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Piggyback drug development: (Molecular docking of Entacapone analogues as direct *M. tuberculosis* InhA inhibitors)

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ABSTRACT

A piggyback or drug repositioning approach to drug discovery and development was applied in finding potential inhibitors ofenoyl reductase (InhA), an enzyme involved in fatty acid and cell wall synthesis of M. tuberculosis. The questsprang from entacapone, a drug for Parkinson's disease, which was also found to inhibit InhA enzyme. A compound database was scoured to search for entacapone-like structures, which were then filtered based on LibDock scores. The hits were subsequently docked into InhA binding site by the use of CDocker protocol and their binding energies were calculated. The results showed that the dimer, and an alcohol and piperazine derivatives of entacapone are potential inhibitors of InhA. H-bonding and π–π interactions with nicotinamide adenine dinucleotide(NAD) at the binding pocket are salient features in binding interactions. Interestingly, the four entacapone analogues exhibited greater binding affinity with InhA compared to entacapone itself and the native ligand,5-pentyl-2-phenoxyphenol.

Keywords: *Mycobacterium tuberculosis*, entacapone analogues, InhA inhibitors, LibDock, CDocker, drug repositioning

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INTRODUCTION

Tuberculosis is an infectious disease caused by the bacillus *Mycobacterium tuberculosis* that most often affects the lungs. It is transmitted from person to person via droplets from the throat and lungs of people with the active respiratory disease [1]. Common symptoms of active lung TB are cough with sputum and blood, chest pains, weakness, weight loss, fever and night sweats. In 2013, almost 9 million people worldwide fell ill and 1.5 million died with TB [1]. In the Philippines, tuberculosis is the sixth leading cause of death in 2009[2].

Standard anti-TB drugs have been used for decades, but resistance to these medicines is remarkably increasing. Multidrug-resistant tuberculosis (MDR-TB) is a form of TB caused by bacteria that do not respond to, at least, isoniazid and rifampicin, the two most powerful, first-line anti-TB drugs [1]. There were about 480,000 people who have developed MDR-TB in2013, and about 9% of these cases were XDR-TB (Extensively Drug-Resistant Tuberculosis) [1]. XDR-TB is a form of TB caused by bacteria that are resistant to isoniazid and rifampicin as well as any fluoroquinolone and any of the second-line anti-TB injectable drugs (e.g. amikacin, kanamycin, orcapreomycin). In2013, 100 countries hadat least one case of XDR-TB [1]. With the rise of MDR-TB and XDR-

TB, the increasing demand for new agents against tuberculosis calls for earnest research efforts on TB drug discovery.

Drug discovery is an expensive and extensive endeavor. Nevertheless, computational techniques such as molecular docking, quantitative structure-activity relationship (QSAR), ADME (absorption, distribution, metabolism, excretion) measurements, and database screenings have been proven valuable in speedy discovery of new therapeutics [3]. Recently, the concept of label extension or the use of so-called off-label drugs, and 'piggyback' strategies are also gaining popularity. The label extension approach involves extending indications of an existing treatment to another disease [4, 5]. This is a fast-track approach that extensively reduces cost and time liabilities for drug development [6]. The 'piggyback' strategy, on the other hand, utilizes identified active compounds that have already been thoroughly evaluated as drugs or leads, as starting points in drug development [7]. This approach led to the identification of entacapone as possible lead in the development of new anti-TB compounds[8].

Entacapone was shown to inhibit the enol-acyl carrier protein reductase or InhA enzyme, which is the target of the first line drugs: Isoniazid [9, 10] and Ethionamide [11]. Isoniazid is activated within the mycobacterial cell by the KatG catalase and the activated molecule suppresses the biosynthesis of mycolic acid, which makes up the cell wall, through the inhibition of InhA, a key enzyme of the type II fatty acid synthesis (FAS) system[12]. Most isoniazid resistance is mediated through mutations in KatG leading to the inability to activate the drug [13,14]. It is therefore, instructive to search for direct inhibitors of InhA to avoid much of the current resistance by bypassing the requirement for KatG activation.

