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Research of new anti-tuberculosis agents by molecular docking's method

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ABSTRACT

The discovery of anti-tuberculosis agents is crucial for effective tuberculosis therapy. The present strategy for new drug development is directed towards identifying essential enzyme systems in the bacteria and developing potent molecules to inhibit them. The aim of this study was to study the inhibition of a peptide deformylase of Mycobacterium tuberculosis using molecular docking FlexX. This study highlighted the actinonin as the better inhibitor of the enzyme. The study of the modelling realized on this inhibitor show that the binding energy can be decreased in a significant way by a judicious choice of fragments to be substitutes. Replacement of the pentyl group in position R1 by a cyclopentylmethyl, the dimethyl-propyl group in position R2 by a pyrrolidine and the hydroxymethyl pyrrolidin in position R3, by hydroxyphenyl group decreases the energy of interaction of 8 units. The study of the pharmacokinetic properties of this proposed molecule shows that they join perfectly the margin of the criteria imposed by the rule of Lipinski. The results of the present study are reported herein so that researchers, who are having required laboratory facilities for synthesizing drugs, can utilize findings of this study for developing new drugs against Mycobacterium tuberculosis with better efficacy.

Keywords: Molecular docking, FlexX, peptide déformylase, anti-tuberculosis.

INTRODUCTION

Tuberculosis, caused by *Mycobacterium tuberculosis*, is the most common infectious disease caused by a single bacterium, and it is estimated that two billion people or one-third of the world's population have been infected by *M. tuberculosis*. More than eight million new cases of active TB disease each year result in two million deaths annually, mostly in developing countries. **[1,2]** The clinical impact and rise of tuberculosis patients with drug-resistant strains of *M. tuberculosis* have made the development of new antimycobacterials urgent. [**3-9]** The increase in multidrug-resistant strains of *M. tuberculosis* has decreased the effectiveness of current standard Tuberculosis treatment options.**[8]**

Thus, the discovery of anti-tuberculosis agents that target new pathways with novel mechanisms of action is crucial for effective short-term tuberculosis therapy that will limit the development of resistance.

Peptide deformylase (PDF) is a bacterial metalloenzyme which deformylates the N-formylmethionine of newly synthesized polypeptides, a key step in protein maturation. [**10, 11]** PDF is essential for bacterial growth **[12-16]** making it an appealing target for the design of novel antibiotics. **[17,18]**

The purpose of this work is to test the ability of molecular docking software FlexX version 2.1.8, 2014 (http://www.biosolveit.de/FlexX/), used in this study by determining the RMSD (root mean square deviation), then

use this program to analyze the interactions between the PDF of *M. tuberculosis* and divers PDF inhibiting drugs, to explore their binding modes and to make tests of modelling, with a view to identify novel and more efficient PDF inhibitors. These results will probably help in the development of an effective therapeutic tool in the fight against the development of tuberculosis.

MATERIALS AND METHODS

1.1. Methods

1.1.1. Molecular docking

Docking is one of the commonly used computational methods in structure based drug design. Docking is the process of fitting of the ligand into the receptor. It not only gives an idea about how the ligand is going to bind with the receptors, but also about up to what extent conformational changes can be brought in the receptor structure. Docking comprises two distinct tasks, the first being the prediction of favorable binding geometries for a small molecule in the binding site of a target protein, and secondly, the estimation of the binding free energy of the complex so formed, also referred to as scoring. **[19, 20]**

1.1.2. RMSD (Root Mean Square Deviation)

Equal to the average of the deviation of each of atoms compared to the original molecule. The best value of mean RMSD between the placing of the ligand calculated by the software and the conformation in the experimental complex is the smallest possible. The ratio accepted is 2 angstroms beyond which the prediction is considered irrelevant. The current standard for evaluating the performance of a docking program is to make a test from hundreds of protein-ligand complexes crystallized. **[21, 22]**

This test was performed on 101 complexes available in the PDB and the RMSD determined. The prediction RMSD is acceptable if the value does not exceed 2 Å.

1.1.3. Lipinski rule

Lipinski's Rule of Five is a rule of thumb to determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans.

