Research Article

Structure-based Design Of Benzophenone Inhibitors Targeting Enoyl-ACP Reductase Enzyme

M. M. V. Ramana*, R. S. Lokhande*[1], Urmila. J. Joshi[2], Gayatri. V. Gadre[1], Akshada. J. Joshi[2]
Department of Chemistry, University of Mumbai, Vidyanagari, Santacruz (East), Mumbai, India.
[1]Department of Chemistry, Jaipur National University, Jagatpura, Jaipur, India.
[2]Prin. K. M. Kundnani College of Pharmacy, 23-Jote Joy Building, R. S. Marg, Cuffe Parade, Colaba, Mumbai, India.
***Corresponding author's E-mail:** mmvramana@yahoo.co.in

Accepted on: 20-02-2014; **Finalized on:** 30-04-2014.

ABSTRACT

Mycobacterium leprae is the causative agent of the disease, leprosy. As *M. leprae* develops resistance against most of the drugs, novel drug targets are required in order to design new drugs. The present study is aimed at finding the active site of the protein, which is used as a strategy in drug design. Enoyl-acyl carrier protein reductase is one of the receptor proteins used in drug discovery for screening anti-leprosy agents. The crystal structure of the inhibited *M. leprae* InhA complex PDB ID: 2NTV provide the details of protein–ligand interactions. Series of compounds having benzophenone moiety were designed. The virtually designed drug candidates have docked well in the active site region of the protein. The prediction of ADME properties was also performed by Qikprop software.

Keywords: Benzophenone, Drug design, Enoyl-acyl carrier protein reductase, *Mycobacterium leprae*.

INTRODUCTION

Leprosy was originated long back, over 5000 years ago, going back to the Neolithic times.[1] The World Health Organization (WHO) estimated that 2 million people worldwide were infected with *Mycobacterium leprae*.[2] The effective treatment for the disease appeared in 1940's with the introduction of Dapsone. Soon the bacteria developed resistance for Dapsone. After 1980's multi drug therapy (MDT) was introduced in which the combination of three drugs Dapsone, Clofazimine and Rifampicin were used and found effective.[3-6] But it was expensive and its long term treatment led to resistance. Therefore, there is an urgent need for the development of novel antileprosy agents.

The drugs Thioamide, Ethionamide (ETH) and Prothionamide (PTH) are clinically effective in the treatment of *M. leprae*, *M. Tuberculosis* complex infections.[7] These are second line drugs for tuberculosis. The crystal structure of the inhibited *M. leprae* InhA complex (PDB ID: 2NTV) provide the details of protein-ligand interactions. It is reported that Prothionamide binds with Nicotinamide adenine dinucleotide (NAD^+) and this adduct inhibits *Mycobacterium leprae* (InhA), the enzyme is the product of InhA gene which plays essential role in Mycolic acid biosynthesis. This crystal structure[7] can be utilized to test new possible inhibitors using drug design techniques. The availability of three-dimensional coordinates for target enzyme enables the use of structure-based drug design techniques. Drug design that relies on the knowledge of the three-dimensional structure of the biomolecular target is known as structure-based drug design. Structure of the biological target can be obtained through methods such as X-ray crystallography or NMR spectroscopy.[8] Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist. X-ray and NMR methods can resolve the protein structure to a resolution of a few angstroms. At this level of resolution, researchers can accurately examine the interactions between atoms in protein targets and atoms in potential drug molecules that bind to the protein. One advantage is, it helps in reducing research cost and saves environment by avoiding wastage of huge number and quantity of chemicals during synthesis of novel compounds in chemistry laboratory.[9,10] This makes structure based drug design one of the potent methods in drug design.[11]

Target-Enoyl-acyl carrier protein reductase enzyme

The enzymes of the fatty acid biosynthesis pathway (FAS II) in bacteria, represent interesting targets for antimicrobial drug design because their mammalian counterpart FAS I uses a single, multifunctional enzyme with low sequence similarity.[12] This provides an opportunity to selectively target this essential bacterial pathway without interfering with mammalian enzymes. Enoyl-acyl carrier protein reductase is a key enzyme in the bacterial FAS II. It is a rate-controlling enzyme in the FAS II pathway, which makes it stand out as an ideal target among the other FAS II enzymes. The final and rate–determining step of chain elongation in the bacterial fatty acid biosynthesis is the reduction of Enoyl–ACP to an Acyl–ACP, which is catalyzed by the Enoyl–ACP reductase. Due to its essential role in metabolism and sequence conservation across many bacterial species[13] it is an attractive target for antibacterial drug discovery. The enzyme is a member of the short-chain alcohol

dehydrogenase/reductase (SDR) superfamily characterized by a catalytic triad of key tyrosine, lysine, and serine residues that reduce a double bond in the enoyl substrate with NAD^+ or $NADP^+$ as an acceptor, a key step in bacterial production of fatty acids.[14] The systematic name of this enzyme class is acyl-[acyl carrier-protein]: $NADP^+$ oxidoreductase (A-specific).

