AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Exploring Fatty Acid Synthase Inhibitors From An Approved Drugs Database Through Receptor-Based Pharmacophore Modelling And Docking

Anup S. Ramdhave[1*], Mukesh Nandave[1]
1.SPP School of Pharmacy & Technology Management, SVKM's NMIMS, Mumbai, INDIA

ABSTRACT

Fatty acid synthase (FASN) is a key enzyme involved in neoplastic lipogenesis and accumulating evidence suggests that overexpression of FASN is common in many cancers. To date, not many compounds are known to inhibit FASN. Thus, our aim was to explore more lead compounds which are able to inhibit FASN. High affinity compounds for FASN were searched among a database of chemical structures comprising 9,127 approved drugs, chemical isolates from traditional medicinal herbs, and regulated chemicals, termed the SWEETLEAD. The screening protocol consisted of a high-throughput pharmacophore screening followed by an extensive docking and scoring study. Three different pharmacophore models and a merged pharmacophore model were generated using three different inactive crystal structures of FASN. Pharmacophore-based screening yielded overall 1,661 hits. Afterwards, all these hit compounds were docked to the inactive form of FASN using docking and scoring protocols. The best pose was further evaluated based on the existence of key residues for antagonist binding in its vicinity which retrieved 38 hits. After final evaluations based on S-score, 12 hits were revealed. Although the experimental validation of these compounds are lacking, computational methods predicts them as strong binders. Further experimental validation of these compounds will confirm this *in silico* study and their potential role of these hits in treatment of cancer with high FASN expression.

Keywords: FASN antagonist, drug repurposing, pharmacophore modelling, docking

*Corresponding Author Email: mukeshnandave@gmail.com
Received 06 February 2016, Accepted 10 February 2016

Please cite this article as: Ramdhave AS *et al*., Exploring Fatty Acid Synthase Inhibitors From An Approved Drugs Database Through Receptor-Based Pharmacophore Modelling And Docking. American Journal of PharmTech Research 2016.

INTRODUCTION

Fatty acid synthase (FASN) is a key enzyme involved in neoplastic lipogenesis and accumulating evidence suggests that overexpression of FASN is common in many cancers[1]. FASN is involved in lipogenesis and the production of long-chain fatty acids from acetyl-coenzyme A (CoA) and malonyl-CoA. Uptake of glucose into cancer cells leads to the production of pyruvate via the glycolytic pathway. Pyruvate is utilized to produce ATP via the Krebs cycle in the mitochondria; in turn, acetyl-CoA, one of the products, acts as a substrate for neoplastic lipogenesis. In rapidly proliferating cancer cells, fatty acids can be synthesized de novo in order to provide lipids for membrane formation and energy production via β-oxidation and lipid modification of proteins[2].

Recently, the crystal structure and catalytically active sites of FASN have been delineated. FASN is made up of a paired multifunctional poly peptide with seven catalytic domains that include an acyl-carrier protein (ACP). These domains (in linear order from the carboxy terminus) are: thioesterase, ACP, β-ketoacyl reductase, enoyl reductase, β-hydroxyacyl dehydratase, acetyl/malonyl-CoA transferase and β-ketoacyl synthase. There are two additional non enzymatic domains: a pseudoketoreductase; and a peripheral pseudomethyltransferase, which is probably a remnant of an ancestral methyltransferase domain maintained in some related polyketide synthases [1,3].

Importantly, inhibitors of the ketoreductase domain and small-molecule inhibitors of the β-ketoacyl synthase and thioesterase domains have been described as having anti-oncogenic properties. To date, not many compounds are known to inhibit FASN. This includes cerulenin derivative C75, the beta-lactone orlistat, the green tea polyphenol epigallocatechin-3-gallate (EGCG) and other naturally occurring flavonoids (i.e., luteolin, quercetin, and kaempferol), as well as the antibiotic triclosan[2,4.]