The rule made by Christopher Lipinski. **[23]** Each drug must comply with several basic criteria, such as low cost of production, be soluble, stable, but must also conform to the schedules associated with its pharmacological properties of absorption, distribution, metabolism, excretion and toxicity(ADME/Tox).

Lipinski's Rule of Five states that an orally active drug must complete three of these fives proprieties:

- No more than 5 hydrogen bond donors
- No more than 10 hydrogen bond acceptors
- No more than 15 rotatable bonds
- The Molecular weight is over 500 g/mol-1
- The Log P is over 5

1.2. Softwares :

1.2.1. FlexX 2.1.8, 2014

FlexX is one of the most established protein-ligand docking tools in the literature, it has proved to be highly successful in numerous drug discovery applications. **[24]** Several subnanomolar inhibitors have been discovered with FlexX and are on the market after having proved their potential as a drug **[25]**. The technology behind FlexX is based on a robust incremental construction algorithm **[26]**. The scoring is done using a modified Böhm scoring function (Equation.1),

$$\Delta G=\Delta G_0+\Delta G_{rot}N_{rot}+\Delta G_{hb}\sum_{H\text{-}bond} f(\Delta R,\Delta\alpha)+\Delta G_{io}\sum_{ionic.\ int} f(\Delta R,\Delta\alpha)+\Delta G_{aro}\sum_{aro.\ int} f(\Delta R,\Delta\alpha)+\Delta G_{lipo}\sum_{cont\ lipo} f^{*}(\Delta R) \quad (1)$$

1.2.2. Arguslab 4.0.1, 2003:

Arguslab is the drawing tool of choice to create 3D structures of proposed compounds in several formats such as pdb, mol, mol2, etc.

1.2.3. Molinspiration:

Molinspiration cheminformatics package (http://www.molinspiration.com/) is used to test the toxicity and other ADMET properties of inhibitors. It is maintained by Bratislava University.

1.3. Data collection

1.3.1. Drug target

In this study, *Mycobacterium tuberculosis* PDF is taken as a drug target. The 3D structure of this enzyme was retrieved from Protein Data Bank (PDB) (http://www.rcsb.org./pdb/). The PDB ID of this enzyme is the 3E3U trained by 167 residues of amino-acids.

1.3.2. Inhibitors

The PDF inhibiting drugs selected for the present investigation were given (Fig. 1). Eight potential drugs were selected from PDB and PubChem compounds for molecular docking against active amino acids pocket in the 3D structure of *Mycobacterium tuberculosis* PDF.

