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ARTICLE

Designing of Nitroimidazole Derivatives as a Promising Target for Treatment of Tuberculosis

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ABSTRACT

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis,* with high level of mortality worldwide, currently with approximately 10 million cases of tuberculosis. These rate of incidence are due to several factors such as bacterial resistance, AIDS, latent tuberculosis that reoccur in patient. Deazaflavin dependent nitroreductase (Ddn) is an emerging target in the field of antitubercular agent. Ddn catalyses the reduction of nitroimidazoles resulting in intra-cellular release of lethal reactive nitrogen species. Nitroimidazole class drug- delamanid and pretonamid are used in the treatment of MDR-TB. In this present study, 26 new nitroimidazole derivatives were designed and docked into Ddn enzyme. In docking study, compounds **3**, **5**, **15**, **16**, **17**, **18** and **21** showed similar interaction with amino acid residues such as Tyr 65, Ser 78, Tyr 136 as pretonamid reference drug and highest docking score and better ADMET compatibility. The ADMET prediction docking study of new designed compound revealed that the compounds **3**, **16**, **17** and **21** showed good binding with Ddn. In future it may be good and effective lead for development of antitubercular agent.

KEYWORDS

Tuberculosis, Deazaflavin dependent nitroreductase, Nitroimidazole, Docking studies, ADMET profile.

INTRODUCTION

Tuberculosis is contagious disease which is transmitted through air and caused by *Mycobacterium tuberculosis*. However, it most commonly affect the lungs (pulmonary tuberculosis) but it can also infect any other parts of body (extra pulmonary tuberculosis) [1]. If it is not treated it can destroy the body tissue by chronic inflammation and may culminate in death. According to WHO tuberculosis report 10 million incident cases of tuberculosis of which 1.6 million died from the disease (including 0.3 million people with HIV) and 5.58 lacs new cases with resistance to rifampicin – the most effective first-line drug – of which 82 % had MDR-TB [2]. So, there is need for the development of drug with newer mechanism of action against mycobacteria. Deazaflavin-dependent nitroreductase (Ddn) is newer and effective target for the treatment of tuberculosis. Ddn catalyzes the reduction of nitroimidazoles resulting in intracellular release of lethal reactive nitrogen species. The N- terminal 30 residues of Ddn are important functionally.

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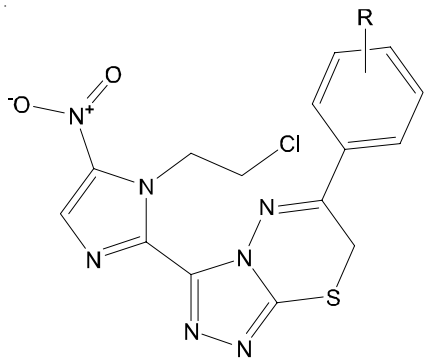
They are flexible or access multiple conformations, preventing structural characterization of full length, enzymatically active enzymes.

Deazaflavin dependent nitroreductase (Ddn) converts prodrug that require for metabolic activation by deazaflavin (cofactor F420)-dependent nitroreductase into three primary metabolites, a des-nitroimidazole and two unstable by-product. Des-nitro metabolite formation generates reactive nitrogen species include nitric oxide (NO) which are effectors of anaerobic mycobacterial activity. Deazaflavin dependent nitroreductase is likely a membrane-bound protein which involves in protective mechanism under oxidative stress. Deazaflavin F420 is structurally analogous to riboflavin based cofactors such as flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Functionally they are closer to nicotinamide (NAD) cofactors which is involved in redox reaction as a hydride carrier involved in two electron transfer mechanisms. F420 exists in both oxidized (F420) and reduced (F420 H2) forms [3].

Nitroimidazoles are imidazole heterocyclic compound with a nitro group. Nitroimidazole can be classified according to the position of nitro functional group in the ring such as 2-nitroimidazoles, 4-nitroimidazoles, 5-nitroimidazoles. Nitroimidazoles belong to nitro-heterocyclic compound group having broad spectrum activity against parasites, mycobacteria, Gram-positive and Gram-negative bacteria. Nitro-imidazoles are the prodrug which require bioactivation of nitro group in order to produce antimycobacterial effect.

