Optimization of In Silico Designed Fatty Acid Synthase Modulators Derived From the Lead Molecule Orlistat

C. Shoemake & R. Sciberras

Molecular Modeling, Department of Pharmacy, University of Malta, Malta

Abstract: Orlistat; or tetrahydrolipstatin has been found to have inhibitory effects on human Fatty Acid Synthase (hFASN), an enzyme overexpressed in many tumors responsible for energy upkeep and fatty acid synthesis. The crystalline structure of hFASN was used to extract Orlistat; analysis it's pharmacophoric moieties and generate a library using Orlistat as a scaffold to be utilized within a database for high throughput screening. Two distinct methods were used; and over 1,200 unique molecules have been generated, all compliant with Lipinski's rule of 5.

INRODUCTION

In silico studies have also established a mechanism for the inhibition of the Thioesterase Domain in the Human Fatty Acid Synthase, an enzyme overexpressed in numerous tumors. Given the natural progression of these carcinomas, overexpression of Fatty Acid Synthase is observed prior to clinical symptoms, therefore Orlistat would be an ideal chemotherapeutic agent acting as a bioactive marker as well as an anti-tumor mediator. Numerous mechanisms have been hypothesized regarding the anti-cancer properties Orlistat possess: including reduction in end-product leading to low levels of phosphatidylcholine – the lipid present in high levels affected by the inhibition of fatty acid synthase inhibition; responsible for the synthesis of new cancer cells' membrane and inhibition of DNA replication during the G1 and S phases in preparation of cell division.

Currently Orlistat is being used for the management of obesity; via the inhibition of pancreatic lipase as it inhibits the formation of absorbable fats within the gastro intestinal system. This mechanism does not need happen a systemic route; which given the physiochemical properties Orlistat possess due to its structure; is a benefit within this situation; as fewer side-effects are expected. For the scope of this study Lipinski's rule of 5 were applied for each and every generated entity; which favor systemic availability as the target site of the new generated entities are available only through a systemic route.

Orlistat shows a significant resemblance in structure to Palmitate; a fatty acid synthesized via the multiple active sites within hFASN. The beta-lactam ring within Orlistat serves as an anchorage point for the inhibition of the Thioesterase domain within hFASN; binding covalently and hydrolyzing Orlistat. This interaction was kept in the process by which the new molecular entities were generated.

METHODOLOGY

Two methodologies where designed around 4 canonical ideas within this study:

1. Ligand binding site identification
2. Ligand preparation
3. Docking process
4. Pose scoring

METHOD ONE

Method one utilized LigBuilderv1.2®. The ligand binding pocket was elucidated using the pocket module; which generated data which describe the ligand binding pocket as well as pharmacophoric regions of importance in the forging of molecular interactions between the ligand and the active site.

Ligand preparation was undergone via the growth module within LigBuilderv1.2®. Orlistat was molecularly altered – removing undesired areas and indicating a site of growth – allowing LigBuilderv1.2 to recognize this site to computationally adhere different structures from within its library.

