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# In silico evaluation of natural bioactive compounds as Mycobacterium leprae enoyl acyl carrier protein reductase inhibitors

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**Abstract:**

**Background and Objectives:**

The present study was designed to assess the antileprotic effectiveness of some bioactive natural compounds towards enoyl acyl carrier protein reductase inhibition. Leprosy still constitutes a global pandemic in spite of long years of discovery. The current therapy option is multi-drug treatment using a combination of Dapsone, Rifampicin and Clofazimine. However, mycobacterium leprae counteracted by mutating the drug targets which necessitates the search for novel targets. One such target is enoyl acyl carrier protein reductase that mediates the fatty acid biosynthesis.

**Materials and Methods:**

Multiple (14) ligands of natural origin were drawn from PubChem database and their ADMET parameters were predicted using ADMETLab 2.0 webserver. After, the ligands were docked against the enzyme (PDB ID: 2NTV) at its active site using iGEMDOCK software.

**Results**

ADMET parameters of the tested ligands proven to be accepted by Lipinski's rule of five except for two ligands. Furthermore, molecular docking results revealed that all of the tested compounds showed better binding energy than the reference drug Dapsone. The best of which was silymarin.

**Conclusion**

The tested natural ligands have the capability to control M.leprae.

**Keywords:** Molecular Docking, mycobacterium leprae, leprosy, ENR, ADMET

## 1. Introduction

Although discovered about a century and half ago, leprosy (also known as Hansen's disease) has been continued to be an ongoing suffering infectious concern. Globally, approximately 250000 is its incidence every year with the majority of cases hitting Africa and the Americas [1]. Leprosy is caused by the obligate intracellular bacteria *Mycobacterium leprae* which favors inhabitance in skin tissue (primarily targeting keratinocytes, histiocytes and macrophages therein) leading to dermatological manifestations [2,3]. Besides skin, *M. leprae* also affects the myelinated and non-myelinated Shwann cells [4] of the central nervous system resulting in disability of sensory neurons and nerve dysfunction [5]. Consequently, the dermato-neurological nature of leprosy makes the inflicted patients unacceptable in the society. However, these symptoms vary considerably among inflicted patients of leprosy. This scenario necessitates the intervention to control the disease.

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The current therapy of leprosy is multidrug therapy (MDT) using a combination of Dapsone, Rifampicin and Clofazimine [6]. In terms of mechanism of action, Dapsone act as an inhibitor to dihydropteroate synthase present exclusively in prokaryotes required for folic synthesis [7]. Some evidences showed that Dapsone can also affect fatty acid biosynthesis [8]. On the other hand, Rifampicin blocks transcription within *M.leprae* via inhibition of DNA-dependent RNA polymerase [9]. Clofazimine acts via copmpetion of menaquine, a cofactor of NADH dehydrogenase involved in the mycobacterial electron transport chain on the outer membrane [10]. Although this strategy has proven to be effective in terms of reducing the number of cases since 1990s up to nowadays, two limiting factors have emerged: (i) the resistance (mainly through amino acid substitution) formed by the bacteria toward the cocktail treatment options and (ii) the demanding compliance of the patient to the treatment which necessitates 1 year continuity [11]. In addition, MDT is bactericidal in its action, meaning that it only kills the bacterium and has no healing effect to the neurodegeneration. In some cases, MDT aggravates the situation by causing acute inflammatory reactions that increase neurodegeneration [6].

One of the available novel options to treat leprosy and thus limiting its transmission is to inhibit new targets of *M.leprae*. One such target is to block fatty acid biosynthesis inside the bacterium via inhibiting enoyl acyl carrier protein reductase (ENR) [12]. ENR (EC. 1.3.1.9) is a homodimeric protein comprised of 268 amino acid residues and an active binding site in each monomer (Fig 1). The enzyme has a deep hydrophobic pocket in which the active site present.