Nitroimidazole compounds have wide variety of application that is ranging from food preservative to antibiotics [4]. The antimicrobial activity of these chemotherapeutic agents inhibits the growth of both anaerobic bacteria and certain anaerobic protozoa such as *Trichomonas vaginalis*, *Entamoeba histolytica* and *Giardia lamblia* [5], antitubercular [6,7]. 2-Nitroimidazoles play a major role as bioreductive markers for tumour hypoxia, as radiosensitizers [8,9] and some also demonstrate antiprotozoan activity [10]. Some dinitro and mono nitroimidazole derivatives have been predicted as notable radiosensitizers, antiepileptic agents [11,12].



Structure of nitroimidazole compounds

EXPERIMENTAL

Protocol for docking study: The compounds were studied for their binding activities to 3R5R receptor and sketched in Chem Draw ultra 8.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2003)]. These structures were converted to 3D structures using Chem3D ultra 8.0 software and the constructed 3D structures were energetically minimized by energy minimization technique Allinger's Molecular Mechanics (MM2) force fields followed by geometry optimization using semi empirical Quantum mechanics based on AM-1 (Austin Model-1). The X-ray structure of deazaflavin-dependent nitroreductase with co-factor F420 from *Mycobacterium tuberculosis* with pretonamid (PA-824) with resolution of 2.1 Å reported [3] was retrieved from the protein Data Bank (Entry code 3R5R) and used as target for modeling studies. Since the position of most water molecules in the crystal structure complex are unlikely to be conserved, water molecules were excluded before the docking protocols. In the receptor, binding site was defined by amino acid included into 2.1 Å. The mode of interaction of substituted nitroimidazole derivatives to 3R5R(PDB ID) was used as standard docked model. Exported receptor which was prepared in last step into iGEMDOCK version 2.1 docking software. Selected prepare binding site. Set the population size 200 with 70 generation and two number of solutions. Then selected ligand in mol file from where saved and select apply and dock, so docking process was started. In the result of docking, there is total binding energy. Save the interaction analysis and interaction profile and exported in excel.

Ramachandran plot: Two torsion angles in the polypeptide chain is also known as Ramachandran angles. A special way for plotting protein torsion angles was also introduced by Ramachandran *et al.* [13] and was subsequently named the Ramachandran plot. This Ramachandran plot provides an easy way to view the distribution of torsion angles in a protein structure. It also provides an overview of excluded regions that show which rotations of the polypeptide are not allowed due to steric hindrance (collisions between atoms). The Ramachandran plot of a particular protein may also serve as an important indicator of the quality of its three-dimensional structures. A Ramachandran plot is also can be used in two somewhat different ways. First way is the top right site in show the theory which values, or conformations, of the ψ and φ angles are possible for an amino-acid residue in a protein. A second way is to show the empirical distribution of datapoints observed in a single structure at the plot in right, here in usage for structure validation, or else in a database of many structures as in the lower 3 plots at left. Either case is usually shown against outlines for the theoretically favoured regions [13].

On the basis of the Ramachandra plot of 3R5R PDB file (Fig. 1) show the theory which values or conformations, of the ψ and φ angles are possible for an amino-acid residue in a protein.

Physico-chemical parameters: As an effective drug, a potent molecule must reach to its target in the body in enough concentration, and stay there in a bioactive form long enough to produce biological activity. Drug development involves assessment of absorption, distribution, metabolism and excretion (ADME) increasing earlier in the discovery process, at a stage when considered compounds are numerous.

Swiss ADME web tool gives free access, to a pool of fast and robust predictive models for physicochemical properties,

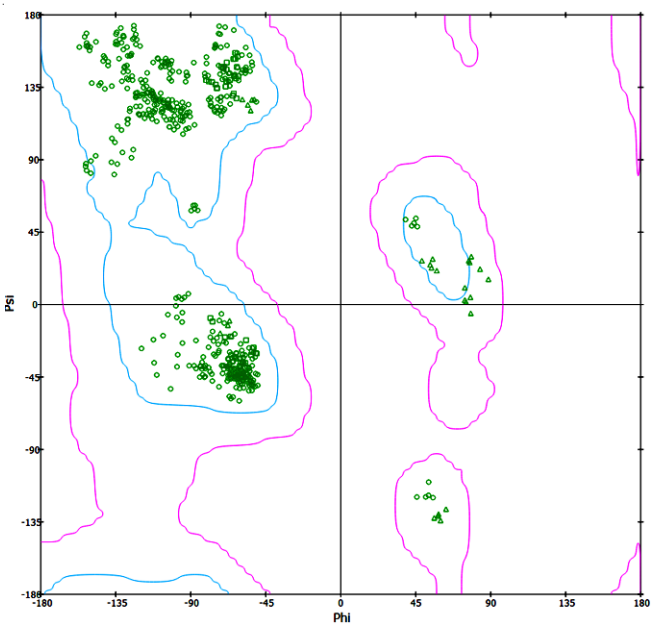


Fig. 1. Ramachandran plot of PDB:3R5R

pharmacokinetics, drug-likeness and medicinal chemistry friendliness, among which in-house proficient methods such as the BOILEDEgg, iLOGP and Bioavailability Radar [14].

