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Synthesis, Molecular Docking and 2HI4 inhibitory activity of functionalized dimethyl 1, 4 – diphenyl naphthalene – 2, 3 – dicarboxylate and Naphthoflavone

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***Abstract*-** Microsomal cytochrome P450 family 1 enzymes (2HI4) play prominent roles in xenobiotic detoxication and procarcinogen activation. P450 1A2 is the principal cytochrome P450 family 1 enzyme expressed in human liver and participates extensively in drug oxidations. This enzyme is also of great importance in the bioactivation of mutagens, including the N-hydroxylation of arylamines. P450-catalyzed reactions involve a wide range of substrates, and this versatility is reflected in a structural diversity evident in the active sites of available P450 structures. Here the presented structure of human P450 1A2 in complex with the inhibitor alpha-naphthoflavone, determined to a resolution of 1.95 A alpha-Naphthoflavone is bound in the active site above the distal surface of the heme prosthetic group. Inhibitors of 2HI4 showed highly adapted for the positioning and oxidation of relatively large, planar substrates and a new series of dimethyl 1,4 – diphenyl naphthalene – 2,3 – dicarboxylate and naphthoflavone which possess good inhibitory activity against 2HI4. This compound showed better binding energy than the compound which has been already co-crystallized with the target protein, (PDB ID 2HI4).

***Index Terms*-** 2HI4inhibitor, molecular docking, protein ligand interaction, naphthoflavone

I. INTRODUCTION

Experimental Procedure

Synthesis

To a stirred solution of **dimethyl 1,4 – diphenyl naphthalene – 2,3 – dicarboxylate** in THF(10ml) was added at room temperature with trietylamine (1mM). After the formation of quaternary ammonium salt and benzimidazole, Cl (minor) was added and stirring was continued for 4 hours. After the completion of the reaction, the reaction mixture was quenched with 0.1 mol of HCl (5ml) and extracted with ethyl acetate. The solvent was evaporated under reduced pressure and the crude mixture was subjected to column chromatography using hexane and ethyl acetate (3:1) as eluent to get the pure compound.

Crystallization

The crystal is obtained by slower evaporation method at room temperature. The sample was taken and it was recrystallised using solvent combination of chloroform and methanol. Good quality crystal was selected for X-ray diffraction studies.

Data collection

A good crystal of dimension 0.2*0.25*0.22 mm was selected for data collection. Intensity data were measured on Bruker SMART APEX II CCD diffractor with graphite monochromatic MoKα radiation (λ = 0.71703Å) at room temperature. The data collection was covered over a hemisphere of reciprocal space by combination of three sets of exposure each having a different phi angle (0, 120 and 240˚) for the crystal and each exposure of 15 seconds covered 0.3˚ in ω. The crystal to detector distance was 5 cm and detector swing angle was -35˚. Coverage of the unit set was over 92% complete.

The cell parameters obtained by this process are:

Cell axes (Å)	15.8021(8)	7.4706(4)	17.8599 (9)
Cell angles (deg)	90	96.581(2)	90
Crystal System	P2(1)/c		
Crystal symmetry	- X, 1/2 + Y, 1/2 - Z		

Crystal decay was monitored by repeating 30 initial frames at the end of the data collection and analyzing duplicate reflections, and was found to be negligible. The intensity data were reduced and Lorentz and polarization corrections were applied. A total of 31675 were collected and among them 8520 reflection were found to be unique. With the criterion of $I > 2\sigma$ (I), 8520 reflections were considered as observed. The computer program XCAD4 reduced the data and the space group was deduced to be P-1 .

Structure solution and refinement

The structure was solved by direct method using SHELXS97 computer program. The trial structure was solved refined isotropically followed by an isotropic refinement using full-matrix least squares procedures based on F2 by SHELXL97 computer program. All H atoms were located in the difference Fourier maps and were refined isotropically. Refinement converged at a final R = 0.0547 and wR2 = 0.1384

Protein Preparation and its function:

The protein three dimensional (3D) structure was taken from the PDB ID 2HI4. It is a 1 chain structure of Cytochrome P450 with sequence from Homo sapiens. Full crystallographic information is available from OCA. Microsomal cytochrome P450 family 1 enzymes play prominent roles in xenobiotic detoxication and procarcinogen activation. P450 1A2 is the principal cytochrome P450 family 1 enzyme expressed in human liver and participates extensively in drug oxidations. This