Thus, our aim was to explore more lead compounds which are able to inhibit FASN. Drug repurposing, particularly of previously approved drugs, is an attractive strategy because, in theory, the drug development cycle time and the most importantly, the cost of a repurposing program are significantly shorter than de novo R&D. Pharmacophore approaches have become one of the major tools in drug discovery. Various ligand-based and structure-based methods have been developed for improved pharmacophore modelling and have been successfully and extensively applied in virtual screening, de novo design and lead optimization [5].

In this article, we have utilized structure-based pharmacophore approach to explore leads from an approved drugs database, which were further docked with FASN to predict them as robust FASN

inhibitors. We have used individual pharmacophores generated from orlistat, triclosan, GSK2194069 and a combined pharmacophore thereof, to screen the approved drugs database. These hits were then docked with 2PX6 (containing orlistat as a ligand) to determine the best fit molecules.

MATERIALS AND METHOD

Generation of the pharmacophore model

Three different inactive states of FASN were used to create a structure-based pharmacophore model using LigandScout® software tool. For each antagonist-bound complex structure extracted from Protein Data Bank (PDB ids: 2PX6, 4PIV and 4W9N), a pharmacophore model was generated. A single merged pharmacophore model was also generated from above three pharmacophores. Excluded volumes representing the sterically occupied regions by the receptor, were taken into account to increase the selectivity of the model.

SWEETLEAD database to be screened

A highly-curated *in silico* database of chemical structures comprising approved drugs, chemical isolates from traditional medicinal herbs, and regulated chemicals, termed the SWEETLEAD was used[6]. This subset was especially selected because it consisted of only dug-like compounds. Database was screened against each structure-based pharmacophore generated as described above.

Evaluations through docking and scoring

The computations for docking analysis were carried out using Molecular Operating Environment (MOE 2009.10) software. The co-crystallized protein structure of 2PX6 was used for docking. The crystal structure was protonated and the energy was minimized using AMBER99 force field. The active site was generated using atom selector and was labelled as the 'binding site'. All the hits of pharmacophore screening were retrieved from SWEETLEAD database and used as ligands for the docking studies. Docking was performed using Alpha triangle placement method with London dG scoring. For each compound, the conformation with the highest S-score was selected and evaluated based on the interacting residues of orlistat of 2PX6 complex.

RESULTS AND DISCUSSION

Generation of the pharmacophore

Three different pharmacophore models were generated using the structural information of inactive crystal structures in complex with three different antagonists (PDB ids: 2PX6, 4PIV and 4W9N). A shared pharmacophore could not be generated as all antagonists had different binding sites and thus, a merged pharmacophore was generated. The pharmacophore model generated had 3-4

hydrophobic features which are depicted with yellow spheres. Besides, each model, except 4W9N holds an average of one hydrogen bond donor and three hydrogen bond acceptors depicted by green and red arrows respectively (Figure 1). In all three ligands, a positive ionizable group located on the backbone Nitrogen atom was absent (which is denoted by blue sphere). The so called "merged" pharmacophore model holds the features of all three individual pharmacophore.