Figure 1. Systematic summery of method one employed.
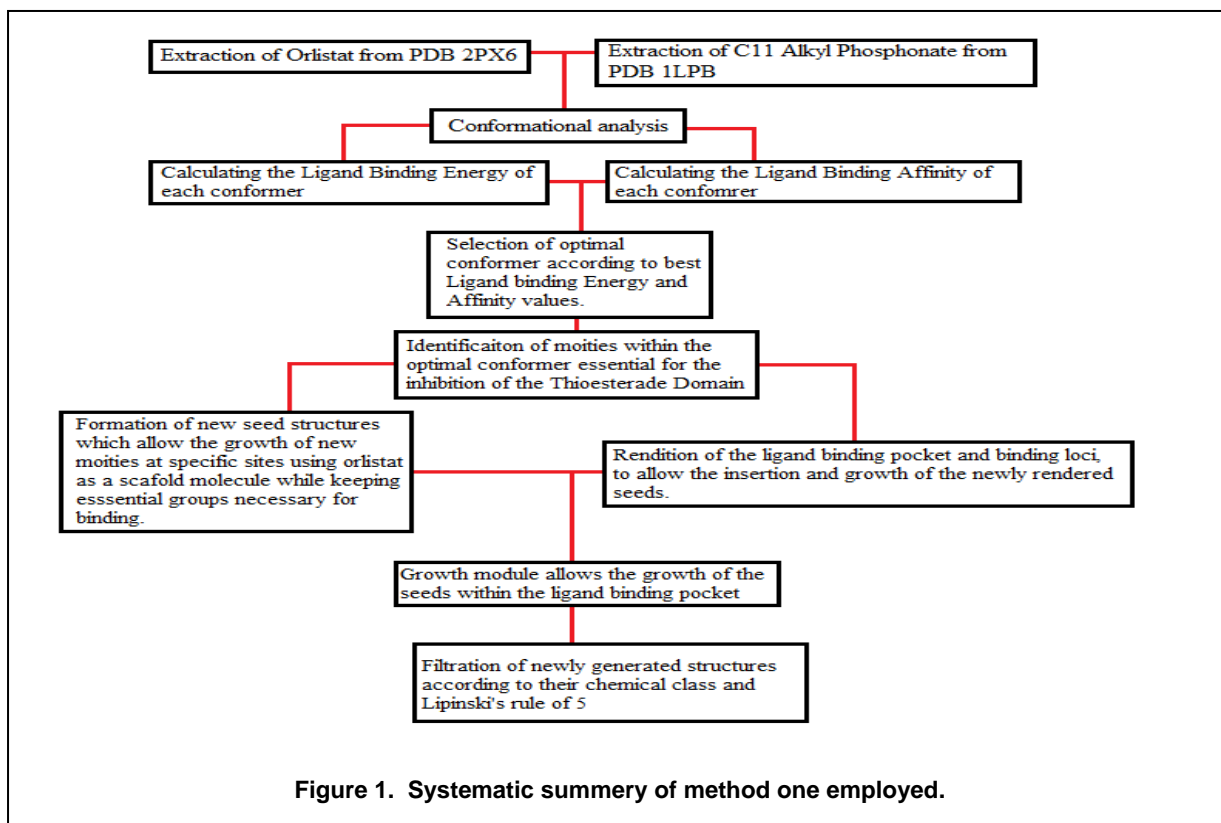
The grow module within LigBuilderv1.2® takes into consideration the geometric shaping of the ligand and it's complementation within the ligand binding pocket. This is done as the pocket module maps the ligand binding site via a grid method, having 5 types of grids, denoting solvent; vacant; hydrogen donor and acceptor and hydrophobic grip types. These grid types describe the thioesterase domain in terms of its surface description, which is compared with the surface of the docked ligand; in this case; the seed molecule. LigBuilderv1.2® does not simulate the docking process, yet uses a shape complementation system; using the grid generated with the pocket module.

This approach is more favorable towards a pharmacophore based approach; as the three dimensional description of the generated structures are taken into consideration for the ideal binding modality.

Method one's scoring method utilizes LigBuilderv1.2® SCOREv2.0; which looks at each individual atom of a particular ligand and adds each value towards a final value to indicate the likelihood

of it being feasible to exist as a ligand-ligand binding site complex.

METHOD TWO

Method two utilized the Surflex-Dock within Sybyl to generate a protomol; an idealized active site; as it also takes into consideration vicinal instability pockets close to the main active site which may improve the stability of the ligand-receptor complex.

Method two generated de novo entities using the online service provided by the University of Hamburg; ViCi®. ViCi® analyzed Orlistat's electrostatic and topological mapping and generated molecules which have similar characteristics to that of Orlistat. Out of 1,001 molecules, 777 were compliant with Lipinski's rule of 5; which was used as a set of characteristics which indicate favorable drug-like characteristics with respect to pharmacokinet and pharmacodynamics properties.

The Surflex-Dock within Sybyl-X® Suite; a protomol is formed which is an idealized ligand to occupy the stipulated ligand binding pocket. This method also uses a shape complementation system; as the protomol is a fixed; idealized volume which the ligand should occupy for inhibition to occur. The advantage of using this approach compared to method one is that; although this method is still considered as a rigid-body; shape complementarity approach – conformers for each introduced ligand

are still generated. This indicates that although the ligand

Surflex output units are represented as –log(KD).