Entacapone is a nitrocathecol drug that has been proven to directly inhibit the action of cathecol-O-methyltransferase (COMT) [15]. It has been widely used for the treatment of Parkinson's disease, which is a degenerative disease caused by the depletion of dopamine in the brain. Entacapone alters the pharmacokinetics of levodopa, an amino acid that can be converted to dopamine, by delaying its breakdown and making it available for dopamine conversion [16]. Interestingly, using chemical systems biology approach, Bourne and coworkers found that entacapone also potentially inhibits the *M. tuberculosis* InhA[8]. Indeed, they found experimentally that entacapone inhibited the growth of *M. tuberculosis* with a minimal inhibitory concentration (MIC_{99}) of 260 μM, and the drug Comtan inhibited InhA activity by 47% atentacapone concentration of 80 μM. Having safe drug profile and inhibitory action against *M. tuberculosis*, entacapone could serve as potential lead for tuberculosis treatment [8].

In this study, we used entacapone as our starting material to find structurally related compounds that can also potentially inhibit InhA and could be pursued as leads in the development of new anti-TB compounds. ChemMine (chemmine.ucr.edu), a compound mining database, was screened for compounds based on the structure of entacapone. The resulting entacapone-like hits were docked into the InhA enzyme. The analogues with LibDock [17, 18] scores greater than that of entacapone were subsequently docked using CDocker [19] to obtain the binding energy, and determine the nature of ligand-target interactions. The high-affinity entacapone analogues identified in this study mayprovide access to a new class of antitubercular agents.

EXPERIMENTAL SECTION

Structural Data for Docking

The crystal data for *M. tuberculosis* InhA enzyme (PDB entry code: **2B36**) was downloaded from the RCSB Protein Data Bank (http://www.rcsb.org/pdb). ChemMine Similarity Comparisons tool (http://chemmine.ucr.edu/) was used in searching for entacapone analogues. The SMILES notation for entacapone was used as input in searching for similar structures from ChemMine database. The entacapone analogues were downloaded and saved as sdf files.

Preparation of Structures for Docking

Molecular docking studies were performed using Discovery Studio (DS) 2.5 (Accelrys, Inc.). The enzyme structure waspreparedthrough removal of water molecules and restoration of missing hydrogen atoms. The pH of the enzyme was adjusted to 7. The docking sphere was positioned around the site where the bound inhibitor was located. The entacapone analogues were prepared by means of adding missing hydrogen atoms and optimizing the structure. The analogues, with Tanimoto coefficients [20, 21]greater than 0.90, were selected for molecular docking studies.

Molecular Docking

Each entacapone analogue was docked into the InhA using the LibDock protocol in DS. For each ligand, the

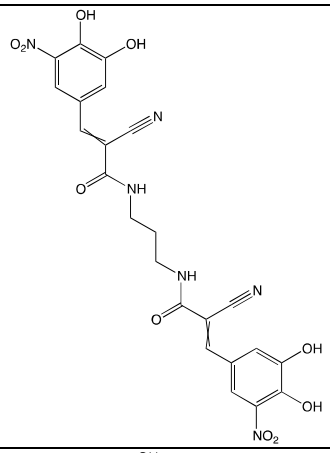
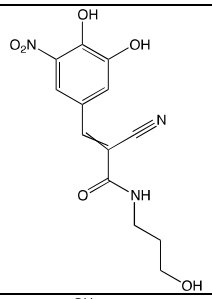
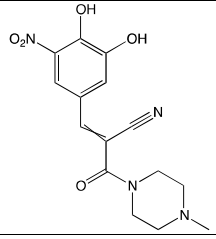
LibDock score was recorded. The analogues that showed greater docking score than entacapone were subsequently docked to the enzyme using the CDocker protocol, which employs the CHARMm force field. The best pose for each ligand wasobtained as well as the corresponding binding energy.