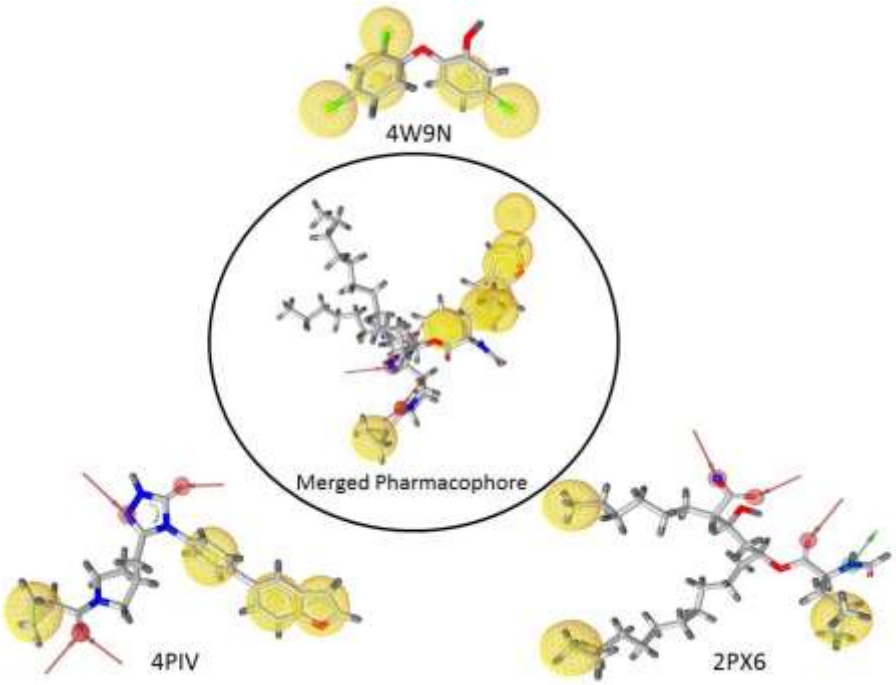


Figure 1: Pharmacophore models of three different X-ray crystal structure of human FASN receptor illustrated with PDB ids and the bound antagonist. Hydrophobic features are depicted with yellow spheres, hydrogen bond donor and acceptors by green and red arrows. All models are illustrated by LigandScout software tool.

Pharmacophore screening

SWEETLEAD database of compounds consisting of 9,127 approved drugs, chemical isolates from traditional medicinal herbs, and regulated chemicals was screened against individual as well as merged pharmacophores using LigandScout default parameters; scoring function taken as "Relative Pharmacophore Fit", the number of omitted features set to 2, and check exclusion volume turned on. Table 1 depicts the number of hit molecules out of 9,127 molecules which passed the screening and were selected for further evaluation through MOE docking tool. Pharmacophore screening helped in narrowing down the exact molecules which possess some of the features required for inhibition of FASN receptor.

Table 1: Number of hits against various pharmacophore models retrieved with default parameters of Ligand Scout

Pharmacophore model	Number of hits with default parameters of LigandScout
2PX6	48
4PIV	90
4W9N	1523
Merged	0

Docking: Evaluations based on binding mode and score values

The docking studies were performed using Molecular Operating Environment (MOE). Hits of pharmacophore screening (1661 hits, which is summation of individual PDB hits) as described above were retrieved from SWEETLEAD database, and were docked to the binding site of inactive crystal structure (PDB: 2XP6). The X-ray crystal of orlistat bound to the active site of truncated FASN revealed that orlistat binds in an extended conformation with covalent attachment to Ser2308 of the catalytic triad within subdomain A. Glu2251 forms a hydrogen bond to the nitrogen atom of the N-formylamide group. The hexanoyl tail, which extends off the C2 carbon atom of Orlistat, interacts with Gly2339, Thr2342, Tyr2343 and Tyr2462[7]. Accordingly, our first criterion for satisfying the correct binding mode was to interact with at least one residue as mentioned above. The compounds that passed this critical binding test were further evaluated based on their S-score value. A total of 38 compounds fulfilled the binding requirements of the first step. Later, a threshold value of 30 was selected for S-score for further elimination and 12 compounds were shortlisted.

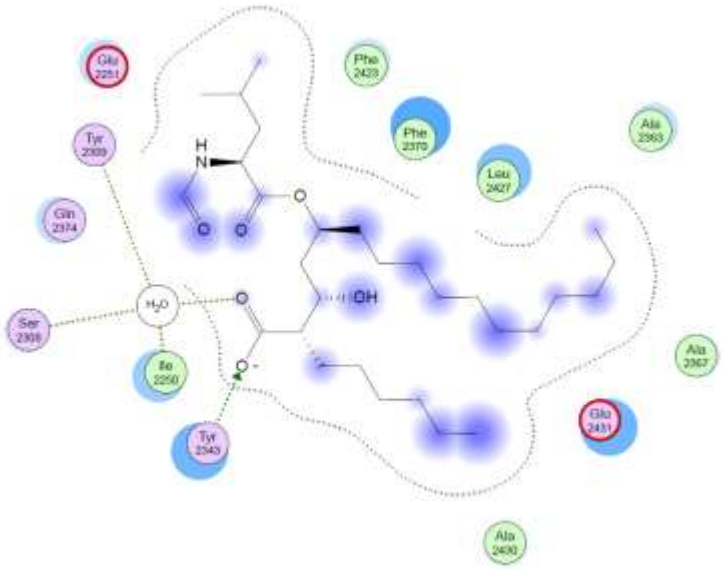


Figure 2: Interaction of orlistat bound to the active site of truncated FASN residues (2PX6).