enzyme is also of great importance in the bioactivation of mutagens, including the N-hydroxylation of arylamines. P450-catalyzed reactions involve a wide range of substrates, and this versatility is reflected in a structural diversity evident in the active sites of available P450 structures. Here the presented structure of human P450 1A2 in complex with the inhibitor alpha-naphthoflavone, determined to a resolution of 1.95 A alpha-Naphthoflavone is bound in the active site above the distal surface of the heme prosthetic group. The structure reveals a compact, closed active site cavity that is highly adapted for the positioning and oxidation of relatively large, planar substrates. This unique topology is clearly distinct from known active site architectures of P450 family 2 and 3 enzymes and demonstrates how P450 family 1 enzymes have evolved to catalyze efficiently polycyclic aromatic hydrocarbon oxidation. This report provides the first structure of a microsomal P450 from family 1 and offers a template to study further structure-function relationships of alternative substrates and other cytochrome P450 family 1 members. The structure was refined using amber force filed and the final model was taken as starting structure for the docking studies.

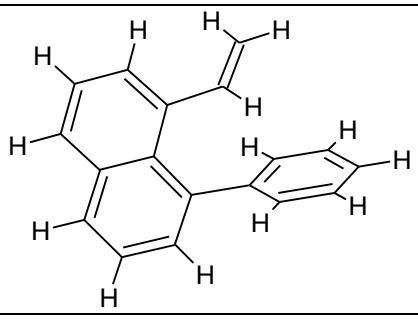
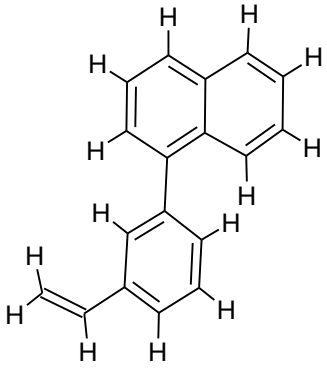
Q – Site finder:

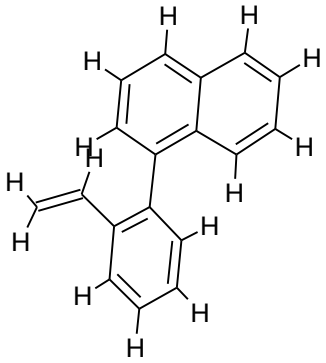
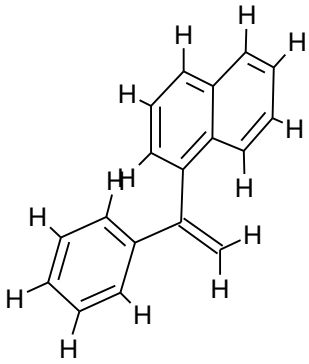
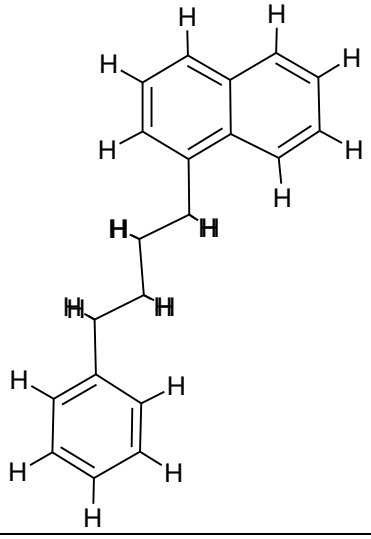
Q – Site finder uses a hierarchical series of filters to search for possible locations of the amino acids as active site region of the receptor for the ligand. The active site molecules are ILE A 117, THR A 118, SER A 122, THR A 124, PHE A 125, THR A 223, PHE A 226, VAL A 227, PHE A 256, ASN A 257, PHE A 256, ASN A 312, ASP A 313, GLY A 316, ILE A 314, ALA A 17, PHE A 319, ASP A 320, THR A 321, LEU A 382, ILE A 386, GLY A 460 and CYS A 458.

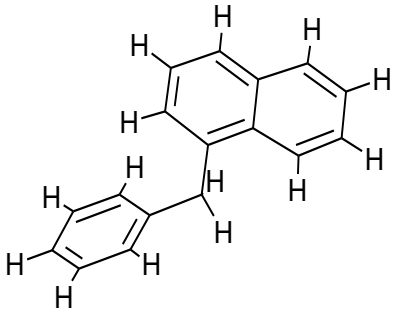
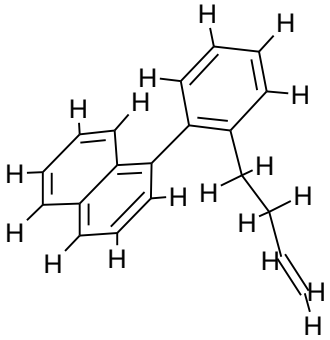
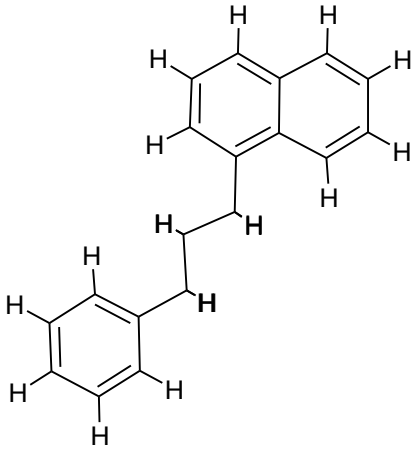
Ligand Preparation:

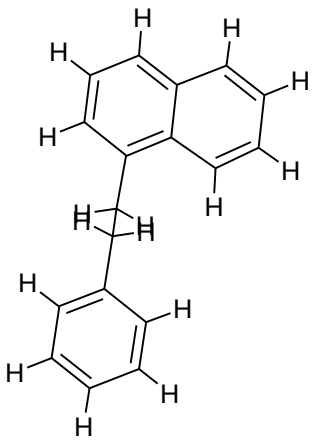
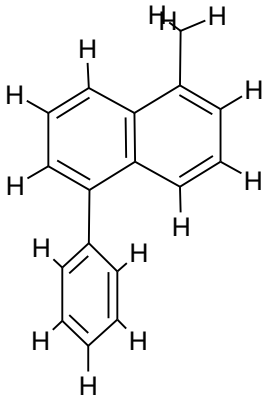
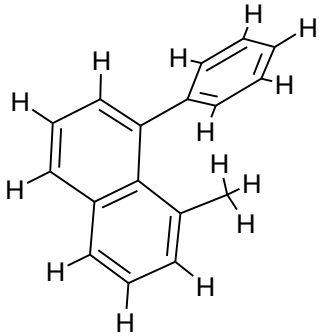
All the small ligand molecule structures were drawn using the builder panel available in the CHEM SKETCH and it was viewed in Argus lab. In order to get the biological conformations of the ligands NAPHTHOFLAVONE structure available in the PDB ID 2HI4 was taken as scaffold. Similar structure were downloaded in PUBCHEM and these stricter were drawn in CHEM SKETCH and these 3D structures were then energy minimized using the Argus lab – force field until it reaches the RMSD 0.001 kcal/mol. The structures were shown in the the following table.

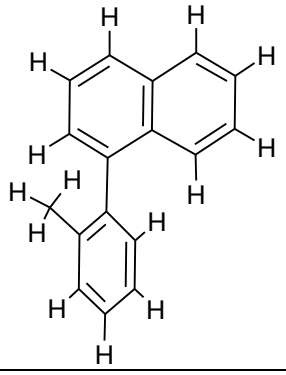
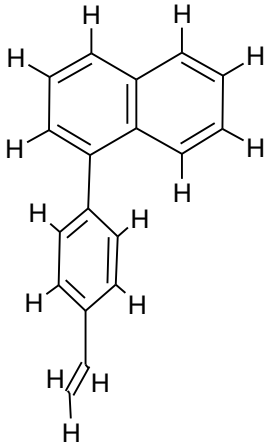
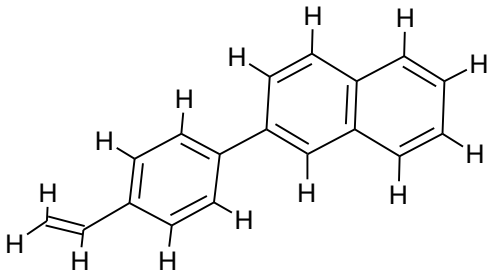
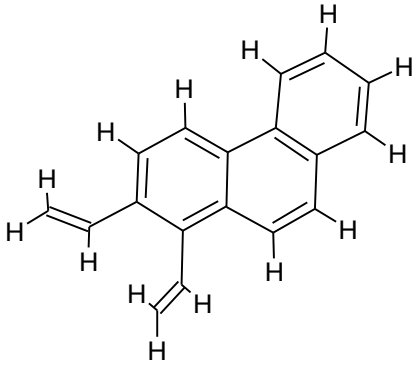
Table 1: Structures of 2HI4 inhibitors