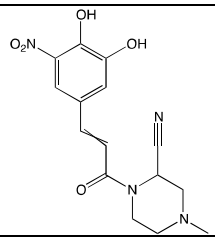
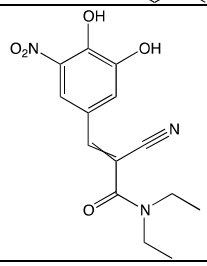
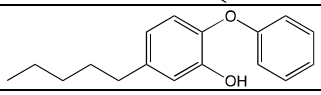
RESULTS AND DISCUSSION

Search and Screening for Potential Inhibitors

The promising bioactivity of entacapone against *M. tuberculosis* InhA [8] stimulates the search for structurally similar compounds with antitubercular activity. Accordingly, we looked for entacapone-like compounds from ChemMine database and evaluated the top hits for their binding ability with the InhA target. Specifically, the entacapone analogues were docked to InhA, initially using LibDock, and the docking score for each ligand was obtained. The LibDock score is a measure of the strength of binding between a receptor and a ligand, a higher score indicates stronger binding interaction between the two [17]. Examination of the LibDock data (Table 1) revealed that the scores for compounds **1** – **4** have exceeded that of entacapone and the bound ligand, 5-pentyl-2-phenoxyphenol, signifying that these four entacapone analogues would bind with the InhA target more strongly than entacapone itself, even the native ligand.

Table 1. Structure similarity and molecular docking data for 5-pentyl-2-phenoxyphenol, entacapone, and itsanalogues with *M. tuberculosis* InhA as drug target

Compound	Database ID	Structure	Tanimoto Coefficient	LibDock Score	Binding Energy (kcal/mol)
1	4370591		0.90	141.82	-98.43
2	18990394		0.93	105.15	-91.44
3	18990375		0.94	109.26	-81.05

4	22161977		0.92	113.79	-56.97
5	4659568 (Entacapone)		0.99	100.18	-45.41
5-pentyl-2-phenoxyphenol (native ligand)	-		-	-	-29.20

Molecular Docking of Top 4 Hits

The top four compounds were then subjected to further docking studies using CDockerto obtain the binding energy. CDockeris a docking algorithm based on CHARMm force field. Molecular docking by CDocker was accomplished by the use soft-core potentials with an optional grid representation. CDocker employs molecular dynamics simulation to generate random ligand conformations. To each of the conformations, rigid-body rotations and translations were applied to obtain ligand poses [19]. Molecular dynamics-based simulated annealing was performed and the energy of the receptor/ligand complex was then minimized. Since CDocker utilizes soft-core potentials, it was able to cover the conformational space of small molecules and macromolecules making CDocker a widely used algorithm in various docking studies [22].

Table 1 also shows the binding energy for compounds **1** – **4** against InhA target. The binding energy is an important factor to consider in an enzyme-substrate interaction. It is the underlying principle that governs proximity, orientation effects, substrate strains, etc. that are thought to effect catalysis as well as enzyme inhibition [23]. A more negative binding energymeans more favorable binding interaction. As expected, the entacapone analogues with high LibDock scores also exhibited greater binding energies compared to that of entacapone.

The PDB file 2B36 used in this study provides the crystal data for InhA in complex with the bound inhibitor,5-pentyl-2-phenoxyphenol.This inhibitor forms hydrogen bonds with NAD coenzyme at the binding site of the enzyme. π-π interaction also exists between the NAD coenzyme and one of the aromatic rings of this compound. The binding energy for 5-pentyl-2-phenoxyphenol with InhAis -29.20 kcal/mol.