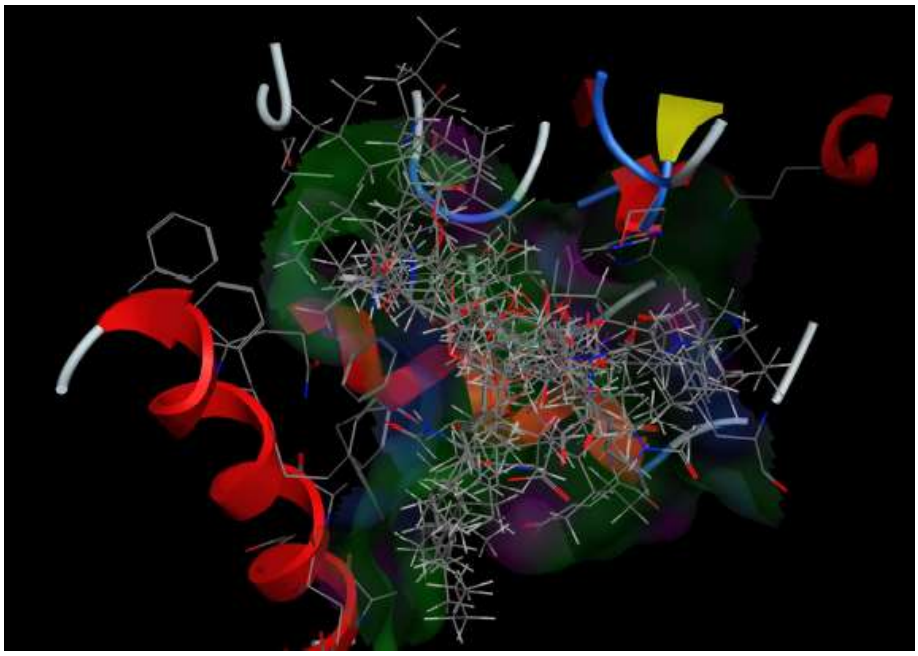
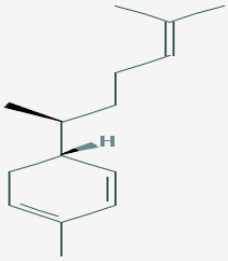
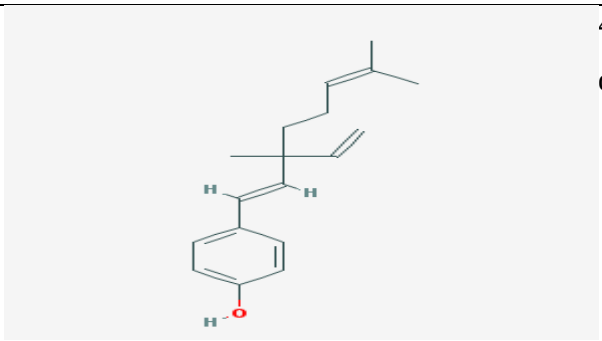
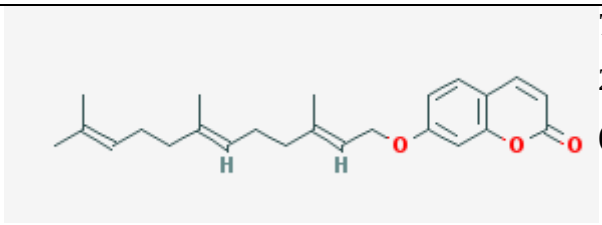
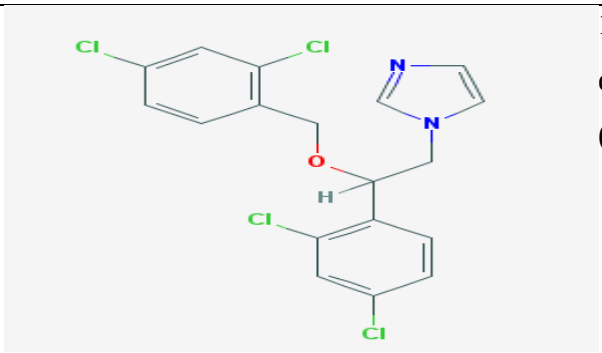
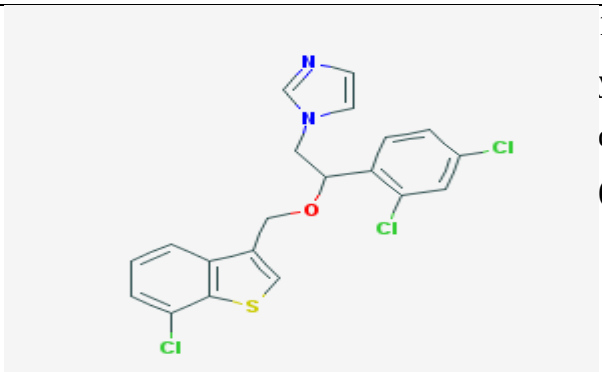
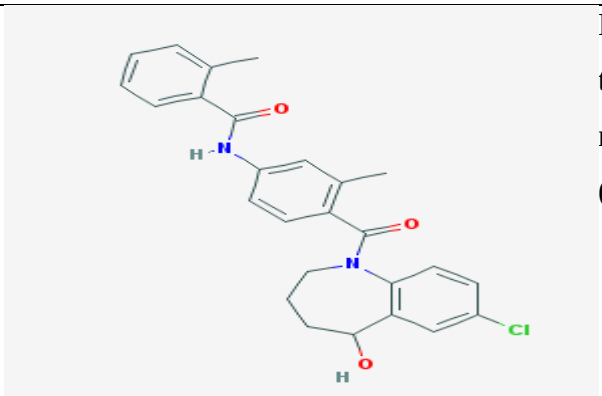