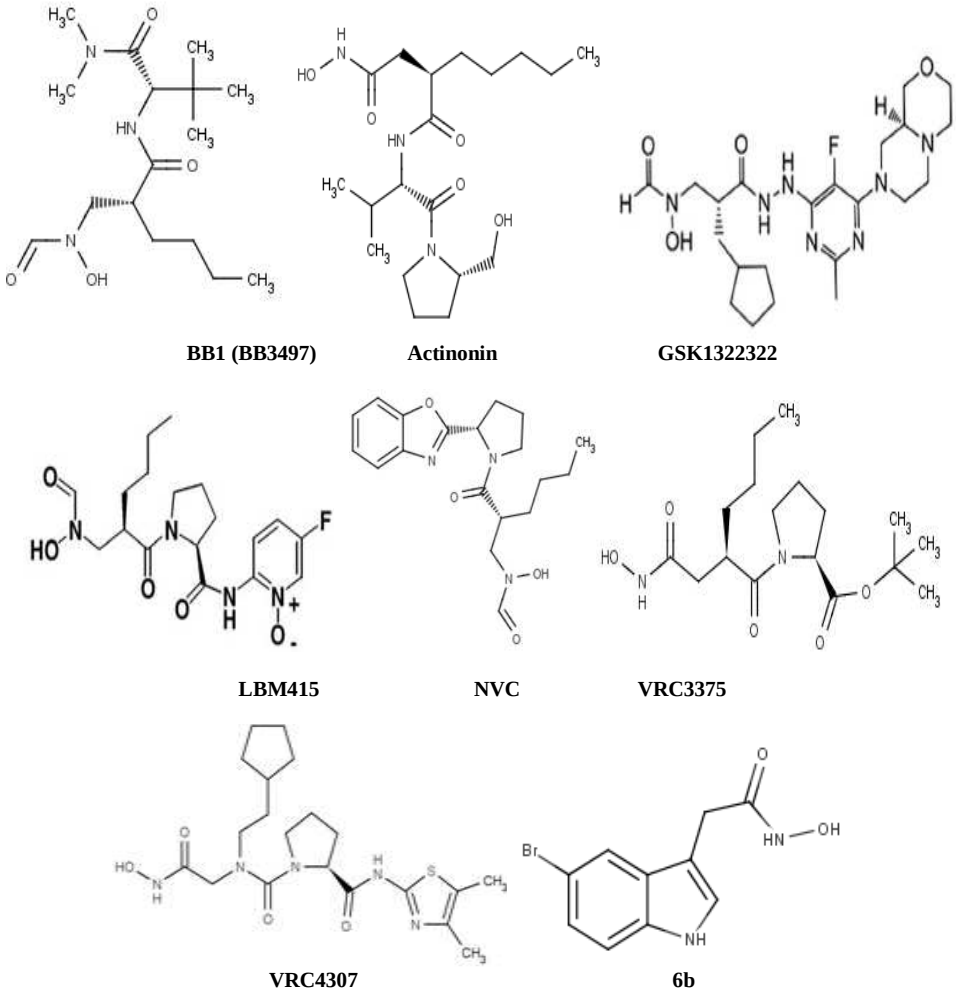


Figure 1: Drugs used in this study.

BB1 (BB3497) : 2-[(formyl-hydroxy-amino)-methyl]- acid hexanoic (1-dimethylcarbamoyl-2,2-dimethyl-propyl)-amide.

Actinonin : (2R)-N'-hydroxy-N-{1-[(2S)-2-(hydroxymethyl)pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl}-2-pentylbutanediamide

LBM415 :N-(5-fluoro-1-oxidopyridin-2-yl)-1-[(2R)-2-{[formyl(hydroxy) amino] methyl}hexanoyl]-L-prolinamide.

NVC : N-{3-[2-(1,3-benzoxazol-2-yl)pyrrolidin-1-yl]-2-butyl-3-oxopropyl}-N-hydroxyformamide

GSK1322322 : N-[(2R)-3-[2-[6-[(9aS)-3,4,6,7,9,9a-hexahydro-1H-pyrazino[2,1-c] [1,4]oxazin-8-yl]-5-fluoro-2-mthylpyrimidin-4-yl]hydrazinyl](cyclopentylmethyl)-3-oxopropyl]-N-hydroxyformamide.

VRC3375 (CID : 488029) : tert-butyl (2S)-1-[(2R)-2-[2-(hydroxyamino)-2oxoethyl]hexanoyl]pyrrolidine-2-carboxylate.

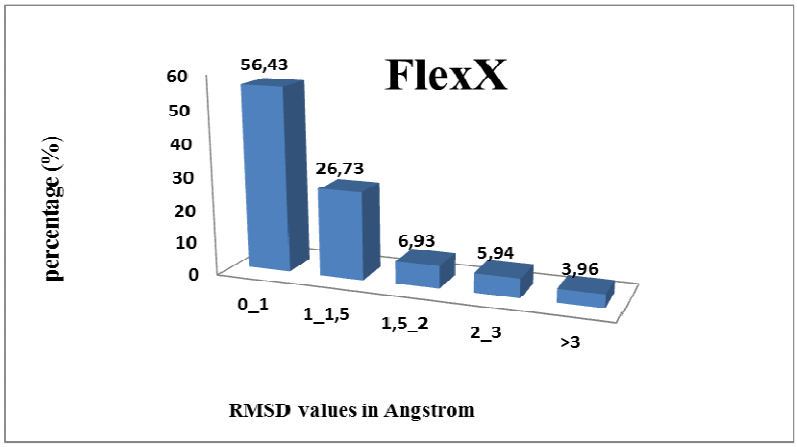
VRC 4307 (CID : 9824395) : (2S)-2-N-(4,5-dimethyl-1, 3-thiazol-2-yl)-1-N-[2-(hydroxyamino)-2-oxoethyl]-1- N-(3-methylbutyl) pyrrolidine-1,2-dicarboxamide.

6b (CID 11572551) : 2- (5-bromo-1H-indol-3-yl)-N-hydroxyacetamide.