Lipinski rule of five: The first analysis of the impact of physico-chemical parameters was performed by Christopher Lipinski at Pfizer in the late 1990s. The rule of five helped to raise awareness about properties and structural features that make more or less drug-like. This Lipinski rule describes molecular properties which is important for drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism and excretion (ADME). This Lipinski rule is important for drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity as well as drug-like properties as described by Lipinski's rule.

RESULTS AND DISCUSSION

Calculated physico-chemical parameters: Calculated physico-chemical parameters are shown in Table-1.

Interaction of molecules with amino acid present in 3R5R receptor: Structure based docking study helps us to know amino acid interaction with protein essential for biological activity. We docked proposed molecules with PDB ID: 3R5R. From this interaction analysis the docking score, amino acid involved in hydrogen bonding and amino acid involved in van der waals interaction are mentioned in Table-2. Interaction profile are shown in Table-3. Docking images of best scored compound with 3R5R are shown in Figs. 2-6.

Fig. 2 shows proposed binding pose of compound **3** to deazaflavin dependent nitroreductase before molecular dynamics carbon atom of compound **3** in green colour, carbon atom of Ddn residue in blue colour and other atoms indicated as follow are nitrogen in blue, oxygen in red and Fig. 3 shows proposed binding pose of compound **5** to deazaflavin dependent nitroreductase before molecular dynamics carbon atom of compound **5** in green colour, carbon atom of Ddn residue in blue colour and other atoms indicated as follow are oxygen in red, bromine in red, sulphur in tints colour.

Fig. 4 shows proposed binding pose of compound **15** to deazaflavin dependent nitroredutase before molecular dynamics carbon atom of compound **15** in green colour, carbon atom of

TABLE-1
CALCULATED PHYSIOCHEMICAL PARAMETER

Compound	R	m.w.	H-bond donor	H-bond acceptor	C log P	Lipinski alert
1	2-Cl	424.26	0	6	2.58	0
2	3-Cl	424.26	0	6	2.67	0
3	4-Cl	424.26	0	6	2.63	0
4	2,4-di-Cl	458.71	0	6	3.24	1
5	2-Br	468.72	0	6	2.78	0
6	3-Br	468.72	0	6	2.80	0
7	4-Br	468.72	0	6	2.82	0
8	2-CH_3	403.85	0	6	2.27	0
9	2,4-di-CH_3	417.87	0	6	2.57	0
10	4-CH_3	403.85	0	6	2.28	0
11	3-CF_3	457.82	0	9	3.17	0
12	4-CF_3	457.82	0	9	3.18	0
13	3-CH_3O	419.85	0	7	1.94	0
14	4-CH_3O	419.85	0	7	1.97	0
15	2-NO_2	434.12	0	8	1.34	1
16	3-NO_2	434.12	0	8	1.46	1
17	4-NO_2	434.12	0	8	1.34	1
18	3,4,5-tri-OCH_3	479.90	0	9	1.88	1
19	2-OH	405.82	1	7	1.61	0
20	3-OH	405.82	1	7	1.50	0
21	4-OH	405.82	1	7	1.53	0
22	2-NH_2	404.83	1	6	1.43	0
23	4-$N(CH_3)_2$	432.89	0	6	1.87	0
24	2-F	407.81	0	7	2.47	0
25	3-F	407.81	0	7	2.47	0
26	4-F	407.81	0	7	2.46	0
Pretonamid	–	359.26	0	9	2.06	0