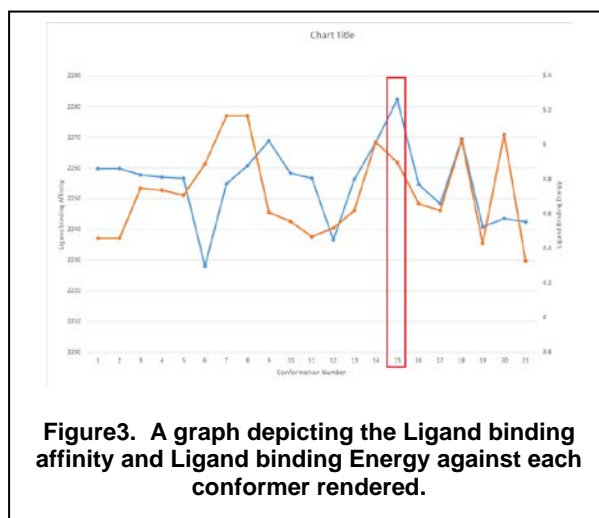**Figure 2. Systematic summery of method two employed.**
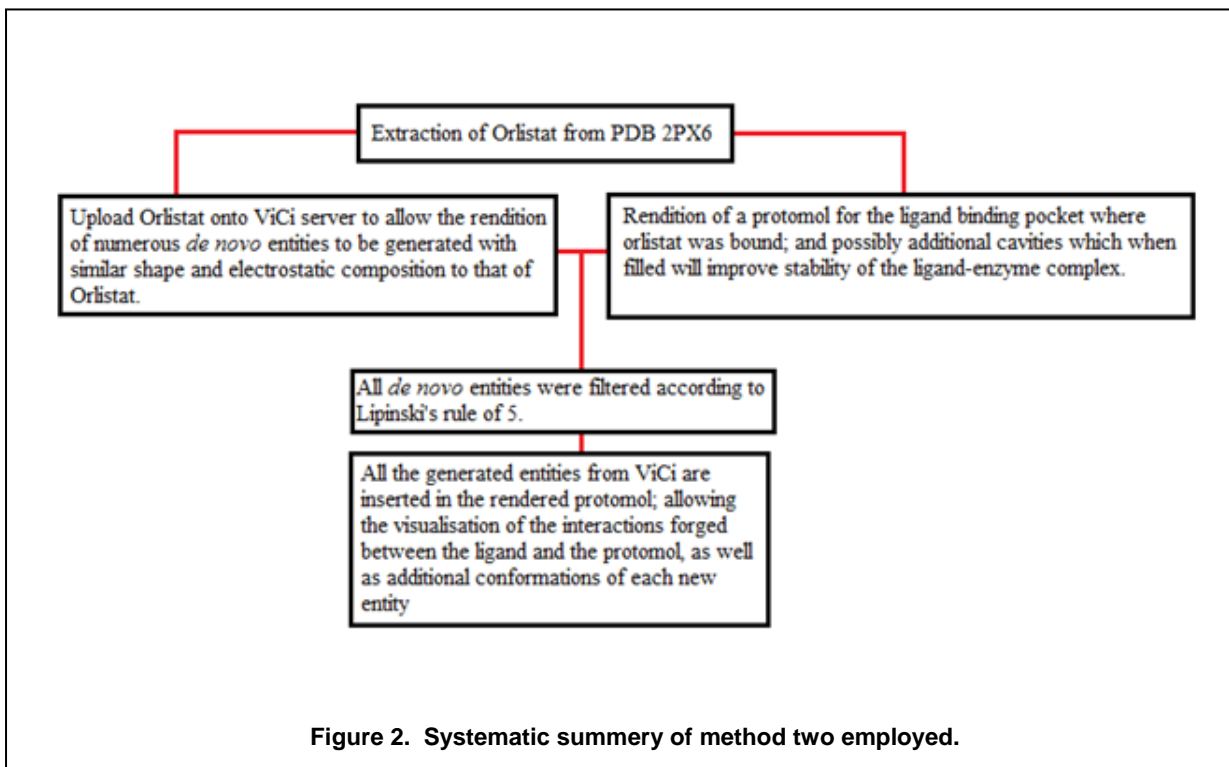
binding pocket is considered to be frozen for all the ligands to bind to; the ligand itself is allowed to rotate; thus generating different conformers.
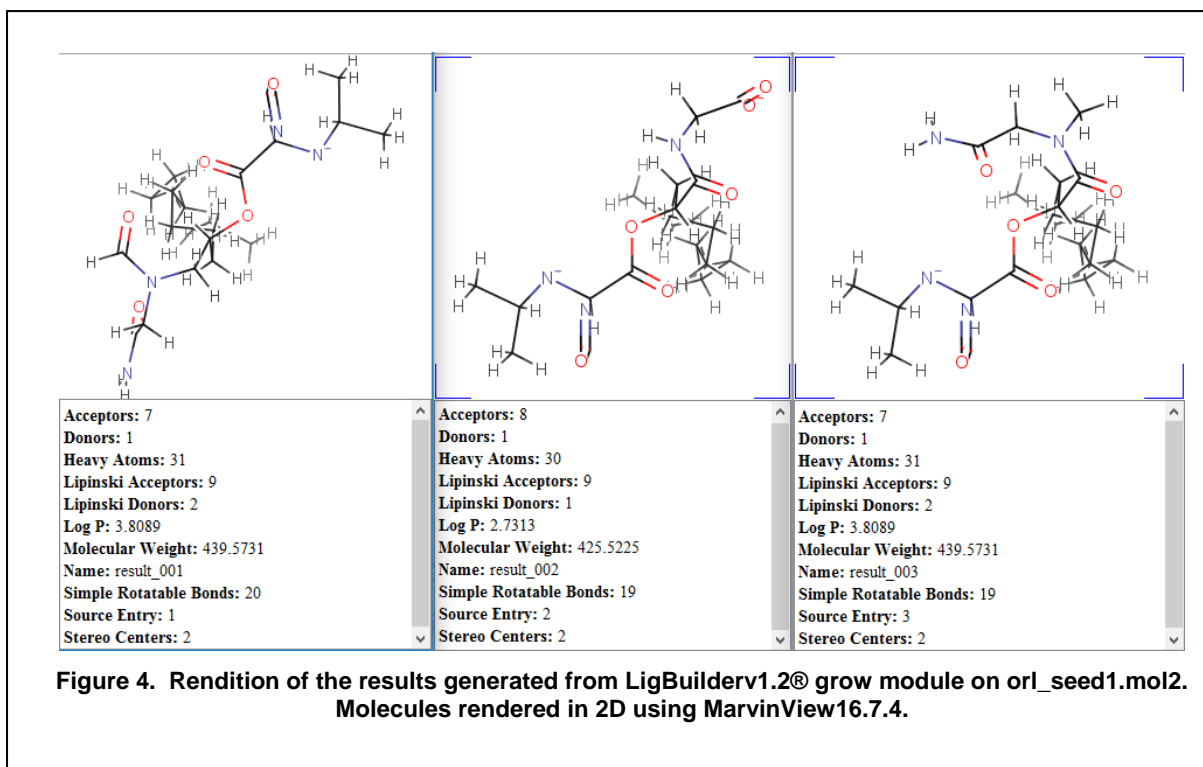
Virtual docking simulation is a more realistic simulation of what happens in vivo, as each ligand is guided towards the ligand binding pocket. This approach takes into consideration that both ligand and the ligand binding pocket are not in reality frozen in time; but are somewhat flexible to accommodate each other to form a stable ligand – enzyme complex. Due to limitations within this study; particularly software and hardware availability; this option was not available.