MATERIALS AND METHODS

Preparation of protein

The selected protein Enoyl-ACP reductase crystal structure was available PDB ID: 2NTV (www.rcsb.org). The selected 3D structure of enzyme was having natural inhibitor *2-propyl-isonicotinic-acyl-nicotinamide-adenine dinucleotide*. It is also referred as PTH-NAD^+ adduct.[7] The PDB structure of enzyme Enoyl-ACP reductase [PDB id: 2NTV] was downloaded, refined, and prepared using Schrodinger protein preparation wizard tool (Glide, version 5.9, Maestro 9.4, Schrodinger), which performed the following steps: assigning of bond orders, addition of hydrogens, optimization of hydrogen bonds, correction of charges, and minimization of the protein complex. All the unnecessary water molecules, ligands, and cofactors were removed (preprocess) from the proteins which were taken in.mae format. The tool neutralized the side chains that were not close to the binding cavity and did not participate in salt bridges. This step was then followed by restrained minimization of co-crystallized complex, which reoriented side-chain hydroxyl groups and alleviated potential steric clashes. The complex obtained was minimized using OPLS_2005 force field (Kaminski and Friesner, 2001).

Preparation of ligands

Structure of the Enoyl-ACP reductase inhibitors were sketched using built panel of Maestro and taken in.mae format. Ligand Preparation is a utility of Schrodinger software suit that combines tools for generating 3D structures from 2D and searching for all possible steric isomers, tautomers, and perform a geometry minimization of the ligands. Molecular Mechanics Force Fields (OPLS_2005) with default settings were used for the ligand minimization.

All designed structures were initiated from substituted benzophenones as benzophenones are reported as biologically active compounds. They exhibit various biological activities such as antibacterial,[15,16] antimalarial,[17] anticancer,[18] antiHiv[19,20] etc. Aminobenzophenones are a new class of biologically active compounds. These compounds showed various types of biological activity such as antitumor and anticonvulsive activities[21] and activity against the hepatitis C RNA virus.[22] Recently, it has been shown that amino benzophenones with an amino group at the ortho position of the benzophenone ring showed fascinating biological activity characteristics.[23,24] So, our present study is aimed at studying the interaction details between Enoyl-ACP reductase and benzophenone class of compounds, which is the basic structure of lead compound. Its general structure is depicted in Figure 1.

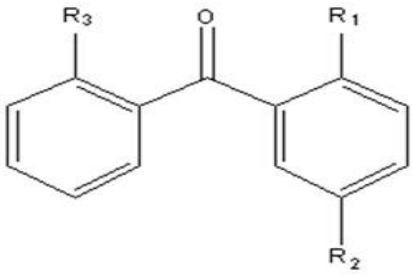


Figure 1:

Docking methodology

The docking studies were carried out using extra precision mode of Glide using default parameters. The active site was defined by generation of a grid box such that the co-crystallized ligand occupied the center of the box. The grid-based ligand docking with energetics (glide) algorithm approximated a systematic search of positions, orientations, and conformations of the ligand in the enzyme binding pocket via a series of hierarchical filters. The shape and properties of the receptor were represented on a grid by several different sets of fields, which provided progressively more accurate scoring of the ligand pose. The inhibitor was extracted from the complex and redocked. The final docked conformation of the inhibitor was aligned to the original conformation and root mean square deviation (RMSD) calculated. RMSD value less than 2 confirmed the accuracy of the docking program. The ligands of the dataset were docked flexibly into the receptor using default parameters. No constraints of similarity scoring were applied. The G-score value was calculated by taking into consideration factors as favorable vander Waals, coulombic, lipophilic and hydrogen-bonding interactions and penalizing for steric and buried polar clashes.

Benzophenone class of compounds were prepared and supplied to the docking software. Their G scores are depicted in Table 1.