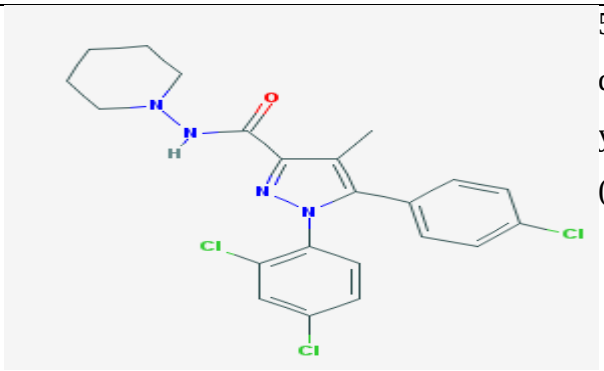
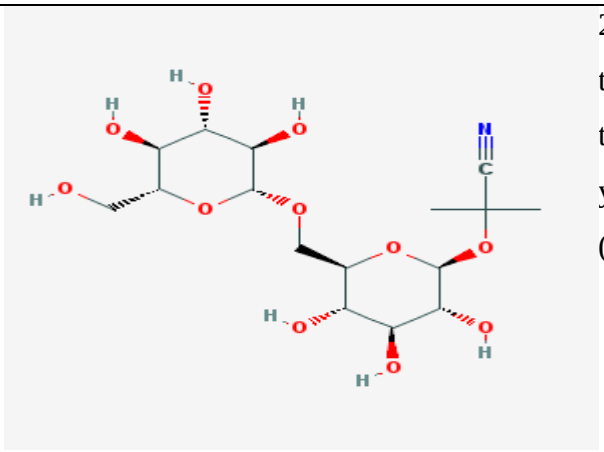
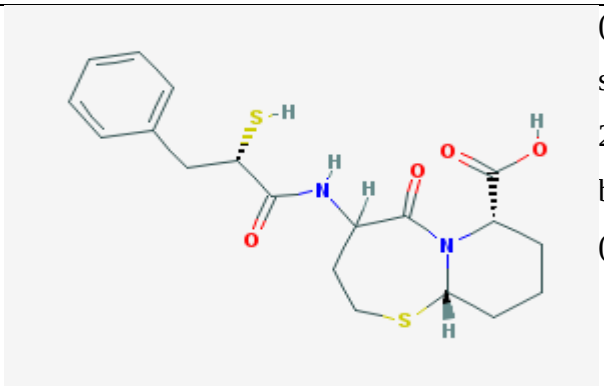
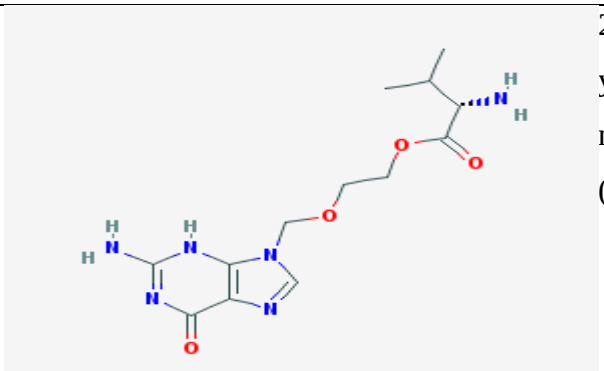
Figure 3: Docked poses of 12 molecules with the highest score obtained from MOE