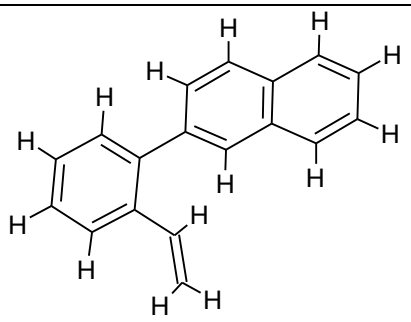
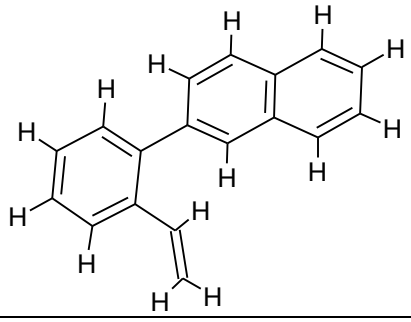
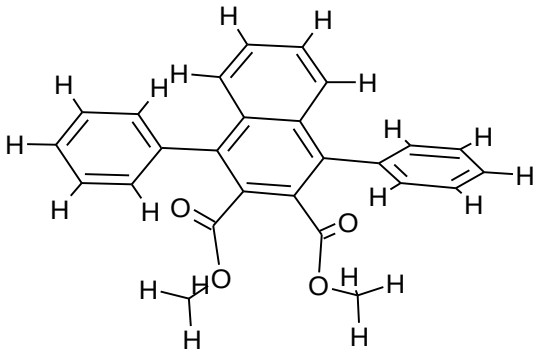
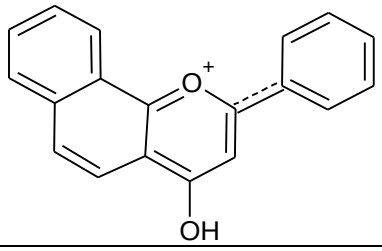
S. No	Compound Name	Structure
1	1-ethenyl-8-phenylnaphthalene	
2	1-(3-ethenylphenyl)naphthalene	

3	1-(2-ethenylphenyl)naphthalene	
4	1-(1-Phenylvinyl)naphthalene	
5	1-(4-phenylbutyl)naphthalene	

6	1-Benzylnaphthalene; Naphthalene	
7	1-(2-but-3-enylphenyl)naphthalene	
8	1-(3-phenylpropyl)naphthalene	

9	1-(2-phenylethyl)naphthalene	
10	1-methyl-5-phenylnaphthalene	
11	1-methyl-8-phenyl-naphthalene	

12	(Methylphenyl)naphthalene; Naphthalene, (methylphenyl)-	
13	1-(4-ethenylphenyl)naphthalene	
14	2-(4-ethenylphenyl)naphthalene	
15	1,2-bis(ethenyl)phenanthrene	

16	2-(2-ethenylphenyl)naphthalene	
17	1-(2-ethylphenyl)naphthalene	
18	dimethyl 1,4 – diphenyl naphthalene – 2,3 – dicarboxylate	
19	Naphthoflavone	

II. RESULT AND DISCUSSION

Table 2: Docking Scores of different inhibitors by Arguslab

S. No	Compound Name	Final Docked Energy (Binding Energy Kcal/mol)
1	1-ethenyl-8-phenylnaphthalene	-13.2465
2	1-(3-ethenylphenyl)naphthalene	-13.9318
3	1-(2-ethenylphenyl)naphthalene	-13.7285
4	1-(1-Phenylvinyl)naphthalene	-13.5066
5	1-(4-phenylbutyl)naphthalene	-14.8642
6	1-Benzylnaphthalene; Naphthalene	-15.8411
7	1-(2-but-3-enylphenyl)naphthalene	-15.4927
8	1-(3-phenylpropyl)naphthalene	-14.7016
9	1-(2-phenylethyl)naphthalene	-14.3659
10	1-methyl-5-phenylnaphthalene	-13.5870
11	1-methyl-8-phenyl-naphthalene	-13.5507
12	(Methylphenyl)naphthalene; Naphthalene, (methylphenyl)-	-13.7834
13	1-(4-ethenylphenyl)naphthalene	-13.8225
14	2-(4-ethenylphenyl)naphthalene	-13.9428
15	1,2-bis(ethenyl)phenanthrene	-13.3537
16	2-(2-ethenylphenyl)naphthalene	-13.7582
17	1-(2-ethylphenyl)naphthalene	-13.6064
18	**dimethyl 1,4 – diphenyl naphthalene – 2,3 – dicarboxylate**	**-14.6659**
19	**Naphthoflavone**	-14.1935