RESULTS AND DISCUSSION

1.4. The ability of molecular docking software FlexX:

Program performance had been evaluated on 101 crystallized protein-ligand complexes available in the PDB. The docked binding mode was compared with the experimental binding mode and a root-mean-square distance (RMSD) between the two was calculated by FlexX; a prediction of a binding mode was considered successful if the RMSD was below 2.0 Å **[27]**. In the following graphs, the results are given in percent (%) at various intervals of RMSD.



Graph 1. Results in % obtained by FlexX at various intervals of RMSD (Å)

The minimum RMSD overall is 0.0651Å, while the maximum is 6.7099Å. The majority of positive results is in the range 0 to 1Å.

In our results we noticed that the program FlexX reproduced the experimental data well. Indeed, 90.09% of RMSD values were less than 2Å. The RMSD values were consistent with the results of Kellenberger *et al.*, (2004) **[22]**, showing that with any program the docking was successful when the RMSD was less than 2 Å. This was also consistent with the results obtained by Chikhi A., 2007 **[28]**, and Merzoug *et al*., (2012) **[29]**, who have shown that the docking program FlexX produces reasonable binding modes.

1.5. Study of the interactions involved in the inhibition of *M. tuberculosis* PDF by various molecules

Binding energies between ligand and receptor play the most crucial role in drug designing. In this work, the peptide deformylase of *M. tuberculosis* was selected as drug target and binding energies with 8 inhibitors were evaluated using the latest version of the program FlexX. The results are shown in table 1:

Table 1: The Interaction energy (kj/mol) of peptide deformylase and drugs obtained by molecular docking

Drugs	Binding energy (kj/mol)
Actinonin	-30.4808
BB1	-17.1813
GSK 13232	-28.5776
LBM	-15.7954
NVC	-27.0172
VRC3375	-29.8137
VRC 4307	-30.2279
6B	-29.2761

In this study, it was observed that these compounds have greater affinity towards *M. tuberculosis* PDF to successfully inhibit this enzyme. Among these inhibitors, l'actinonin showed the highest docking score of -30.4808 Kj/mol showed highest binding affinity with the peptide deformylase–drug interaction, followed by compound VRC4307 and VRC3375 (ΔG = -30.2279 Kj/mol and -29.8137 Kj/mol respectively).

Interesting interactions were detected between *M. tuberculosis* PDF and actinonin with high number of matches (11 matches) and high interaction energies values (-38.8091 kj/mol of the matched interacting groups, -11.0194 kj/mol of the lipophilie contact area and -8.5317 kj/mol of the lipophilie-hydrophilie area), but the effect of rotatable bonds has been carried out, they decreases the total score of the docking solution to -30.4808 kj/mol (Table I). The interactions are represented in the Fig 2

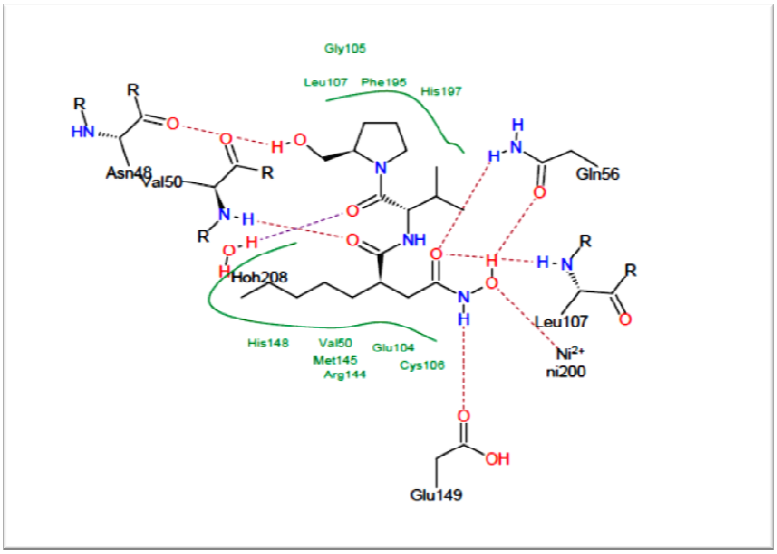


Figure 2: Docking results for actinonin. Hydrogen bonding is represented by dotted lines. Hydrophobic interactions are shown as green lines. The numbers represent the interaction energy.