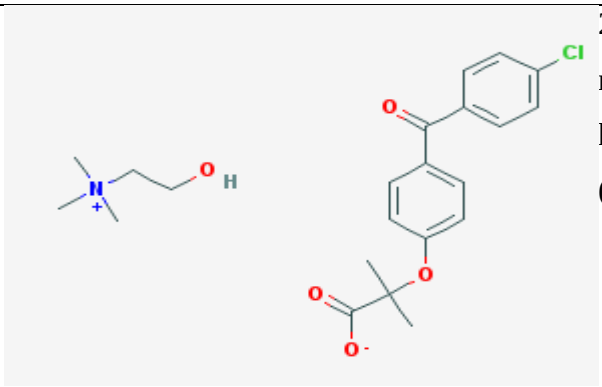
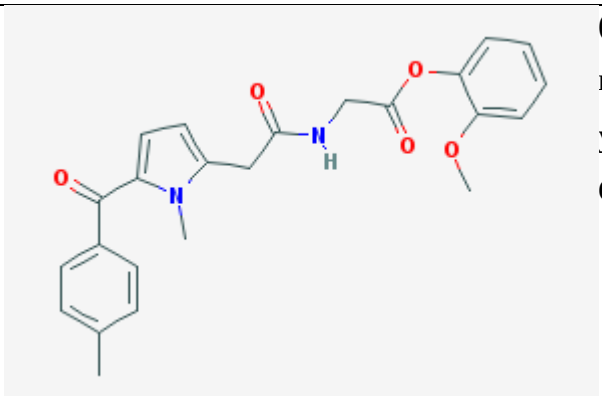
Figure 3 illustrates 12 molecules best poses from MOE to validate that they bind properly in the binding pocket surrounded by key interacting residues. The compounds which were selected based on the binding and scoring criteria are described in Table 2. Furthermore, the pharmacophore model, which was based on the structure of receptor–antagonist complex, increases the probability of these compounds to function as antagonists than as agonists[8].

Table 2: Compounds selected based on the binding and scoring criteria, which are predicted to be FASN inhibitors.