QikProp descriptors

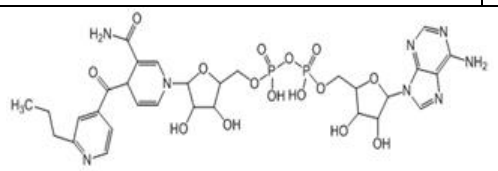
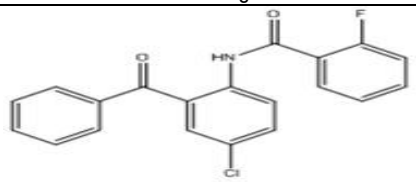
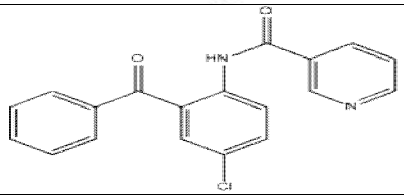
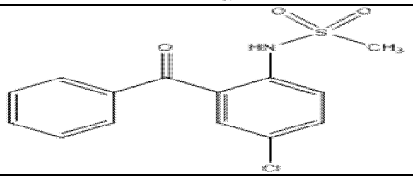
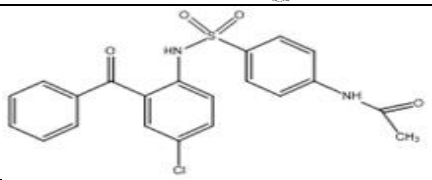
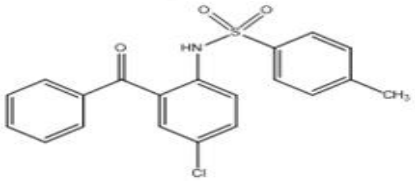
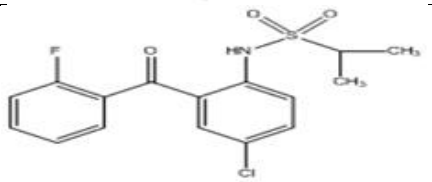
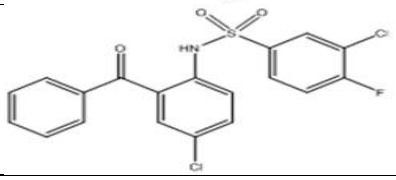
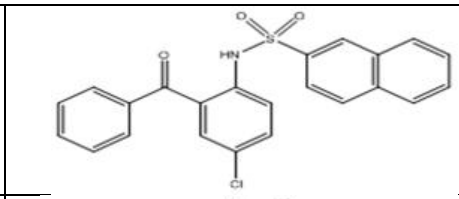
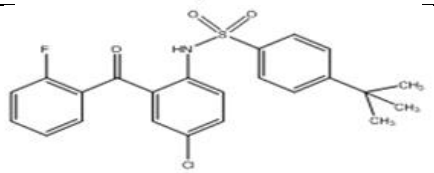
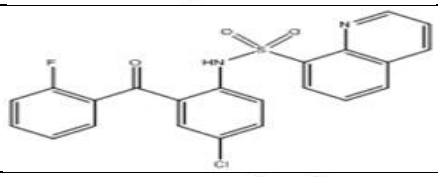
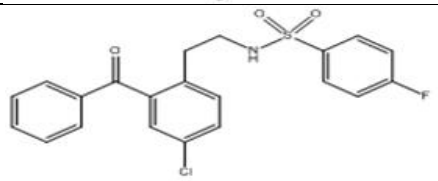
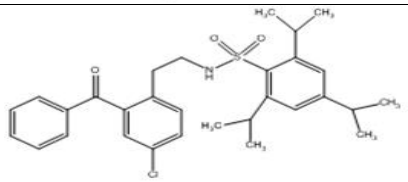
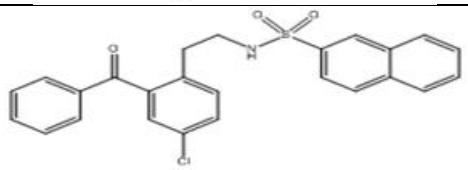
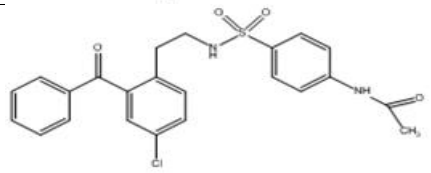
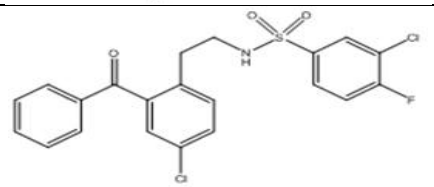
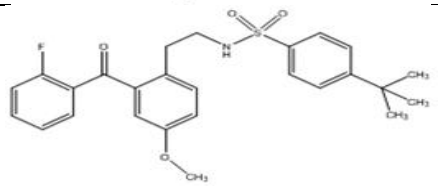
These molecules were also subjected to further filter via Lipinski's rule of five to identify compounds with favorable absorption, distribution, metabolism and excretion (ADME) properties. They were calculated using QikProp. In the present study, QikProp was run in normal processing mode with default options. The molecules were analyzed for drug-likeness by assessing their physicochemical properties and by applying Lipinski's rule of five.