The figure 2 shows that the actinonin penetrates well into the active site of the enzyme, forming several hydrogen bonds with amino acid residues of the binding pocket. In particular, the metal binding group was involved in five hydrogen bonds (Fig. 2), two by its carbonyl group with NH of Leu107 and lateral amine of Gln76, one by its NH with carbonyl of Glu179 and two by its hydroxyl with metal ion and carbonyl of Gln56.

The two carbonyl groups of actinonin are hydrogen bonded to the NH of Val50 and HOH208. An additional hydrogen bond of the hydroxyl of pyrrolidin ring with the carbonyl of Asn48 further stabilizes the inhibitor in the binding pocket.

Hydrogen bonds are not solely responsible for the interaction of the ligand with *M. tuberculosis* PDF. The role of hydrophobic interactions is also important in explaining. The actinonin is stabilized by hydrophobic interactions with the region S1 residues which is the binding site for the methionine side chain of the substrate: Val50, Met145, Leu107, His197, His148, Gly105, Glu104, Cys106, Arg144, His148 and Phe195 (Fig. 2).
The results obtained in our study were consistent with those found by other authors, e.g., Singh,V and Somvanshi, P, (2009) **[3.]** who found that the actinonin was highest binding affinity with PDF and Sharma, A *et al*, (2009) **[31]** who had shown that actinonin was a powerful inhibitor of *M. tuberculosis* peptide deformylase.

1.6. Design of new potent inhibitors

In the perspective of developing new potent and more effective inhibitors of the PDF, the structure of the actinonin was taken and several types of substitutions were carried on several positions (Fig 3).

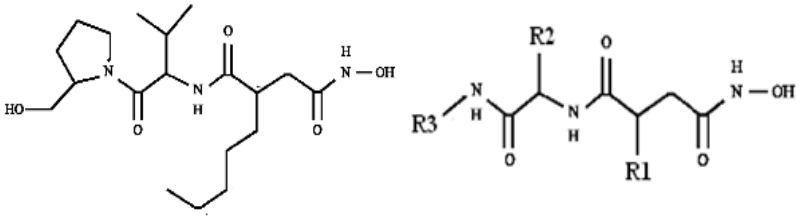
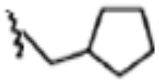
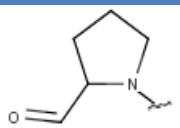
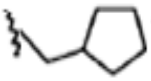
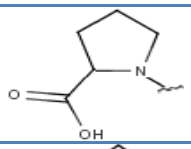
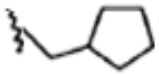
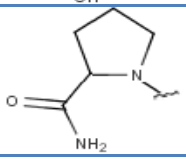
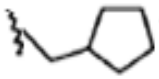
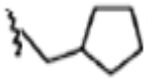
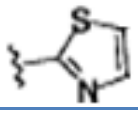
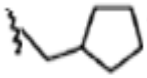
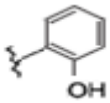
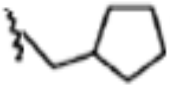
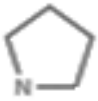
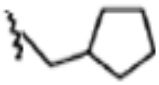
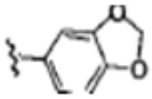
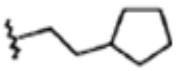
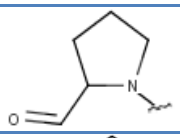
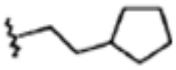
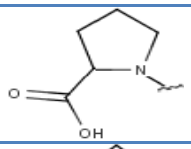
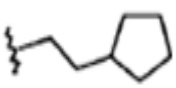
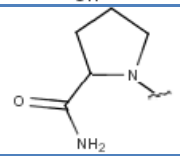
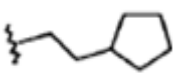
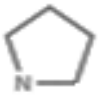
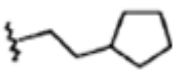
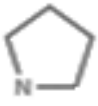
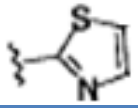
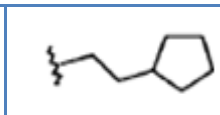
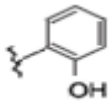
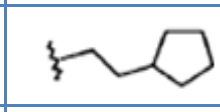
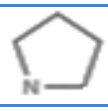
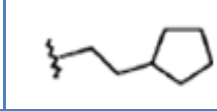
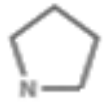
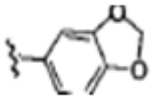
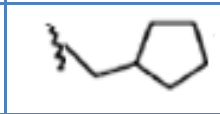
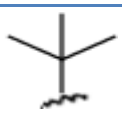
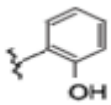
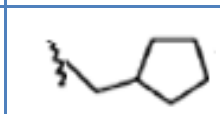
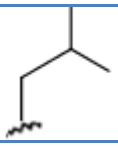
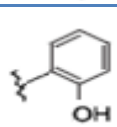
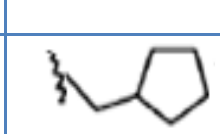
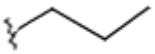
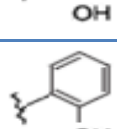
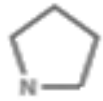
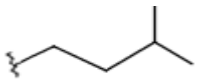
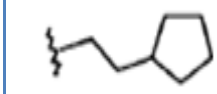
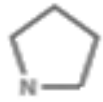
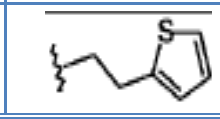
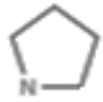
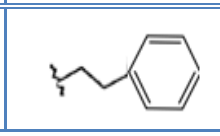
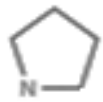