ID	Structure	Identity
1.		(5R)-2-methyl-5-[(2S)-6-methylhept-5-en-2-yl]cyclohexa-1,3-diene (ZINGIBERENE)

2.		4-[(1E)-3-ethenyl-3,7-dimethylocta-1,6-dienyl]phenol ((+)-BAKUCHIOL)
3.		7-[(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienoxy]chromen-2-one (UMBELLIPRENIN)
4.		1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl]imidazole (MICONAZOLE)
5.		1-[2-[(7-chloro-1-benzothiophen-3-yl)methoxy]-2-(2,4-dichlorophenyl)ethyl]imidazole (SERTACONAZOLE)
6.		N-[4-(7-chloro-5-hydroxy-2,3,4,5-tetrahydro-1-benzazepine-1-carbonyl)-3-methylphenyl]-2-methylbenzamide (TOLVAPTAN)

7.		5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-piperidin-1-ylpyrazole-3-carboxamide (RIMONABANT)
8.		2-methyl-2-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-2-yl]oxypropanenitrile (LINUSTATIN)
9.		(7S,10aS)-5-oxo-4-[[(2S)-3-phenyl-2-sulfanylpropanoyl]amino]-2,3,4,7,8,9,10,10a-octahydropyrido[2,1-b][1,3]thiazepine-7-carboxylic acid (OMAPATRILAT)
10.		2-[(2-amino-6-oxo-3H-purin-9-yl)methoxy]ethyl (2S)-2-amino-3-methylbutanoate;hydrochloride (VALACYCLOVIR)

11.		2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoate;2-hydroxyethyl(trimethyl)azanium (CHOLINE FENOFIBRATE)
12.		(2-methoxyphenyl) 2-[[2-[1-methyl-5-(4-methylbenzoyl)pyrrol-2-yl]acetyl]amino]acetate (AMTOLMETIN GUACIL)

CONCLUSION

Three different pharmacophore and a merged pharmacophore thereof were generated from known inactive crystal structures of FASN. These pharmacophores were used to screen SWEETLEAD database of chemical structures comprising 9,127 approved drugs, chemical isolates from traditional medicinal herbs, and regulated chemicals. Pharmacophore-based screening yielded overall 1,661 hits that were docked to 2XP6. Following docking, a total of 38 compounds were found to satisfy the requirements for key residues, which were further screened with highest docking score to yield 12 final hits. Although the experimental validation of these compounds are lacking, computational methods predicts them as strong binders. Further experimental validation of these compounds will confirm the *in silico* studies results and their potential role in treatment of cancer with high FASN expression.

ACKNOWLEDGEMENT

Work was supported by the Doctoral Fellowship from SVKM's NMIMS University to Anup S. Ramdhave. Authors would like to acknowledge the technical help and expert advice provided by Dr. Prashant Kharkar, Associate Professor, SPP SPTM, SVKM's NMIMS, Mumbai.

REFERENCES

1. Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. Nat Rev Cancer. 2007;7(10):763-77.
2. Flavin R, Peluso S, Nguyen PL, Loda M. Fatty acid synthase as a potential therapeutic target in cancer. Future oncology (London, England). 2010;6(4):551-62.
3. Maier T, Leibundgut M, Ban N. The crystal structure of a mammalian fatty acid synthase. Science. 2008;321(5894):1315-22.
4. Lupu R, Menendez JA. Pharmacological inhibitors of Fatty Acid Synthase (FASN)--catalyzed endogenous fatty acid biogenesis: a new family of anti-cancer agents? Curr Pharm Biotechnol. 2006;7(6):483-93.
5. Yang SY. Pharmacophore modeling and applications in drug discovery: challenges and recent advances. Drug Discov Today. 2010;15(11-12):444-50.
6. Novick PA, Ortiz OF, Poelman J, Abdulhay AY, Pande VS. SWEETLEAD: an in silico database of approved drugs, regulated chemicals, and herbal isolates for computer-aided drug discovery. PLoS One. 2013;8(11):e79568.
7. Pemble CW, Johnson LC, Kridel SJ, Lowther WT. Crystal structure of the thioesterase domain of human fatty acid synthase inhibited by Orlistat. Nat Struct Mol Biol. 2007;14(8):704-9.
8. Yakar R, Akten ED. Discovery of high affinity ligands for β2-adrenergic receptor through pharmacophore-based high-throughput virtual screening and docking. J Mol Graph Model. 2014;53:148-60.