RESULTS AND DISCUSSION

The ligand fitting into the active site of protein and hence higher interactions depends on the binding affinity and possibilities of number of hydrogen bonds between ligand atoms and amino acids. The hydrogen bond gives stable conformation to the complex and so generally gives

better drug-like properties. When the benzophenone molecules were docked into the active site of the protein, it was observed that H-bonds which the actives formed were with Trp222, Ile194, Gly96, Phe41. The other amino acids Tyr158, Lys165, Phe149, Ile21, Gly192 were taking part in making H-bonds with ligand atoms. Most actives were able to form at least two H-bonds with the receptor. Ile194 was the most prone amino acid to make H-bond with most ligand atoms. Table 2 shows the 2D images of some of the active compounds docked into enzyme.

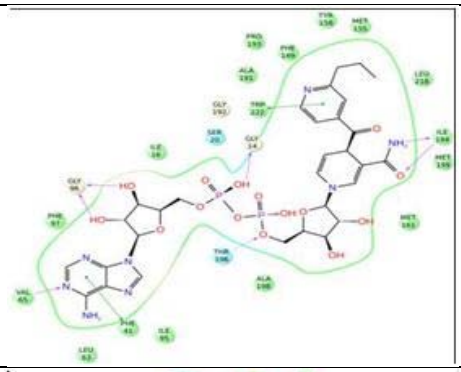
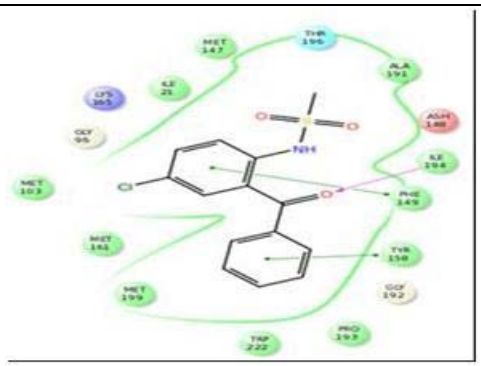
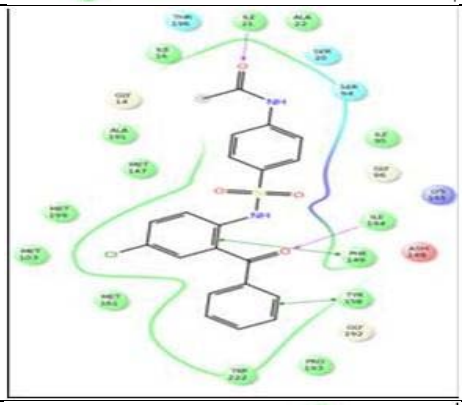
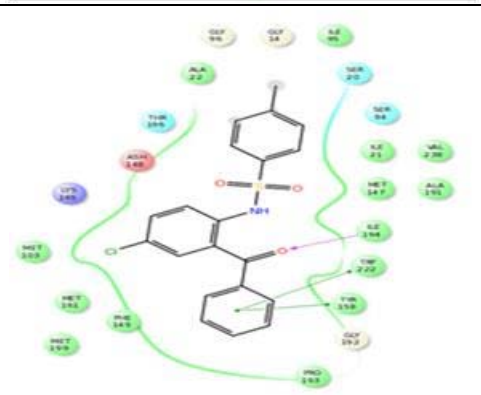
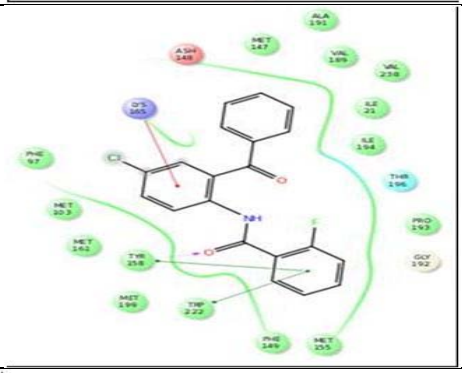
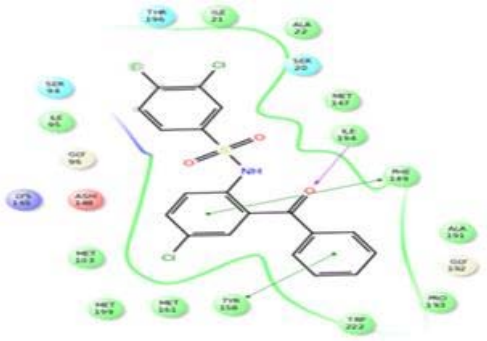
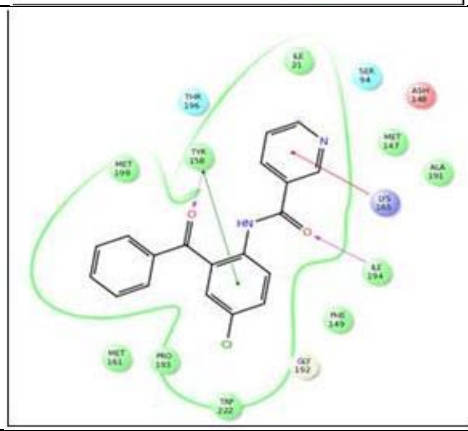
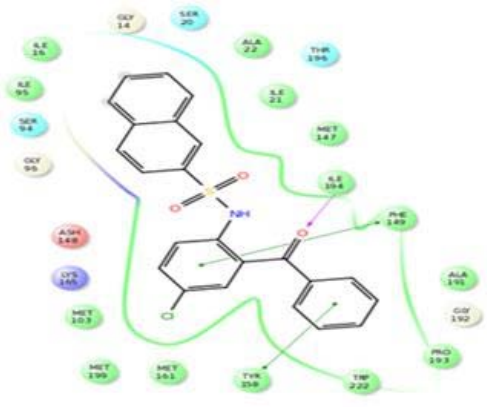
Table 1: Docking results of standard ligand and designed molecules