Figure 3: Chemical structure of actinonin and targeted positions

Interactions between actinonin derivatives and *M. tuberculosis* PDF were studied using FlexX software. Results of substitution are shown in Table 2. Docking calculations reveal that binding energy increase with all designed inhibitors. The best binding energy was obtained by compound 6

Table 2: Binding energy between *M. tuberculosis* PDF and several actinonin derivatives

Substituted compound	R1	R2	R3	Score (kj/mol)
Compound 1				**-34.7344**
Compound 2				**-34.5168**
Compound 3				**-33.0464**
Compound 4				**-36.9258**
Compound 5				**-35.5503**
Compound 6				**-38.6051**
Compound 7				**-34.4550**
Compound 8				**-31.3201**
Compound 9				**-31.0775**
Compound 10				**-33.3040**
Compound 11				**-35.2535**
Compound 12				**-33.8202**
Compound 13				**-33.5394**

Compound 14				**-35.3624**
Compound 15				**-32.1860**
Compound 16				**-33.4223**
Compound 17				**-32.1238**
Compound 18				**-30.7198**
Compound 19				**-31.2019**
Compound 20				**-31.5643**
Compound 21				**-33.7622**
Compound 22				**-33.8546**
Compound 23				**-31.6532**
Compound 24				**-35.2322**

In the case of compound 6, the binding energy was increased from -30.4808 Kj/mol to -38.6051 Kj/mol by introduction of a cyclopentyl methyl group in R1 position, a pyrrolidine ring in R2 position, and a hydroxy phenyl group in R3 positions. The chemical structure of proposed compound is presented in the Fig 4

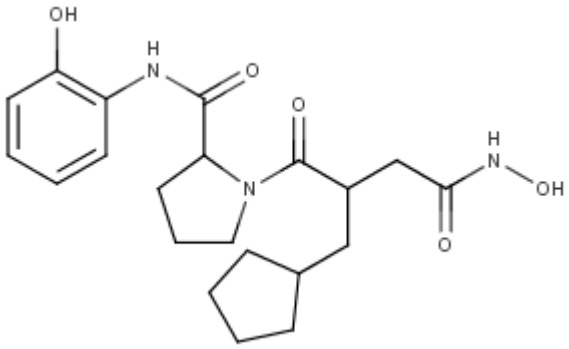


Figure 4: Chemical structure of compound 6

Compound 6: 1-[2-(cyclopentylmethyl)-3-(hydroxycarbamoyl)propanoyl]-N-(2-hydroxyphenyl)pyrrolidine-2-carboxamide

The inhibition mechanism of this new compound toward *M. tuberculosis* PDF was studied using FlexX Tools. Visual analysis shows that compound 6 is well positioned on the active site of PDF (Fig 5).