The scoring function utilized with Surflex follows closely the approach Bohm takes. Bohm's approach includes the parameters of hydrophobic contact; polar interactions and entropic fixation costs for loss of torsional; translational and rotational freedoms.

Only the details in the parameters of optimization of the functions mention vary within Bohm's approach.

Figure3. A graph depicting the Ligand binding affinity and Ligand binding Energy against each conformer rendered.

Both methods utilized within this study use an intendent and separate scoring system; thus one score set cannot be compared with the other.

Acceptors: 7
Donors: 1
Heavy Atoms: 31
Lipinski Acceptors: 9
Lipinski Donors: 2
Log P: 3.8089
Molecular Weight: 439.5731
Name: result_001
Simple Rotatable Bonds: 20
Source Entry: 1
Stereo Centers: 2

Acceptors: 8
Donors: 1
Heavy Atoms: 30
Lipinski Acceptors: 9
Lipinski Donors: 1
Log P: 2.7313
Molecular Weight: 425.5225
Name: result_002
Simple Rotatable Bonds: 19
Source Entry: 2
Stereo Centers: 2

Acceptors: 7
Donors: 1
Heavy Atoms: 31
Lipinski Acceptors: 9
Lipinski Donors: 2
Log P: 3.8089
Molecular Weight: 439.5731
Name: result_003
Simple Rotatable Bonds: 19
Source Entry: 3
Stereo Centers: 2

Figure 4. Rendition of the results generated from LigBuilderv1.2® grow module on orl_seed1.mol2. Molecules rendered in 2D using MarvinView16.7.4.
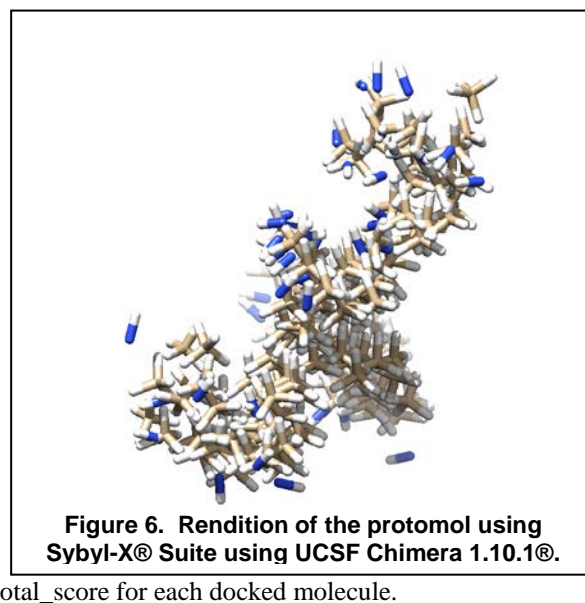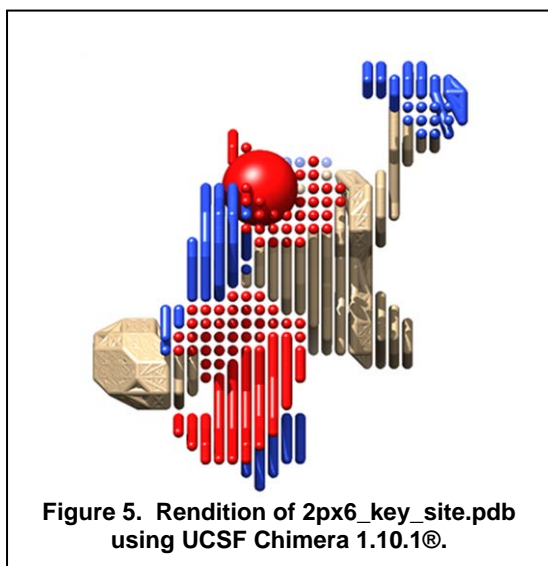
RESULTS

Results generated from method one:

3 seeds were input into the Growth module within LigBuilderv1.2® to allow the rendition of 403 new molecular entities, only 173 of which were Lipinksi Rule compliant.

Figure 5. Rendition of 2px6_key_site.pdb using UCSF Chimera 1.10.1®.

Results generated from Method two:

Using ViCi'S database which generated 1000 new molecular entities, only 777 were Lipinski rule compliant. These entities superimposed on the protomol generated using Sybyl-X® Suite to give a
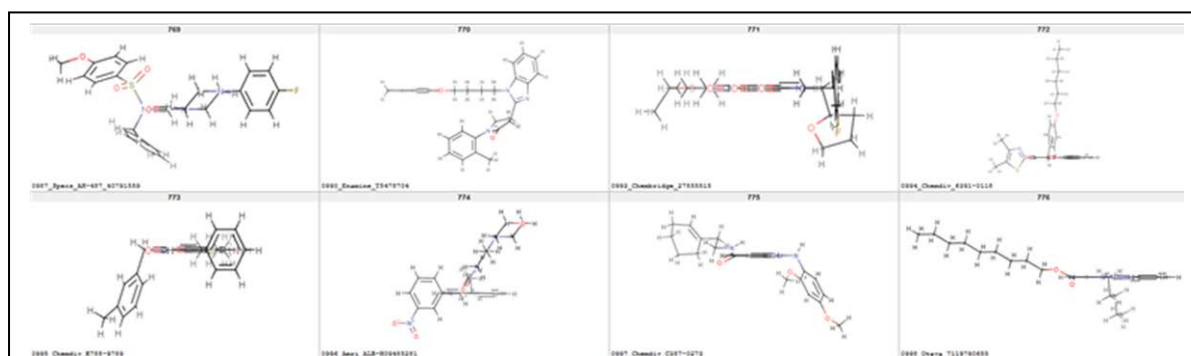

Figure 6. Rendition of the protomol using Sybyl-X® Suite using UCSF Chimera 1.10.1®.

total_score for each docked molecule.