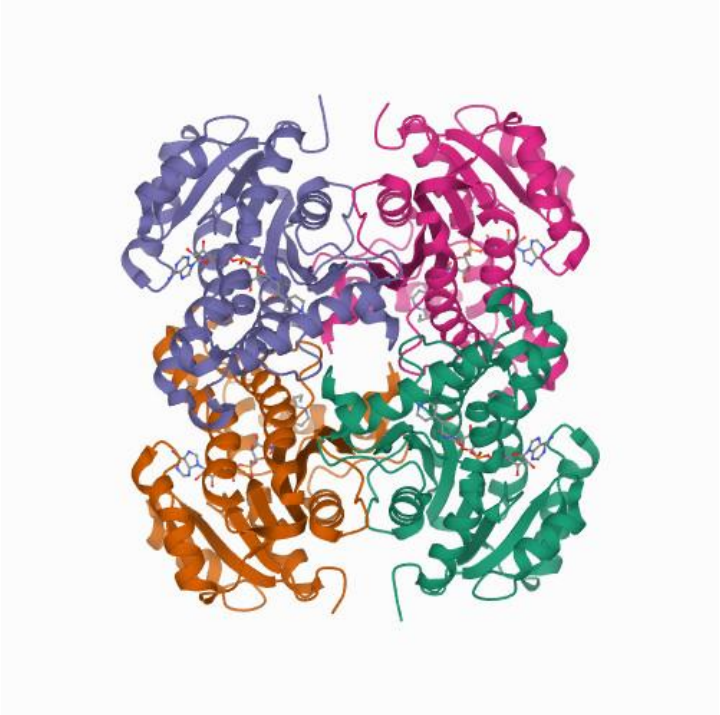

**Figure 1.** the 3D structure of M.leprae ENR in complex with a thiomide drug derivative Prothinomide (PDB ID: 2NTV)

ENR is an oxidoreductase catalyzing the last reaction in the fatty acid elongation via the reduction of c-c double bond to the enoyl intermediate as depicted by Fig 2. Fortunately, there is no mammalian counterpart of the ENR [8] making it an ideal target for leprosy therapy.

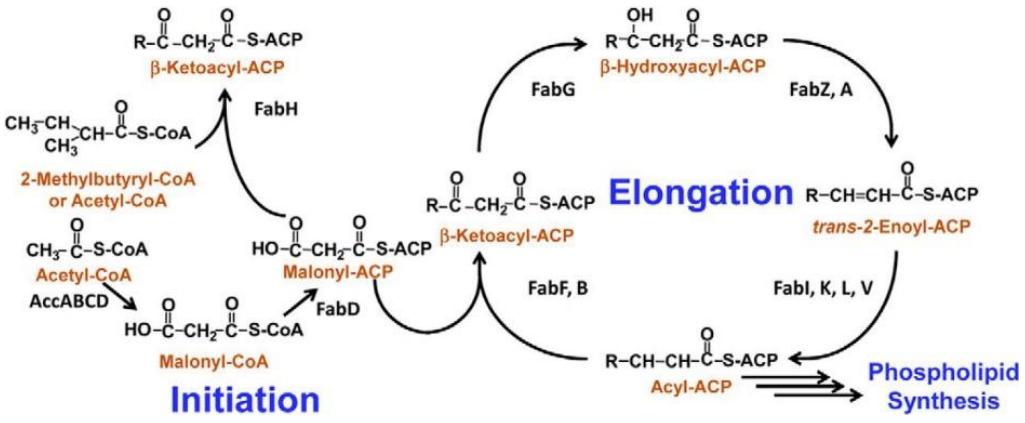


**Figure 2.** Fatty acid biosynthesis cycle using prokaryotic fatty acid synthase complex II. FabG – β-ketoacyl-ACP reductase; FabZ – β-hydroxyacyl-ACP dehydratase; FabI – enoyl-ACP reductase; FabF – β-ketoacyl-ACP synthase.

Nowadays, the investigation of wet lab assays and subsequent *in vivo* evaluation to determine the ability of a synthetic drug or natural bioactive compound as a drug candidate is preceded by the computational (i.e. *in silico*) virtual screening approach. This important step not only lowers the financial costs of undesired tests and experiments, but also improves the easiness as well as the efficiency of finding a lead candidate to a selected target [13]. Recently, many methods and strategies are available to enhance the findings of computational-aided drug design as a preliminary and inclusive action and many of which are available for free [14,15].

Previously, plenty of natural antioxidants and phytochemicals have proven their efficacy as antimicrobial agents. As compared to classical antibiotics, natural products and natural antioxidants exert their antibacterial action with no obvious resistance developed as well as less or no side effects noticed [16,17].

Nonetheless, according to my knowledge, no evidence of bioactive natural products has been used to control leprosy incidence. Therefore, this study aims to screen a set of natural bioactive compounds against ENR as a preliminary step prior to *in vitro* and *in vivo* assays to control *M.leprae*.

## 2. Materials and Methods

### 2.1. Preparation of receptor

*M.leprae* ENR protein structure data was retrieved from protein data bank (https://www.rcsb.org). It has the PDIB ID 2NTV. The protein was crystallized by [18] with a resolution of 2.10 Å and R-value of 2.44.

