also showed that the studied compounds fit better within the enzyme cavity. Most of the molecules could effectively bind to the substrate binding site of ENR. The key H-bonding interactions with Tyr158, and cofactor NAD+ , as well as hydrophobic amino acid residues, stabilized the ligand-receptor complex to conclude that molecules are efficiently bound at the active site of ENR and, hence, can be better ENR inhibitors. All the compounds also satisfied the physicochemical parameters range established by the Lipinski's rule.

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