Figure 7. Rendition of a set of newly generated entities using ViCi's online database; rendered in MarvinView16.7.4®

DISCUSSION

Table 1. Molecules generated with the optimal parameters from each different method compared with Orlistat; the parent molecule.

Molecule Docked	Software used to generate molecule docked	Total_Score
Orlistat	Sybyl-X® Suite (extracted from PDB 2PX6)	-1.6
result_01	Ligbuilderv1.2® growth module	3.21
result_047	Ligbuilderv1.2® growth module	5.13

It is worth comparing and contrasting both ligand binding site mapping methods employed. Ligbuilderv1.2® uses a ligand which is already docked within the ligand binding site to guide the software locate the main site. The software algorithm uses a box system which entraps the ligand and surrounding residues forming a grid with spacing of 0.5 Amstrong; as set by default. Proving via hydrogen atoms is used to scan the volume occupied by the grid; and where "bumps" are observed; creating an empty volume where no hydrogen has bumped, and an occupied region, where the protein surface is interfering with the hydrogen probe, causing a "bump". This method of active site elucidation and docking is considered as a rigid-body docking method – which does not consider the flexibility of neither the ligand nor the receptor; setting a major drawback with this method.

Method two utilized the Surflex-Dock Protomol is referred to as the "binding pocket", a computational representation of the proposed binding site in which target small molecules are aligned.

Generally, the protomol was constructed by the following processes. First, the selected protein surface is coated with certain types of probes representing potential hydrogen bonds and favorable hydrophobic interactions with protein atoms. The probes are positioned and oriented by a score function representing the binding contribution of a similar atom on a ligand.

Furthermore, the probes are filtered by score resulting in a cluster of high-scoring probes which identify the "sticky parts" of the protein's surface. Disconnected sticky spots are discarded with the rest form spheres on a 1 Å cubical grid. Each spheres grows until it reaches the van der Waals surface of a protein atom; sphere with a radius less than 0.5 Å are discarded. Finally, the sticky spots are merged into a pocket by accretion on the set of remaining protein-free spheres. This method is considered as a flexible docking method; allowing a more holistic method of active site elucidation and docking as it allows the rotation of both ligand and receptor. The protomol generated via the second method was observed to occupy a bigger volume and area; which gives a more realistic idea of what the thioesterase domain active site is found in nature; when compared to the key site created by LigBuilderv1.2®.

Both methods employed to generate de novo structures used Orlistat as a scaffold structure keeping intact key pharmacophoric moieties intact while altering other areas believed not to have significant value in the stability of the ligand-enzyme complex.

The docking process employed in this study is also referred as protein docking; the docking of molecular structures chosen are to a main protein at a specific site. Different problems may arise from the selected docking partner.
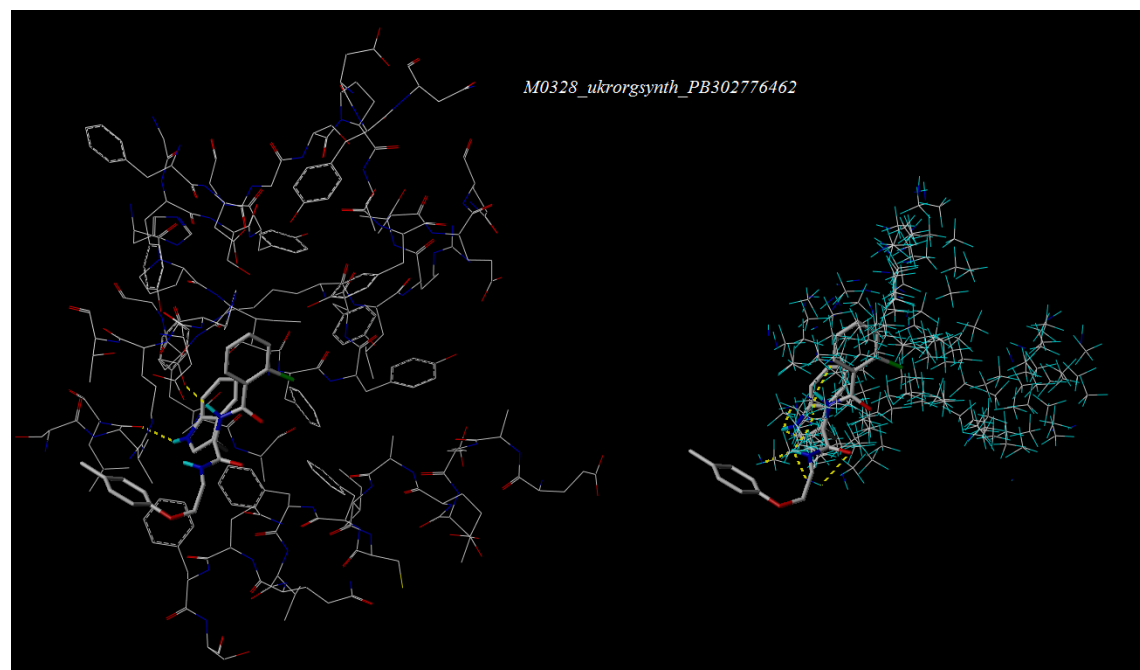
Figure 8. The docking of M0328_ukrorgsynth_PB302776462 within the docking site and superimposition of the docked M0328_ukrorgsynth_PB302776462 both generated by Sybyl-X® Suite ®