### Preparation of ligands

A set of 14 ligands were selected from literature known to have antimicrobial and anti-inflammatory effect. The selected ligands were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov) as SDF files and then converted to MOL2 using Open Babel software (http://openbabel.org) [19]. The considered ligands were selected upon antimicrobial bioactivity towards bacterial targets [16,17]. Table 1 lists the tested compounds.

**Table 1.** The selected ligands to be screened as potential inhibitors of M.leprae ENR.

| N | Compound |
|---|---|
| 1 | Silymarin |
| 2 | Trilobatin |
| 3 | Phlorizin |
| 4 | Epigallocatechin gallate |
| 5 | Mangiferin |
| 6 | Curcumin |
| 7 | Polydatin |
| 8 | (E)-Resveratrol 3-(4"-acetyl)-O-beta-D-xylopyranoside |
| 9 | Elatericin B |
| 10 | Schisantherin A |
| 11 | 22-Hydroxytingenone |
| 12 | Capsaicin |
| 13 | Hesperetin |
| 14 | Quercetin |

### 2.2. Docking process

The docking process was performed using iGEMDOCK software [20]. The docking was chosen based on the bounded ligand of the retrieved receptor file (P1H) given that it occupies the active site of the enzyme. The tested compounds were compared with the reference inhibitor of ENR Dapsone. Docking accuracy setting was chosen to be stable docking (300 population size, 80 generations and 10 solutions). Upon completion of the docking process, post docking analysis was performed to figure out the best docking pose and its corresponding energy values. The empirical scoring function of iGemdock software was estimated using the formula:

Energy = vdW + Hbond + Elec

2D diagram visualization of docking output was performed using Discovery Studio Client 21.

### 2.3. ADME properties

Pharmacokinetics as well as Lipinski's rule of five of the all ligands were calculated by the webserver ADMETLAB 2 [21]. ADMETLAB 2 is a quick, accurate, simple interface, and freely available online tool for the prediction of pharmacokinetic and toxicity ADMET properties (absorption, distribution, metabolism, excretion and toxicity).

## 3. Results

Leprosy can infect people with all age groups, but it typically appears at less than 35 years. Household contact ranks first as the main mode of transmission of leprosy (28%), albeit tattooing and zoonotic exposure are other risk factors. It has between 5-10 years latency period before skin and neurological signs overt. Skin lesions appear first and, if untreated, can proceed to a severe form of neurological disease with debilitating complications. [22]. So, exploring the chemical databases to find a likely drug against unprecedented targets of *M.leprae* is urgent [23]. Here I screened 14

ligands of natural origin to assess their inhibition to the fatty acid biosynthesis enzyme ENR and examined the druggability as well.

### 3.1. Docking process

The molecular docking of all the 14 ligands tested exhibited better binding energies than the standard drug Dapsone which gave 98.21 kcal/mole. The difference between reference inhibitor Dapsone and top ranked of the tested compounds (silymarin) is approximately 53 kcal/mole, reflecting the feasibility of the examined bioactive ligands as ENR inhibitors. Table 2 lists the energy values of the screened compounds.

**Table 2.** Docking energy profile of the selected ligands against ENR.

| Compound | Energy | VDW | H Bond | Elec |
|---|---|---|---|---|
| Silymarin | -151.246 | -124.399 | -26.8468 | 0 |
| Trilobatin | -149.438 | -113.807 | -35.6305 | 0 |
| Phlorizin | -148.212 | -123.199 | -25.0127 | 0 |
| Epigallocatechin gallate | -143.312 | -118.658 | -24.6536 | 0 |
| Mangiferin | -132.413 | -97.1822 | -35.2313 | 0 |
| Curcumin | -131.677 | -100.248 | -31.4285 | 0 |
| Polydatin | -131.618 | -112.109 | -19.509 | 0 |
| (E)-Resveratrol 3-(4"-acetyl)-O-beta-D-xylopyranoside | -131.233 | -94.8239 | -36.4095 | 0 |
| Elatericin B | -129.682 | -108.41 | -21.2718 | 0 |
| Schisantherin A | 121.953 | 107.377 | -14.576 | 0 |
| 22-Hydroxytingenone | -112.576 | -106.576 | -6 | 0 |
| Capsaicin | -110.45 | -104.655 | -5.79452 | 0 |
| Hesperetin | -110.206 | -95.3824 | -14.824 | 0 |
| Quercetin | -109.497 | -88.3647 | -21.1323 | 0 |

With regard to ligand-enzyme interaction, the best ligand (silymarin) formed H-bonds with Ile 15, Val 65, Gly 96, Lys 165, Asp 148 and Ile 194 (Fig 3A). Trilobatin on the other hand H-bonded with Gly 14, Ile 16, Ser 20, Asp 64, Val 65, Gly 96 and Lys 165 (Fig 3C). Similarly, phlorizin H-bonding was found to be with Ile 15, Asp 42, Asp 64, Leu 63, Val 65 and Gln 66 as depicted in Fig 3E. This indicates the highly polar nature of the top 3 ligands that rendered them superior in terms of binding energy to the binding pocket of the ENR enzyme.