No	Molecule	G score
1	 Standard Ligand	-12.1
2		-8.35
3		-8.21
4		-8.1
5		-10.25
6		-9.3
7		-7.66
8		-9.27
9		-9.09
10		-9.4
11		-8.05
12		-7.95
13		-9.3
14		-9.32
15		-9.32
16		-8.99
17		-9.95

The Lipinski's rule for drug like molecules states that the molecule should have molecular weight <500 Daltons, H-bond donors <5, H-bond acceptors <10 and a logP of <5. The compound can be considered a probable drug candidate even if it violates one of the above-mentioned criteria. For molecules, as given in Table 4 the partition coefficient (QPlogPo/w) critical for estimating the absorption of drugs within the body, ranged between 2.752-6.797. Crossing the blood–brain barrier (BBB), which is a prerequisite for the entry of drugs to CNS, was

found to be in the acceptable range ((-3)-1.2) indicating that the compounds may be considered for further development. Caco-2 cell permeability (QPPCaco), a model governing gut-blood barrier, ranged from 251.518 to 1991.679. MDCK cell permeability (QPPMDCK), a model that mimics blood brain barrier, ranges from 274.118 to 9421.084. Further, the predicted percentage human oral absorption for all molecules ranged from 88.648 to 100 %. All these pharmacokinetic parameters were found to be within the acceptable range (Table 4 footnote).

Table 2: 2D structures of standard ligand and some benzophenones

Molecule No.	2D Structure	Molecule No.	2D Structure
1		4	
5		6	
2		8	
3		9	

H-bond interactions are indicated with purple lines; pi-pi stacking is indicated with green line; pi-cation is indicated with red line.