TABLE-2
INTERACTION OF MOLECULE WITH AMINO ACID PRESENT IN 3R5R FILE

Compound	Total binding energy	Hydrogen bond with amino acid	van der Waals bond with amino acid
1	-120.132	GLN 125, GLN 134, GLN 137	GLN 125, GLN TRP 123, GLN 137
2	-116.070	SER 78, TRP 88, TYR 133	LEU 64, TYR 65, LYS 79, TRP 88, TYR 133
3	-122.594	SER 78, TYR 130, TYR 133, TYR 136	TYR 65, TYR 130, TYR 133, TYR 136
4	-111.492	SER 78, TRP 88, TYR 133	LEU 64, TYR 65, TRP 88, TYR 133, TYR 136
5	-121.178	TYR 65, SER 78	PRO 45, PRO 63, TYR 65, TYR 130
6	-115.756	GLN 125, GLN 134, GLN 137, SER 138	TRP 123, GLN 137
7	-117.080	GLN 125, GLN 134, GLN 137	GLN 137, THR 143
8	-120.695	TYR 65	LEU 64, TYR 65
9	-124.252	GLN 125, GLN 134, GLN 137, SER 138	ASP 69, GLN 125, GLN137, THR 143
10	-113.083	ASP 69	ASP 69, GLN 125, GLN 137
11	-117.178	SER 78, TYR 130, TYR 136	LYS 79, TYR 130, TYR 133, TYR 136
12	-112.063	TYR 65	TYR 65
13	-124.817	ASN 62	LYS 104, ARG 60, ASN 62
14	-114.454	ASN 62	ARG 60, MET 87, GLN 101
15	-127.652	TYR 65, TYR 130	PRO 63, TYR 65, TYR 130, TYR 136, VAL 46
16	-139.586	TYR 89, LYS 93, ASP 113	TYR 89, ASP 112, ASP 133,ARG 112
17	-129.509	ARG 54, TYR 89, ASP 113, ARG 119	ARG 54, TYR 89, LYS 93, ASP 113
18	-122.303	SER 78, TYR 130	PRO 63, TYR 65, TYR 130, TYR 136
19	-123.190	SER 78, TRP 88, TYR 133	LEU 64, TYR 65, LYS 79, TRP 88, TYR 133
20	-112.861	TYR 65, SER 78, TYR 130, TYR 136	TYR 65, TYR 130, TYR 133, TYR 136
21	-130.387	ARG 68, GLU 105, GLU 63, GLU 83, ASN 85, THR 140, ASP 141	GLU 105, GLU 83, LYS 84, ASP 141, ARG 142
22	-114.256	TYR 65	TYR 65, TYR130, TYR 133, PRO 45
23	-119.751	SER 78, TYR 133	LEU 64, TYR 65, LYS 79, TRP 88, TYR 133
24	-110.662	SER 78, TYR 133	LEU 64, TYR 65, LYS 79, TRP 88, TYR 130
25	-113.528	SER 78, TYR 133	LEU 64, TYR 65, LYS 79, TRP 88, TYR 133
26	-104.235	SER 78, TRP 88, TRP 133	LEU 64, TYR 65, LYS 79
Pretonamid	-96.959	TYR 65, SER 78, TYR 130, TYR 136	TYR 65, TRP 88, TRP 130, TYR 133, TYR 136

TABLE-3
INTERACTION PROFILE

Compound	Total binding energy	VDW	H-bond	Elec
1	-120.132	-95.1849	-24.9468	0
2	-116.070	-89.6156	-26.4544	0
3	-122.594	-93.9828	-28.6108	0
4	-111.492	-89.2448	-23.1168	0.869938
5	-121.178	-95.1307	-26.2841	0.236291
6	-115.756	-93.9997	-21.7567	0
7	-117.080	-91.5504	-25.7676	0.238205
8	-120.695	-89.5502	-31.1444	0
9	-124.252	-100.778	-23.7278	0.253323
10	-113.083	-96.9365	-16.1465	0
11	-117.178	-81.7645	-35.414	0
12	-112.063	-95.1033	-16.96	0
13	-124.817	-89.9581	-34.8592	0
14	-114.454	-97.172	-17.5239	0.2418
15	-127.652	-89.4099	-38.2423	0
16	-139.586	-93.5147	-46.0718	0
17	-129.509	-86.2239	-44.447	1.16237
18	-122.303	-98.2601	-24.0433	0
19	-123.190	-89.5717	-33.6182	0
20	-112.861	-82.9563	-29.9049	0
21	-130.387	-90.3056	-40.0815	0
22	-114.256	-88.9504	-25.3056	0
23	-119.751	-93.8131	-26.1817	0.243672
24	-110.662	-86.145	-24.7975	0.280749
25	-113.528	-84.7804	-28.9802	0.232991
26	-104.235	-84.6821	-19.8018	0.249137
Pretonamid	-96.959	-70.8353	-26.1237	0

Ddn residue in blue colour and other atoms indicated as follow are oxygen in red, sulphur in tints colour, nitrogen in blue colour.