The three-dimensional (3D) interaction diagram **(**Figure 1**)** for the 2B36 enzyme-entacapone complex shows the spatial orientation of the inhibitor within the active site of the enzyme. The spatial orientation of entacapone (shown in pink) has also been compared to that of 5-pentyl-2-phenoxyphenol(shown in orange). It is worthy of note that entacapone and the native inhibitor both occupy the same region in the enzyme. Some parts of the two inhibitors are superimposed on each other, showing the degree of similarity of the binding of the two inhibitors to InhA enzyme. Like the nativelig and, entacapone participates in hydrogen bonding with NAD coenzyme. The hydroxyl moieties act as hydrogen bond donors. The NAD coenzyme also interacts with the aromatic ring of entacapone via π-π interactions. Interestingly, entacapone yielded a binding energy of -45.41 kcal/mol, which is better than that of the bound ligand. This is not surprising since entacapone has been demonstrated to inhibit *M. tuberculosis* as mentioned above [8].

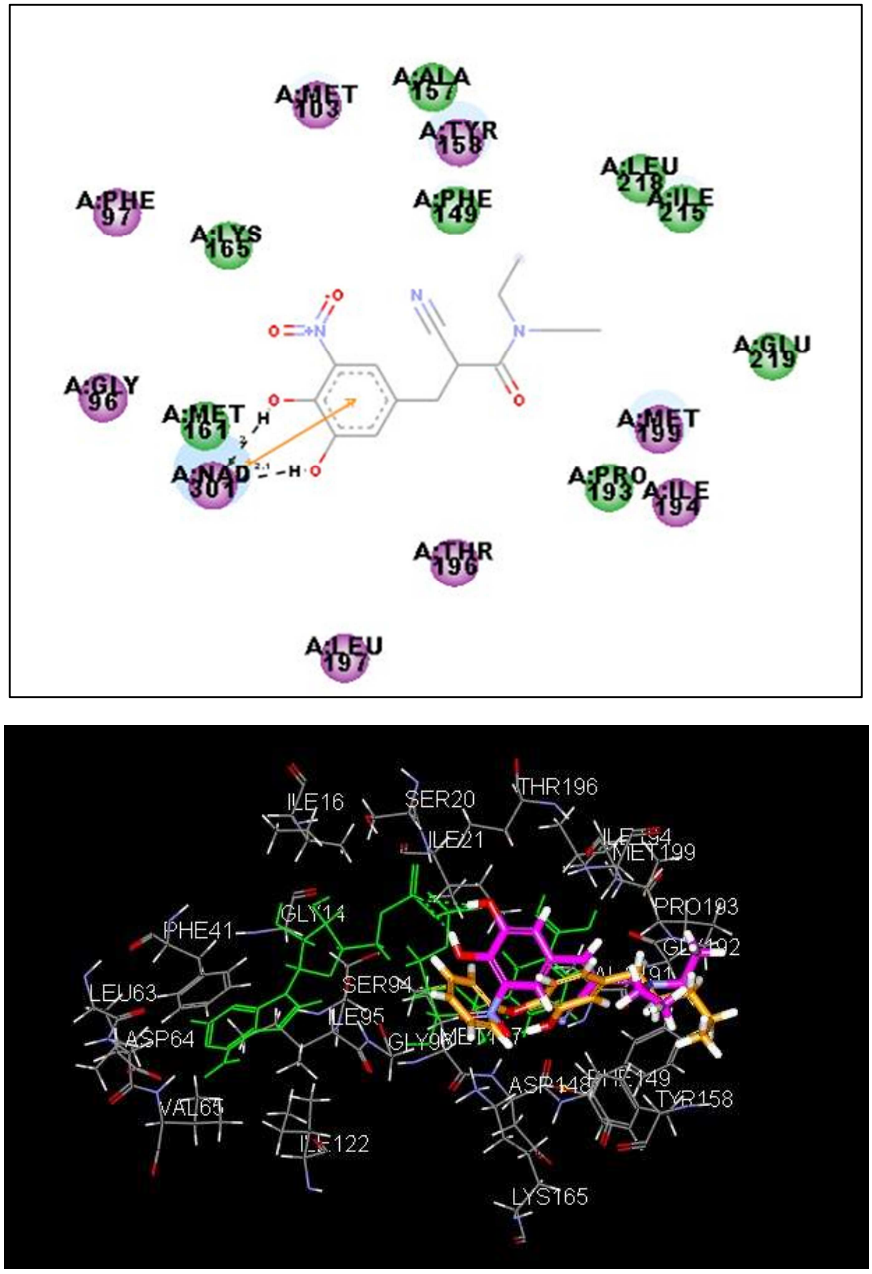


Figure 1. 2D (top) and 3D (bottom) representations of Entacapone (4659568) docked onto InhA receptor. Entacapone (shown in pink) and the native ligand 5-pentyl-2-phenoxyphenol (shown in orange) are overlaid at the binding pocket of InhA (bottom)

The entacapone derivative Compound 4370591 or **1(**Figure 2**)** has the greatest (most negative) binding energy of -98.43 kcal/mol. Compound **1** is a kind of dimer of entacapone. As seen from the 2D diagram, there is a strong π-π interaction between the nitro group of the ligand and PHE149. In addition, H-bonding also occurs between NAD301:H24 (donor) and 4370591:N15 (acceptor). Obviously, **1** interacts with much more residues at the binding site compared to the native ligand and entacapone, resulting in a stronger binding interaction with the InhA enzyme.