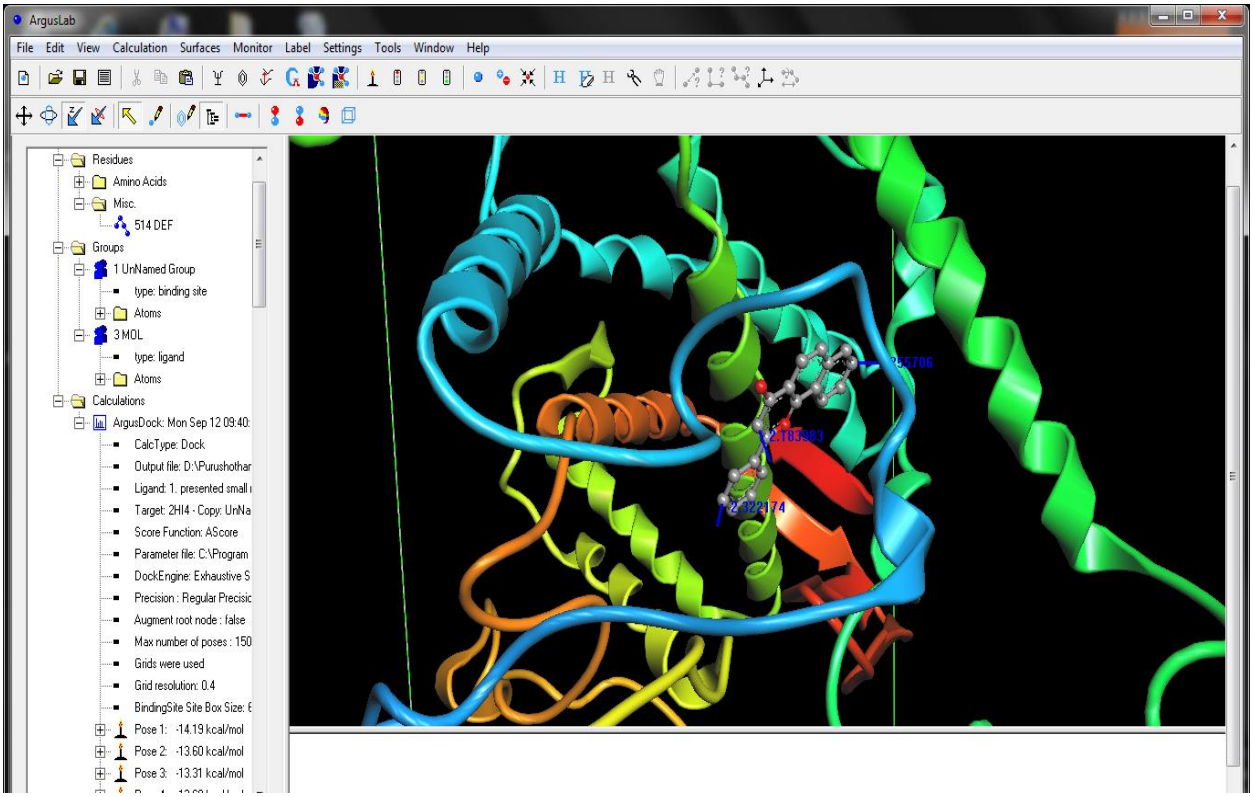


Figure 1: dimethyl 1,4 – diphenyl naphthalene – 2,3 – dicarboxylate with active site of 2HI4

From the experiment it is clear that the crystallographically solved compound, dimethyl 1,4 – diphenyl naphthalene – 2,3 – dicarboxylate can be used as a inhibitor. This compound showed better binding energy than the compound which has been already co-crystallized with the target protein, (PDB ID 2HI4). The binding energy for the ligand when it was removed from the PDB structure and docked with the protein, after minimization, was -14.1935 whereas the binding energy for dimethyl 1,4 – diphenyl naphthalene – 2,3 – dicarboxylate was found to be – 14.6659.

Table: 3 Interactions between dimethyl 1,4 – diphenyl naphthalene – 2,3 – dicarboxylate and protein 2HI4

Pose	**Hydrogen bond D-H…A**	**Distance(å)**	**Binding Energy Kcal/mol**
1	0721 C ------ 125 PHE 2811 C ------ 386 1LE 1734 C ------ 256 PHE	2.172 2.495 2.121	-14.6659
2	3409 N ------ 460 GLY 3400 S ------ 458 CYS	2.999 2.503	-14.67
3	3409 N ------ 460 GLY 3400 S ------ 458 CYS	2.829 2.988	-13.34
4	3409 N ------ 460 GLY 3400 S ------ 458 CYS	3.727 2.157	-11.91

5	3409 N ------ 460 GLY 3400 S ------ 458 CYS	3.919 2.268	-11.71
6	3409 N ------ 460 GLY 3400 S ------ 458 CYS	3.717 2.166	-11.50
7	3409 N ------ 460 GLY 3400 S ------ 458 CYS	3.716 2.162	-11.50
8	3409 N ------ 460 GLY 3400 S ------ 458 CYS	3.858 2.166	-11.49
9	3409 N ------ 460 GLY 3400 S ------ 458 CYS	3.621 2.057	-11.33