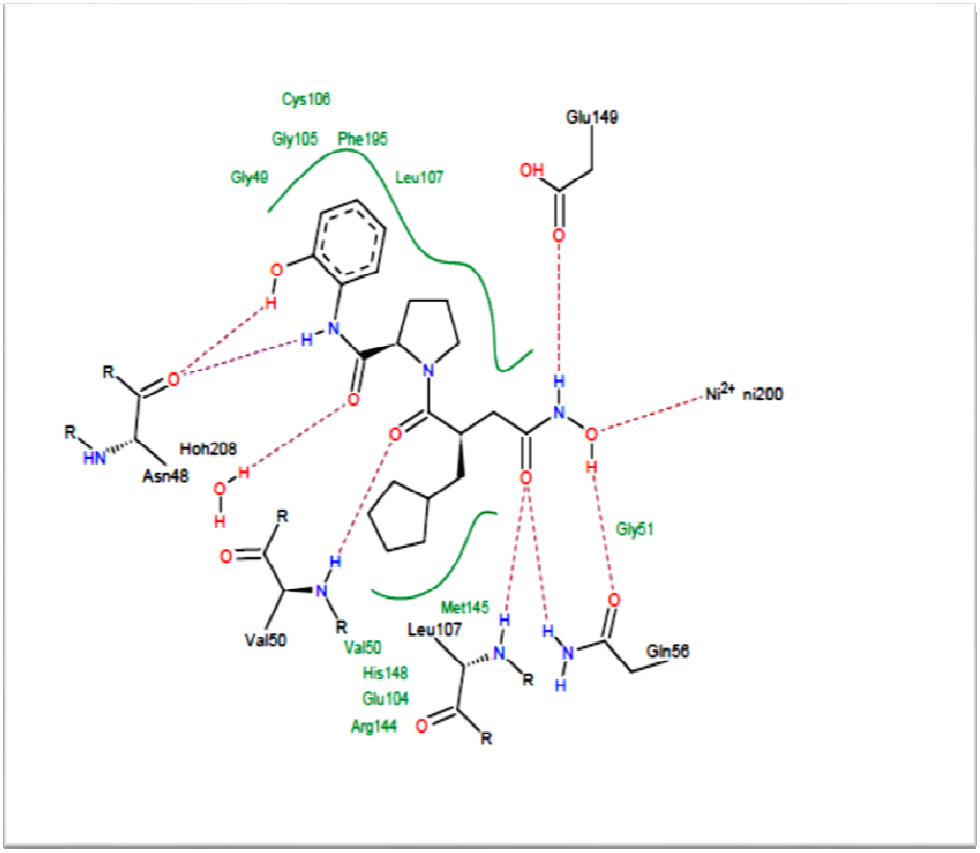


Figure 5: Binding modes of compound 6 with the active site of *M. tuberculosis* PDF

The compound 6 established interactions similar to that of our original inhibitor, actinonin. The binding energy increase might be attributed to the formation of new hydrogen bond between the NH of inhibitor and the carbonyl of Asn48 and supplementary hydrophobic interactions with the Gly49, Gly51 et Phe195 (Fig. 5).

1.7. Prediction of pharmacological properties

The ADME/Tox screening of the proposed inhibitor, compound 6, has not shown any negative result:
- Molecular weight = 402.47g/mol
- Partition coefficient log P = -0.98
- Hydrogen bond donors = 3
- Hydrogen bond acceptors = 7
- Flexible bonds = 8

It could be seen that the molecular weight of this compound is less than 500g/mol which fulfill the criteria of Lipinski's rule, the values of the hydrogen bond donors, acceptors and flexible bonds were also less than the maximum extent permitted by Lipinski's rule; which indicates the potentiality of this molecule to become drug, it is accepted to be orally bioavailable.

CONCLUSION

FlexX can be considered sufficiently effective since it reproduces quite well the experimental results, we can suggest the substituted compound 1-[2-(cyclopentylmethyl)-3-(hydroxycarbamoyl)propanoyl]-N-(2-hydroxyphenyl)pyrrolidine-2-carboxamide as a new potent peptide deformylase inhibitor with an interaction energy a bit less to that of actinonin and with interesting pharmacokinetic properties.

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