Table 3: Amino acid interactions of standard ligand, designed molecules

Molecule No.	Interacting Residues											
	Val 65	Trp 222	Gly 96	Gly 14	Phe 41	Thr 196	Ile 194	Phe 149	Tyr 158	Gly 192	Ile 21	Lys 165
1. Standard ligand	✓	✓	✓	✓	✓	✓	✓					
2		✓							✓			✓
3							✓		✓			✓
4							✓	✓	✓			
5							✓	✓	✓		✓	
6		✓					✓		✓			
7							✓			✓		
8							✓	✓	✓			
9							✓	✓	✓			
10												✓
11			✓		✓							
12								✓				
13		✓							✓			
14								✓				
15								✓		✓		
16		✓						✓				
17			✓		✓							

Table 4: Physiochemical descriptors and ADME properties of designed molecules

Molecule[a]	QPlogPo/w[b]	QPlogS[c]	QPlogBB[d]	QPPCaco[e]	QPPMDCK[f]	% Human oral absorption[g]
2	4.691	-5.501	-0.25	1464.931	2889.555	100
3	3.452	-4.295	-0.548	878.25	1055.803	100
4	2.752	-3.972	-0.676	747.19	976.224	94.488
5	3.2	-5.276	-1.368	251.518	274.118	88.648
6	3.893	-4.914	-0.723	748.729	880.277	100
7	3.733	-5.545	-0.329	1066.52	2338.391	100
8	4.34	-5.425	-0.375	905.578	4359.791	100
9	4.615	-5.891	-0.799	732.365	869.85	100
10	5.067	-6.123	-0.608	940.821	1806.514	96.873
11	4.078	-4.877	-0.521	949.228	1762.475	100
12	5.745	-7.985	-0.533	1658.858	4427.534	100
13	6.797	-7.916	-0.656	1991.679	2544.898	100
14	6.524	-8.947	-0.694	1658.858	2317.022	100
15	4.924	-8.368	-1.728	349.971	431.017	100
16	6.155	-8.598	-0.4	1658.858	9421.084	100
17	5.137	-6.854	-1.09	943.039	734.353	100

[a] Molecules; [b] Predicted octanol/water partition coefficient log p (acceptable range 2.0 to 6.5); [c] Predicted aqueous solubility; S in mol/L (acceptable range (-6.5) to 0.5); [d] Predicted BBB permeability (acceptable range (-3) to 1.2); [e] Predicted Caco-2 cell permeability in nm/s (acceptable range: <25 is poor and >500 is great); [f] Predicted apparent MDCK cell permeability in nm/s (acceptable range: <25 is poor and >500 is great); [g] Percentage of human oral absorption (acceptable range:<25 % is poor and >80 % is high).

CONCLUSION

It can be concluded by docking results, that molecules show good docking values, the docking scores indicate that some molecules like no. 5, 2, 3, 4, 6, 8, 9 are well docked molecules and their hydrogen bond interactions reflect the possibilities of antileprosy drug-likeness. Also the drug likeness can be supported by ADME properties of molecules which are in acceptable range and better than the standard ligand. It can be concluded that the designed benzophenone class of molecules are positively

interacting with Enoyl-ACP reductase protein and hence can be further processed as anti-leprosy drug canditates.