Table: 4 Interactions between dimethyl 1,4 – diphenyl naphthalene – 2,3 – dicarboxylate and protein 2HI4 and co crystallized Naphthoflavone with 2HI4

Compound	**Hydrogen bond D-H…A**	**Distance(å)**	**Binding Energy Kcal/mol**
Dimethyl 1,4 – diphenyl naphthalene – 2,3 – dicarboxylate	0721 C ------ 125 PHE 2811 C ------ 386 ILE 1734 C ------ 256 PHE	2.172 2.495 2.121	-14.6659
Naphthoflavone	0721 C ------ 125 PHE 2811 C ------ 386 ILE 1734 C ------ 256 PHE	2.183 2.322 2.255	-14.1935

Cytochromes P450 (P450s) play a major role in the clearance of drugs, toxins, environmental pollutants, xenobiotic detoxication and procarcinogen activation.. Additionally, metabolism by P450s can result in toxic or carcinogenic products. The metabolism of pharmaceuticals by P450s is a major concern during the design of new drug candidates. Determining the interactions between P450s and compounds of very diverse structures is complicated by the variability in P450-ligand interactions. Understanding the protein structural elements and the chemical attributes of ligands that dictate their orientation in the P450 active site will aid in the development of effective and safe therapeutic agents.

The goal of this review is to describe P450-ligand interactions from two perspectives. The first is the various structural elements that microsomal P450s have at their disposal to assume the different conformations observed in X-ray crystal structures. The second is P450-ligand dynamics analyzed by docking studies and identified flexible inhibitor.

The crystal structure of 2HI4 shows the enzyme in complex with Dimethyl 1,4 – diphenyl naphthalene – 2,3 – dicarboxylate, which is a competitive inhibitor of family 1 P450s. In general, α-naphthoflavone (ANF) substrates and inhibitors are relatively large molecules that contain aromatic, planar regions with various chemical side groups that may or may not be planar as well.

The 2HI4 structure contains 12 α-helices designated A–L and four β-sheets designated 1–4 (12). As observed with other mammalian CYPs of known structure, the most conserved regions are the core of the protein forming the heme binding site and the proximal surface that is considered to offer binding sites for cytochrome P450 reductase and cytochrome b5. The most distinct regions between known CYP structures are the portions that constitute the distal surfaces of the substrate binding cavity, the helix B–C and F–G regions, and the C-terminal loop following helix L.

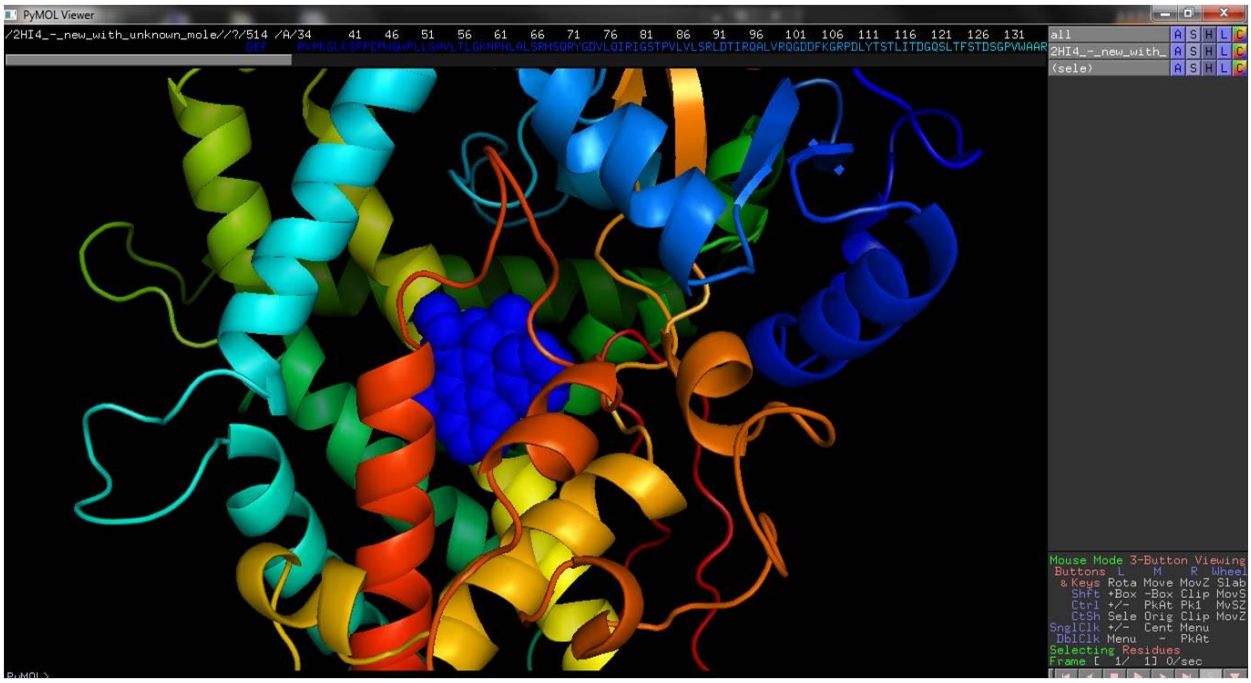


Figure 2: dimethyl 1,4 – diphenyl naphthalene – 2,3 – dicarboxylate with active site of 2HI4