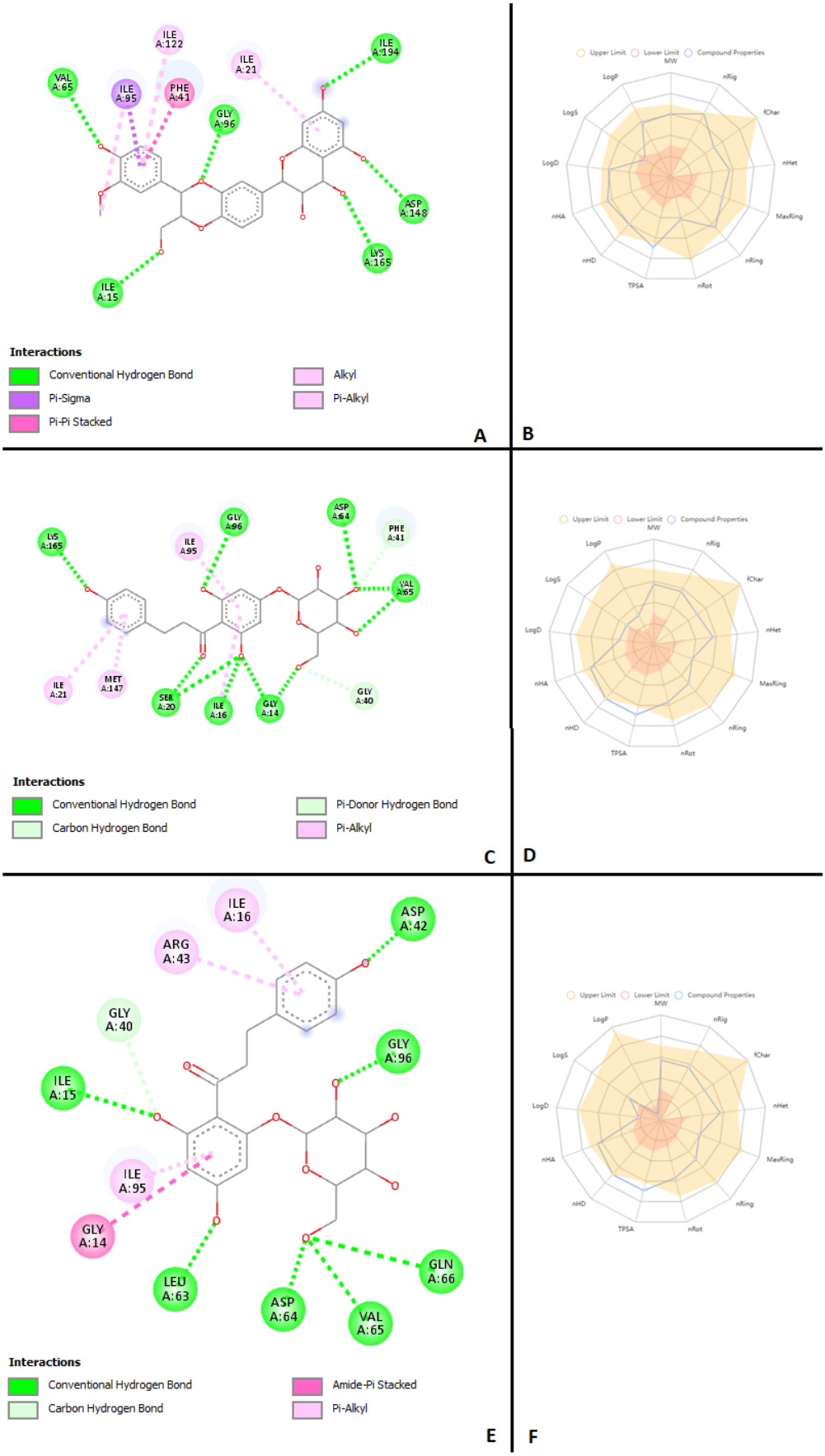


**Figure 3.** 2D Diagram of top 3 docked ligands along with their corresponding ADMET radar chart.

### 3.2. ADMET properties

After getting highly enough values of inhibition based upon docking process, evaluation of the behavior of the lead candidate as a potential drug in parameters such as lipophilicity and reactivity and possible toxicity which can be figured out via prediction of ADMET properties. According to the results of ADMET profile in Table 3, all but two (Epigallocatechin gallate and mangiferin) fit well in the Lipinski's rule of five. Therefore, the tested bioactive compounds suggest themselves to be highly applicable lead inhibitors of *M.leprae* ENR. Furthermore, all of the examined compounds met the criteria of Golden Triangle except for Elatericin B as well as Schsiantherin A. These data suggest that the ligands of the current study will behave well pharmacokinetically. Fig 3B, 3D, 3F also illustrates the graphical ADMET summary of the top 3 ligands.

**Table 3.** ADMET properties of the examined compounds along with their fitness to Lipinski's rule of five and Golden Triangle.

| Ligand | MW | LogP | #HA | #HD | TPSA | #RB | Oral absorption%* | Lipinski | Golden Triangle |
|---|---|---|---|---|---|---|---|---|---|
| Quercetin | 302.04 | 2.155 | 7 | 5 | 131.36 | 1 | 77.207 | Accepted | Accepted |
| Hesperetin | 302.08 | 2.473 | 6 | 3 | 96.22 | 2 | 70.277 | Accepted | Accepted |
| Capsaicin | 305.2 | 3.426 | 4 | 2 | 58.56 | 10 | 90.075 | Accepted | Accepted |
| 22-Hydroxytingenone | 436.26 | 4.311 | 4 | 3 | 77.76 | 0 | 93.537 | Accepted | Accepted |
| Schisantherin A | 536.2 | 4.137 | 9 | 1 | 101.91 | 7 | 100 | Accepted | Rejected |
| Elatericin B | 514.29 | 2.15 | 7 | 3 | 128.97 | 4 | 84.685 | Accepted | Rejected |
| (E)-Resveratrol 3-(4"-acetyl)-O-beta-D-xylopyranoside | 402.13 | 2.052 | 8 | 4 | 125.68 | 6 | 67.277 | Accepted | Accepted |
| Polydatin | 390.13 | 1.112 | 8 | 6 | 139.84 | 5 | 51.086 | Accepted | Accepted |