REFERENCES

1. Wheeler PR, Leprosy–clues about the biochemistry of Mycobacterium leprae and its host dependency from the genome World, Journal of Microbiology and Biotechnology, 2003.
2. Leprosy-global situation, Wkly Epidemiol Rec, 75, 2000, 226-231.
3. Chemotherapy of leprosy, Report of a WHO study group, World Health Organ Tech Rep Ser, 847, 1994, 1-24.
4. Norman G, Joseph G, Ebenezer G, Rao SP, Job CK, Secondary rifampin resistance following multi-drug therapy—a case report, Int J Lepr Other Mycobact Dis, 71, 2003, 18-21.
5. Guelpa-Lauras CC, Grosset JH, Constant-Desportes M, Brucker G, Nine cases of rifampin-resistant leprosy, Int J Lepr Other Mycobact Dis, 52, 1984, 101-102.
6. Ji B, Rifampicin-resistant leprosy: a review and a research proposal of a pilot study, Lepr Rev, 73, 2002, 2-8.
7. Wang F, Langley R, Gulten G, Dover LG, Besra GS, Jacobs WR, Sacchettini JC, Mechanism of thioamide drug action against tuberculosis and leprosy, J Exp Med, 204, 2007, 73-78.
8. Leach AR, Harren J, Structure-based Drug Discovery, Berlin: Springer, 2007.
9. Keenan SM, Geyer JA, Welsh WJ, Prigge ST, Waters NC, Rational inhibitor design and iterative screening in the identification of selective plasmodial cyclin dependent kinase inhibitors, Comb Chem High Throughput Screen, 8, 2005, 27-38.
10. Richards WG, Computer aided drug design, Pure & Appl Chem, 66, 1994, 1589-1596.
11. Warshel A, Computer Modeling of Chemical Reactions in Enzymes and Solutions, Wiley, New York, 1991.
12. Wright HT, Reynolds KA, Antibacterial targets in fatty acid biosynthesis, Curr Opin Microbiol., 10, 2007, 447-453.
13. Kapoor M, Gopalakrishnapai J, Surolia N, Surolia A, Mutational analysis of the triclosan-binding region of Enoyl-ACP (acyl-carrier protein) reductase from Plasmodium falciparum., Biochem J, 381, 2004, 735-741.
14. White SW, Zheng J, Zhang YM, Rock, The structural biology of type II fatty acid biosynthesis, Annu Rev Biochem, 74, 2005, 791-831.
15. Vooturi, SK, Dewal MB, Firestine SM, Examination of a synthetic benzophenone membrane-targeted antibiotic, Organic & Biomolecular Chemistry, 9(18), 2011, 6367-6372.
16. Naldoni FJ, Claudino ALR, Cruz JW Jr, Chavasco JK, Faria e Silva PM, Veloso MP, Dos Santos MH, Antimicrobial activity of benzophenones and extracts from the fruits of Garcinia brasiliensis, Journal of Medicinal Food, 12(2), 2009, 403-407.
17. Kaushik N, Pradhan P, A review on various scaffolds active against PfPFT target, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 1(3), 2010, 271-283.
18. Sakowski J, Bohm M, Sattler I, Dahse HM, Schlitzer M, Synthesis, Molecular Modeling, and Structure-Activity Relationship of Benzophenone-Based CAAX-Peptidomimetic Farnesyl transferase Inhibitors, J. Med. Chem, 44, 2001, 2886-2899.
19. Wyatt PG, Bethell RC, Cammack N, Charon D, Dodic N, Dumaitre B, Evans DN, Green DVS, Hopewell PL, Humber DC, Lamont RB, Orr DC, Plested SJ, Ryan MD, Sollis SL, Storer R, Weingartent GG, Benzophenone Derivatives: A Novel Series of Potent and Selective Inhibitors of Human Immunodeficiency Virus Type 1 Reverse Transcriptase, J. Med. Chem, 38, 1995, 1657-1665.
20. Ma XD, Zhang X, Dai HF, Yang SQ, Yang LM, Gu SX, Zheng YT, He QQ, Chen FE, Synthesis and biological activity of naphthyl-substituted (B-ring) benzophenone derivatives as novel non-nucleoside HIV-1 reverse transcriptase inhibitors, Bioorganic & Medicinal Chemistry, 19, 2011, 4601-4607.
21. Doss S, Baghos V, Abdelhamid A, Halim MM, Synthesis of sultam derivatives with expected biological activity, Molecules, 6, 2000, 816.
22. Wyss D, Arasappan A, Senior M, Wang YS, Beyer B, Njoroge G, McCoy M, Non-peptidic small-molecule inhibitors of the single-chain hepatitis C virus NS3 protease/ NS4A cofactor complex discovered by structure-based NMR screening, J. Med. Chem, 47, 2004, 2486.
23. Jing-Ping L, Chun-Wei C, Jeng-Shin S, Yung-Ning Y, Ching-Fang Y, Huan-Yi T, Yu-Kang L, Yi-Ling C, Chung-Ming C, Hsing-Pang H, Synthesis and structure–activity relationship of 2-aminobenzophenone derivatives as antimitotic agents, J. Med. Chem, 45, 2002, 2556.
24. Hsing-Pang H, Jing-Ping L, Ying-Ting L, Neeraj M, Jang-Yang C, Yung-Ning Y, Shuenn-Shing C, Uan-Kang T, Chun-Wei C, Tung-Wei C, Chi-Hung L, Ying-Ying C, Chiung-Chiu W, Structure–activity and crystallographic analysis of Benzophenone derivatives the potential anticancer agents, Bioorg. Med. Chem. Lett., 13, 2003, 101.

Source of Support: Nil, **Conflict of Interest:** None.