In the 2HI4 structure, the α-helical hydrogen-bonding manner is lost at residues Val220 and Lys221, resulting in one helical turn in the middle of helix F to unwind. Two water molecules fill the space and thus form water-bridged contacts between Val220 carbonyl oxygen and Thr223 Oγ, and Lys221 carbonyl oxygen and His224 amide nitrogen, respectively.

Sansen et al., have reported that the substrate binding cavity of CYP1A2 is lined by residues on helices F and I that define a relatively planar binding platform on either side. Helix I bend while it crosses the heme prosthetic group, placing its residues in one flat side of the substrate binding cavity. Coplanarity is thus formed through the Ala317 side chain, the Gly316–Ala317 peptide bond, and the Asp320–Thr321 peptide bond. On the other side of the cavity, the side chain of Phe226 of helix F forms a parallel substrate binding surface. The active site cavity of CYP1A2 is stabilized through a strong hydrogen-bonding interaction between the side chain of Thr223 on helix F and the side chain of Asp320 on helix I. Both Thr223 and Asp320 play a role in forming an extensive network of hydrogen-bonded water molecules and side chains, including Tyr189, Val220, Thr498, and Lys500.

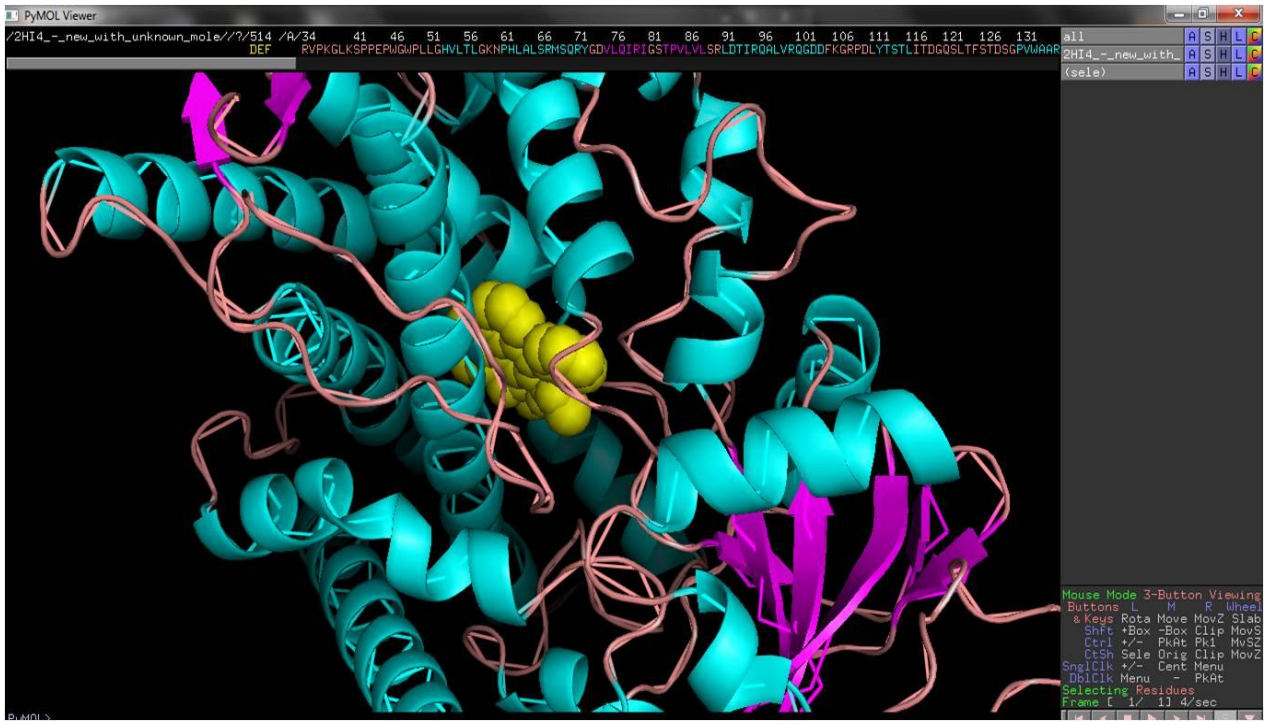


Figure 3: Naphthoflavone with active site of 2HI4

Our docking studies using Arguslab have identified Phe125, Phe256, and Ile 386 as the most important residues to influence the inhibitory potency of an enzyme. Based on the structure of 2HI4, we have successfully docked two substrates (Dimethyl 1,4 – diphenyl naphthalene – 2,3 – dicarboxylate and naphthoflavone) into its active site.