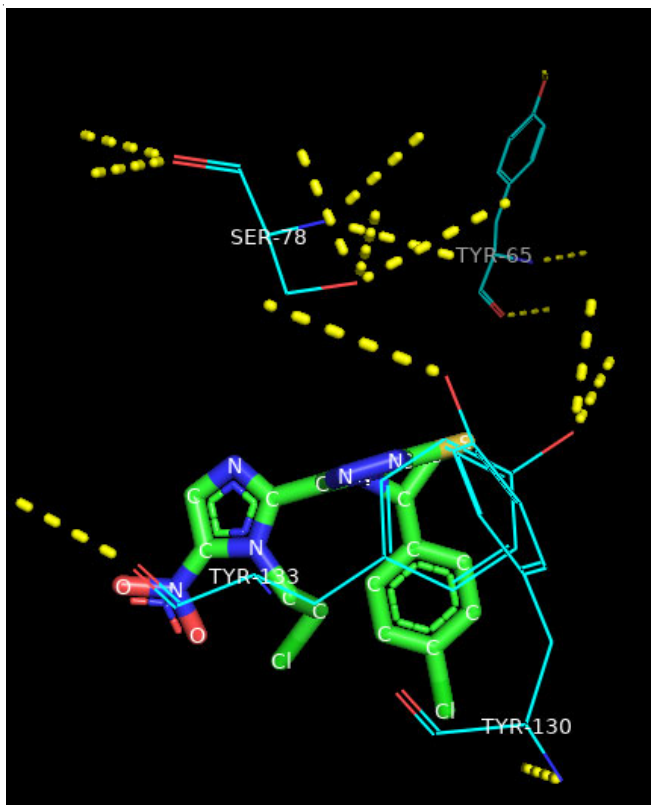


Fig. 2. Compound **3**

Fig. 5 shows proposed binding mode of compound **18** to deazaflavin dependent nitroreductase before molecular dynamics carbon atom of compound **18** in green colour, carbon