One of two concepts are mainly used within a docking process:

1.   Shape Complementation
2.   Virtual docking simulation

There is a noticeable significance in the binding of the newly generated ligands with the generated docking site from both methods, when compared with Orlistat. This indicates that at least one molecule from each method in the generated library shows improvement to both affinity and stability to the thioesterase domain within fatty acid synthase.

The above comparison also shows that ViCi® created at least one molecular entity with a higher total_score than any of the best molecules docked within the docking site generated by Sybyl-X® Suite.

ACKNOWLEDGMENTS

REFERENCES

1.   Chirala SS, Wakil SJ. Structure and function of animal fatty acid synthase. Lipids. 2004;39(5):1045–53.
2.   Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis.

Nat Rev Cancer [Internet]. Nature Publishing Group; 2007 Oct:763–77.
3.   Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. Nat Rev Cancer. Nature Publishing Group; 2007:763–77.
4.   Berndt J, Kovacs P, Ruschke K, Klting N, Fasshauer M, Schn MR, et al. Fatty acid synthase gene expression in human adipose tissue: Association with obesity and type 2 diabetes. Diabetologia. 2007;50(7):1472–80.
5.   Loftus TM. Reduced Food Intake and Body Weight in Mice Treated with Fatty Acid Synthase Inhibitors. Science (80-). 2000;288(5475):2379–81.
6.   Data Sheet - Xenical (R) Source: Internet http://www.medsafe.govt.nz/profs/datasheet/x/Xenicalcap.pdf
7.   Fako VE, Zhang JT, Liu JY. Mechanism of orlistat hydrolysis by the thioesterase of human fatty acid synthase. ACS Catal. 2014;4(10):3444–53.
8.   GM Morris, Lim-Wilby M. Molecular docking. Methods Mol Biol. 2008;443:365–82.

9. Wang R, Gao Y, Lai L. LigBuilder: A Multi-Purpose Program for Structure-Based Drug Design. J Mol Model. 2000;6(7-8):498–516.

10. Ruppert, J., Welch, W. & Jain, A. N. Automatic identification and representation of protein binding sites for molecular docking. Protein Sci 6, 524-533 (1997).

11. Lengauer T, Rarey M. Computational methods for biomolecular docking. Curr Opin Struct Biol. 1996 Jun;6(3):402–6. Morris RJ, Najmanovich RJ, Kahraman A, Thornton JM. Real spherical harmonic expansion coefficients as 3D shape descriptors for protein binding pocket and ligand comparisons. Bioinformatics. Oxford University Press; 2005 May 15

12. Morris RJ, Najmanovich RJ, Kahraman A, Thornton JM. Real spherical harmonic expansion coefficients as 3D shape descriptors for protein binding pocket and ligand comparisons. Bioinformatics. Oxford University Press; 2005 May 15.

13. Jain AN. Surflex-Dock 2.1: Robust performance from ligand energetic modeling, ring flexibility, and knowledge-based search. J Comput Aided Mol Des. 2007;21(5):281–306.