III. Conclusion

In conclusion, we have demonstrated synthesis, molecular modeling studies of a new series of dimethyl 1,4 – diphenyl naphthalene – 2,3 – dicarboxylate and naphthoflavone which possess good inhibitory activity against 2HI4. Molecular docking studies showed that hydroxyl and carbomethoxy functionalities at adjacent positions on dimethyl 1,4 – diphenyl naphthalene – 2,3 – dicarboxylate and Naphthoflavone are crucial for inhibitory activity as they are involved in a number of hydrogen bond interactions with Phe125, Ile386, Phe256 and hydrogen bump with Cys458 and Gly460. The newly crystallized compound dimethyl 1,4 – diphenyl naphthalene – 2,3 – dicarboxylate which showed docked energy -14.6659 Kcal/mol demonstrated 95% inhibition against 2HI4. A series of naphthalene derivatives demonstrated good inhibition against 2HI4 and are useful candidates leads for the development of potential inhibitor of Hydrocarbon Cytotoxicity and Microsomal Hydroxylase.

References

[1] ***"Small Molecule Crystallization"*** (PDF) at Illinois Institute of Technology website

[2] Bijvoet JM, Burgers WG, Hägg G, eds. (1969). ***"Early Papers on Diffraction of X-rays by Crystals (Volume I)".*** Utrecht: published for the International Union of Crystallography.

[3] Bragg W L, Phillips D C and Lipson H (1992). ***"The Development of X-ray Analysis".*** New York: Dover. ISBN 0486673162.

[4] Bragg WL (1912). ***"The Specular Reflexion of X-rays".*** Nature 90 (2250): 410

[5] Cho US, Park EY, Dong MS, Park BS, Kim K, Kim KH. "Tight-binding inhibition by α-naphthoflavone of human cytochrome P450 1A2. Biochim Biophys Acta." 2003; 1648:195–202

[6] Ewald, P. P., editor ***"50 Years of X-Ray Diffraction"*** (Reprinted in pdf format for the IUCr XVIII Congress, Glasgow, Scotland, International Union of Crystallography).

[7] Geankoplis, C.J. (2003) "Transport Processes and Separation Process Principles". 4th Ed. Prentice-Hall Inc.

[8] Glynn P.D. and Reardon E.J. (1990) "Solid-solution aqueous-solution equilibria: thermodynamic theory and representation". Amer. J. Sci. 290, 164-201.

[9] Jorgensen WL (1991). "Rusting of the lock and key model for protein-ligand binding". Science 254 (5034): 954–5.

[10] Kearsley SK, Underwood DJ, Sheridan RP, Miller MD (October 1994***). "Flexibases: a way to enhance the use of molecular docking methods"***. J. Comput. Aided Mol. Des. 8 (5): 565–82.

[11] Lengauer T, Rarey M (1996). ***"Computational methods for biomolecular docking"***. Curr. Opin. Struct. Biol. 6 (3): 402–6.

[12] McCabe & Smith (2000). "***Unit Operations of Chemical Engineerin"*** McGraw-Hill, New York

[13] Meng EC, Shoichet BK, Kuntz ID (2004). ***"Automated docking with grid-based energy evaluation"***. Journal of Computational Chemistry 13 (4): 505–524.

[14] Mersmann. A., ***"Crystallization Technology Handbook "***(2001) CRC; 2nd ed. ISBN 0-8247-0528-9

[15] Nocent M, Bertocchi L, Espitalier F. & al. (2001) "Definition of a solvent system for spherical crystallization of salbutamol sulfate by quasi-emulsion solvent diffusion (QESD) method", Journal of Pharmaceutical Sciences, 90, 10, 1620-1627.

[16] Sansen S, Yano JK, Reynald RL, et al. "Adaptations for the oxidation of polycyclic aromatic hydrocarbons exhibited by the structure of human P450 1A2". J Biol Chem. 2007; 282:14348–14355. doi: 10.1074/jbc.M611692200

[17] Tavare, N.S. (1995). "***Industrial Crystallization"***, Plenum Press, New York

[18] Tine Arkenbout-de Vroome, ***"Melt Crystallization Technology"*** (1995) CRC ISBN 1-56676-181-6

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