| Curcumin | 368.13 | 2.742 | 6 | 2 | 93.06 | 8 | 82.19 | Accepted | Accepted |
| Mangiferin | 422.08 | -0.521 | 11 | 8 | 201.28 | 2 | 46.135 | Rejected | Accepted |
| Epigallocatechin gallate | 458.08 | 1.862 | 11 | 8 | 197.37 | 4 | 47.395 | Rejected | Accepted |
| Phlorizin | 436.14 | -0.263 | 10 | 7 | 177.14 | 7 | 37.825 | Accepted | Accepted |
| Trilobatin | 436.14 | 0.714 | 10 | 7 | 177.14 | 7 | 25.425 | Accepted | Accepted |
| Silymarin | 482.12 | 2.015 | 10 | 5 | 155.14 | 4 | 61.861 | Accepted | Accepted |

HA: hydrogen acceptor, HD: hydrogen donor, TPSA: topological polar surface area, RB: rotatable bonds. * calculated using pkCSM webserver [24].

Only 2 of the tested compounds showed violation of the Lipinski's rule of five and another 2 showed rejection based on Golden Triangle criteria. Upon excluding those bioactive compounds, 10 of the 14 ligands are superior ENR inhibitors based on both docking energy as well as ADMET profiles. Moreover, these compounds are also superior in terms of being natural products.

## 4. Conclusion

This was an *in silico* virtual screening study examined the possibility of some natural products as inhibitors of the fatty acid biosynthesis in the leprosy-causing agent *M.leprae*. The molecular docking revealed a powerful inhibition of the ENR by the selected compounds far better than the

classic inhibitor Dapsone. What is more, the pharmacokinetic and ADMET properties of the examined compounds fall well in both Lipinski and Golden Triangle rules. Taken together, this proves that the tested ligands are potent and pharmacokinetically good lead candidates of the ENR inhibition and should be further assayed in *in vitro* wet lab setting.

Given that M.tuberculosis and *M.leprae* share high extent of DNA sequence homology and exhibit high similarity of ENR in both sequence and 3D structure of the enzyme. Hence, I would recommend screening the established inhibitors of *M.tuberculosis* ENR as inhibitors of *M.leprae* ENR counterpart as evidenced by thioamides.

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