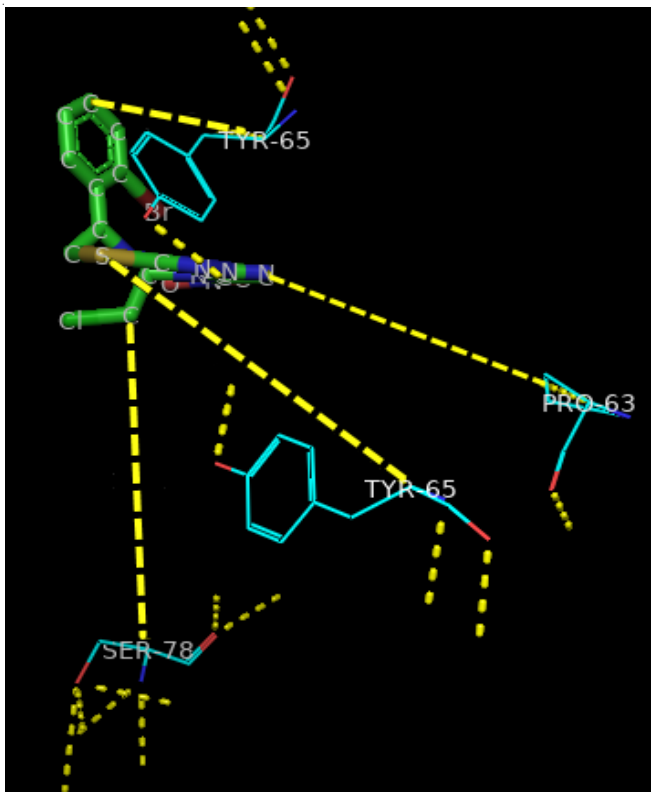


Fig. 3. Compound **5**

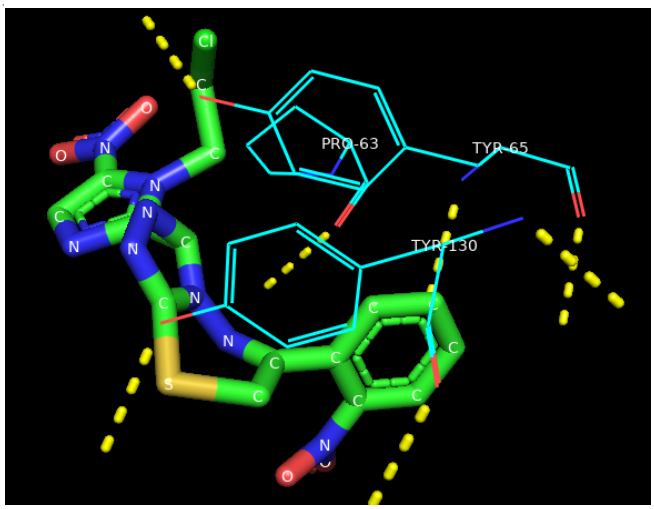


Fig. 4. Compound **15**

atom of Ddn residue in blue colour and other atoms indicated as follow are oxygen in red, sulphur in tints colour, nitrogen in blue colour. Fig. 6 shows proposed binding pose of pretonamid to deazaflavin dependent nitroreductase before molecular dynamics, carbon atom of pretonamid drug in green colour, carbon atom of Ddn residue in blue colour and other atoms indicated as follow are oxygen in red, nitrogen in blue colour.

In present work, molecular docking studies were performed to explore possible binding modes of proposed nitroimidazole derivatives as antitubercular agents with (PDB ID-3R5R) iGEM dock version2.1. Software was used to dock nitroimidazole derivatives as antitubercular agents. Our docking results showed that tyr65, trp88, tyr133 made an important contribution in van der waals interaction and ser78, tyr 65 made an important

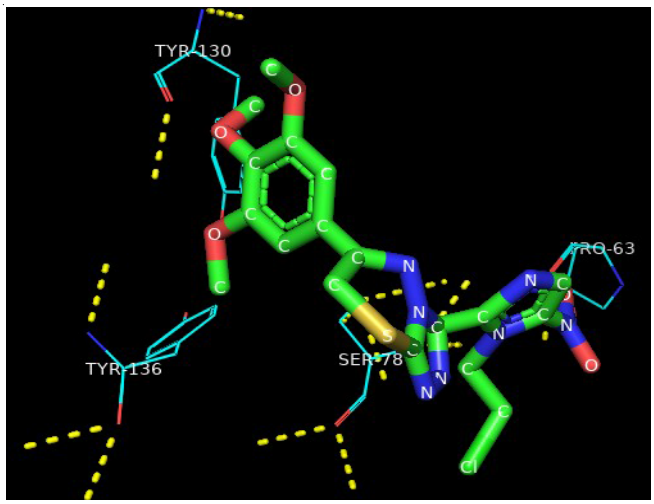


Fig. 5. Compound **18**

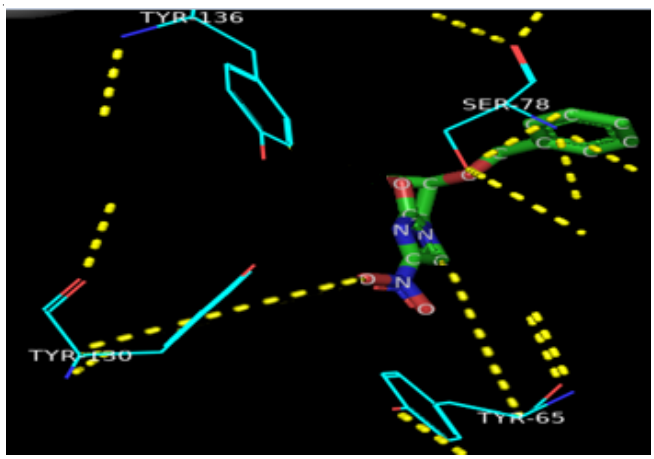


Fig. 6. References drug pretonamid

contribution in hydrogen bond formation. Our docking results suggested the possible conformation of binding site of nitroimidazole derivatives as antitubercular molecules to 3R5R. Further, identified favoured binding modes of 3R5R to nitroimidazole derivatives leading for the development of new antitubercular molecules and also provided valuable insights interaction of molecules with different amino acids.

Conclusion

It concluded that nitro, halo functional groups are showing most desirable docking results compared to other functional groups. Amino acid such as tyr65, trp88, tyr133 made an important contribution in van der waals interaction and ser78,tyr 65 made an important contribution in hydrogen bond formation . The docking studies results showed that compounds **3**, **5**, **15** and **18** showed more binding energy with Ddn than that of reference compound pretonamid. Hence these proposed molecule may give better activity than that of reference compound.

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