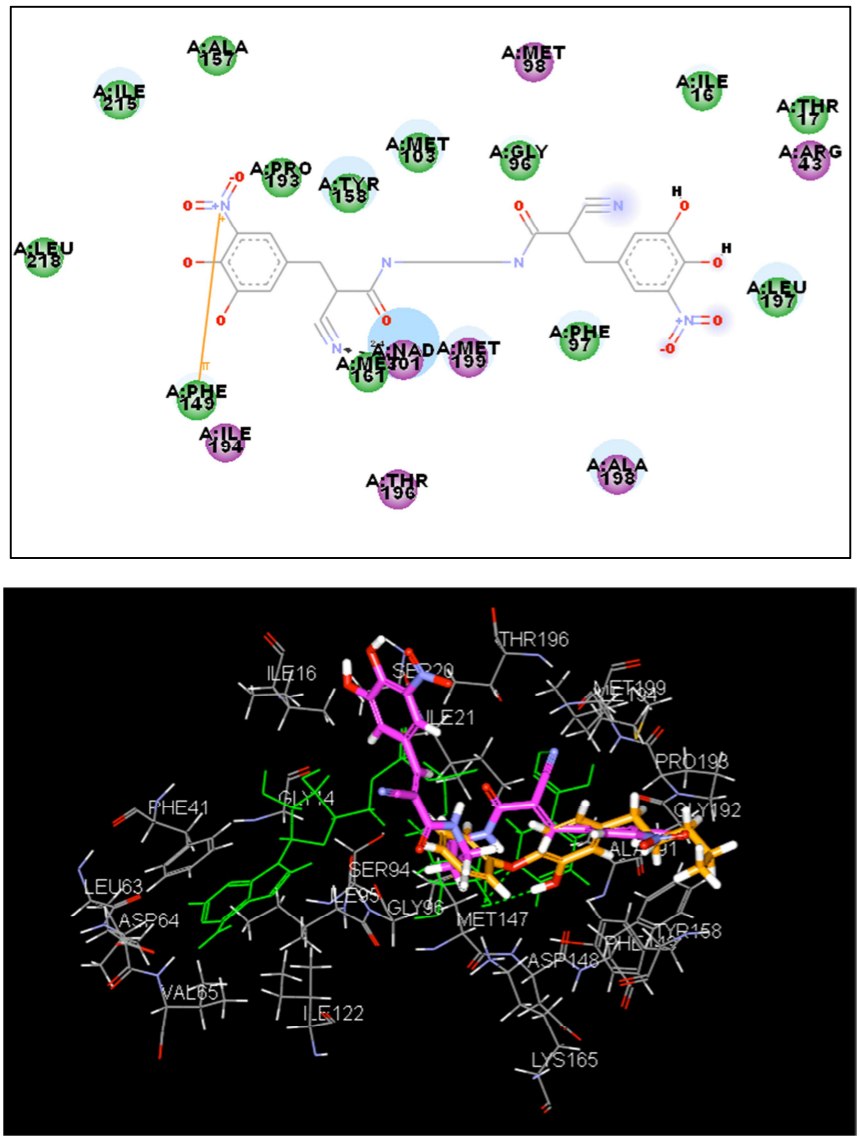


Figure 2. 2D (top) and 3D (bottom) representations of Compound 4370591 or 1 docked onto InhA receptor. Compound 1 (shown in pink) and the native ligand 5-pentyl-2-phenoxyphenol (shown in orange) are overlaid at the binding pocket of InhA (bottom)

The second-rank compound, Compound 18990394 or **2**,is a piperazine derivative of entacapone. When overlaid to the native ligand, **2** forms considerable overlap with 5-pentyl-2-phenoxyphenol. They occupy the same space in the binding site. A number of H-bond interactions were noted with ligand **2** including those between hydrogens of the aromatic ring and NAD301, and between the oxygen of the nitro group and ALA198. π-π interaction is also formed between NAD301 and the aromatic ring of the ligand. These interactions contributed predominantly to the binding energy of -91.44 kcal/mol, a value that is much greater than that of the native ligand and entacapone.

The third high-affinity analogue of entacapone is Compound 18990375 or **3**, with a binding energy of -81.05 kcal/mol. Compound **3** is a hydroxyl derivative of entacapone. Similarly, **3** forms more interactions with InhA compared to the native ligand and entacapone. H-bond interaction was observed between the oxygen of the nitro group of the ligand and the MET199 residue. The ligand, through its aromatic ring, interacts with NAD301 at the binding site. Additional interactions with MET103, ILE215, and ALA198 also contributed to the more favorable binding energy. It is also noteworthy that upon superimposition, there is a significant overlap between the native ligand and **3** (not shown), indicating structural similarity and binding orientation.

Lastly, Compound 22161977 or **4**,returned a binding energy of -56.97 kcal/mol. Like **1** – **3**, it has more ligand interactions compared to the native ligand and entacapone. Analogue **4** is a nitrile-substituted piperazine derivative of entacapone. The 2D interaction diagram involving**4**displays a π-π interaction between NAD301 and the aromatic ring of the ligand. NAD301 also established H-bonding with the same aromatic ring of the ligand. Moreover, both ILE197 and ALA198 formed H-bond interactions with the oxygen of the nitro group.

In general, one common remarkable feature in the interactions of entacapone analogues with InhA is the involvement of NAD coenzyme. NAD significantly contributes to the H-bonding patterns of the ligands. It also allowsπ-π interactions that stabilize the ligand at the active site. The NAD coenzyme, when present at the binding site, is apparently crucial in direct inhibition of InhA enzyme.

CONCLUSION

The structure analogues of entacapone, an Alzheimer's drug that also exhibits antimycobacterial activity, have been identified from ChemMine database by the use of its Similarity Comparisons tool. The analogues were subsequently docked to *Mycobacterium tuberculosis* enol-acyl carrier protein (InhA) enzyme, which is the target of the TB drug Isoniazid, and known ligands such as entacapone and 5-pentyl-2-phenoxyphenol. Out of a score of entacapone analogues identified from ChemMine, four exhibited greater binding energy than entacapone itself and the InhA-bound ligand, 5-pentyl-2-phenoxyphenol. Specifically, the dimer of entacapone as well as a hydroxyl and two piperazine variants of entacapone are potentially more active as direct InhA inhibitors compared with the bound ligand and entacapone. Examination of the interaction diagrams involving these high-affinity entacapone derivatives revealed that the binding predominantly involved strong π–π and H-bonding interactions with the bound NAD cofactor. The results of this work encourage further development of a new class of direct InhA inhibitors based on entacapone structural motif.

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