Inhibition of *Wb-iPGM* using analogues of Clorsulon and co-administration with DEC for bancroftian filariasis treatment

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Abstract. *Wucherria bancrofti* (*Wb*) is the main causative agent of Filariasis. Lymphatic Filariasis (LF) is a disabling, disfiguring, and a poverty promoting disease which is caused by the parasitic filarial nematode worms. The identification of essential nematode genes has shown that independent phosphoglycerate mutase (iPGM) is an excellent drug target against parasitic nematodes. In this current study, we have used our previously modeled structure of *Wb-iPGM* to inhibit the Embden Mayerhoff pathways of *Wb* using the Clorsulon and their analogues. Further, these analogues were scrutinized based on the pharmacokinetics and best complex were proposed to inhibit the *Wb-iPGM* enzyme. This current study provides an insight towards the process of design and development of novel drug target and their mode of interaction with the potent anthelmintic drugs.

Keywords: Lymphatic filariasis: phosphoglycerate mutase: Clorsulon: Co-administration: drug metabolism.

1 Introduction

Lymphatic Filariasis (LF) is a neglected tropical disease which promotes permanent disability, disfiguring and poverty in many countries. It is caused by the parasitic filarial nematode worms which are being transmitted to human through the mosquitoes bite (Sharma, OP et al., 2013, Hoti, SL et al., 2009). Currently more than 120 million people in 83 countries of the world are infected with this disease. It is estimated that 20% of the world population are at risk of acquiring the infection (Ottesen, EA et al., 2008). A global program for the elimination of LF has been launched employing mass drug administration (MDA) of annual single dose of a combination of diethylcarbamazine citrate (DEC)/ Ivermectin and Albendazole (ABZ) (Mani, TR et al., 2004; Sharma, OP et al., 2013) in endemic regions treating the entire at risk population.

Albendazole is the derivative of BZs. Recently, It has been reported that BZs are resistant to β-tubulins in a number of nematode species (Sharma, OP et al., 2012;

Skuce, P et al., 2010). The emergence of drug resistance in to β-tubulins is a potential threat to the latest public health concern around the world. This scenario has made it imperative to identify a new drug targets and develop novel anti-filarial drugs which could co-administrate with DEC which will help to cure this disease efficiently by clearing the microfilaria from the patient's body.

The identification of essential nematode genes has shown independent phosphoglycerate mutase (iPGM) is an excellent drug target against parasitic nematodes because it is non-homologous in human and other vertebrate hosts (Besteiro, S et al., 2005). Independent PGMs are essential for glucose metabolism, it catalyzes three types of reactions, including inter-conversion of 1,3-phosphoglycerate and 2,3-phosphoglycerate (2,3PGA) and of 3-phosphoglycerate (3PGA) and 2-phosphoglycerate (2PGA) as well as synthesis of 3PGA from 2,3PGA (Jedrzejas, MJ et al., 2000; Kumar, S et al., 2007; Zhang, YH et al., 2004). Therefore, inhibition of Wb-iPGM can prevent the full breakdown of glucose intake in nematodes by the Emden-Meyerhoff pathway and inhibits their glucose utilization.

Several studies suggest that Clorsulon could be an effective drug against phophoglycerate kinase and mutase of filarial nematodes (Gonzalez-Diaz, H et al., 2011; Martin, RJ, 1997). In this current study, a putative iPGM gene was identified in the protein sequence of the *Wucherria bancrofti* (*Wb*) and their tertiary structure was built to dock with analogues of Clorsulon to get an insight into their inhibition mechanisms. Since, DEC is highly effective against filarial parasite and currently it is in practice with ABZ. Clorsulon analogues were investigated for co-administration with DEC for the treatment of bancroftian filariasis.

2 Material and Methods

2.1 Receptor protein identification and preparation

We have used our previously modeled structure of Wb-iPGM for the current docking study (Sharma, OP et al., 2013). Receptor protein was prepared using Protein Preparation Wizard in maestro9.1. Protein was imported to workspace and bond order was assigned. Since, hydrogen bonds play an important role to determine the specificity of ligand binding (Wade, RC et al., 1989), hydrogens were added to the receptor protein and protein was minimized using Optimized Potential for Liquid Simulations (OPLS) 2005 force field.

2.2 Ligand identification and preparation.

It has been proposed by several investigators that Clorsulon can inhibit the glycolytic enzymes 3-phosphoglycerate kinase and phosphoglycerate mutase by block-

ing the Emden-Myerhof glycolytic pathway (Martin, RJ, 1997). Consequently, Clorsulon and their similar compounds were identified and retrieved from the PubChem Compound database from NCBI (Figure 1). Selected ligands were prepared using a LigPrep module in masestro9.1 (SchrÖdinger LLC, New York, NY, USA) by modifying the torsions of the ligands and assigning them appropriate protonation states. The OPLS 2005 force field at the pH ±7.2 was chooses to generate 32 conformers for each ligand by retaining ligands specific chiralities.

2.3 Pharmacokinetics prediction of the compounds

Toxicity is the main issue for many compounds which makes them failure during the clinical trials (Zenie, FH, 1994). It is estimated that ~20- 40 % of investigational drugs, during drug development are failing to reach the market due to the toxicity concern (van de Waterbeemd, H et al., 2003). Hence, Pharmacokinetics prediction of any compound is necessary to optimize and enhance the drug discovery process. QikProp is a tool to calculate the Absorption, Distribution, Metabolism, and Excretion (ADME) of any drug molecule. It predicts significant descriptors and pharmacokinetically applicable properties. It provides ranges for comparing specific molecule's properties with those of 95% of known drugs. Li-pinski's rule of five and drug toxicities were also calculated to avoid the toxic drug molecules.

2.4 Receptor grid generation and Docking experiment

Receptor grid was calculated for prepared proteins such that most of the ligand poses bind within the predicted active site. The grid box of 14 Å x 14 Å x 14 Å was generated around the centre of the characteristic residues (Asp14, Lys55, Asn62, Ser63, Glu64, Gly121, Gly122, Val123, His124, Arg155, Arg192, Asp193, Arg259, Asp261, Lys335, Ser360, Asp442, His443, and His460) (Sharma, OP et al., 2013)for docking experiment.

The molecular docking study was performed on the previously calculated receptor grids and ligands structure using Ligand Docking program on Glide for predicting best protein-ligand complexes and the ranking of ligands based on their Gscore (Repasky, MP et al., 2007). The ligands were docked flexibly using two different scoring functions SP (standard precision) docking and then XP (extra precision) docking to write at most 10,000 poses per docking run. For post docking minimization we included 5 posses per ligand.

SP docking is a method for screening the ligands of unknown quality in large numbers. For ligand atoms in SP scoring functions, van der Waals scaling factor and the partial charges cut-off was selected to be 0.80 and 0.15 respectively.

XP scoring function is more powerful and discriminating procedure to identify the ligand poses based on well-known principles of chemistry. It has additional terms over the SP scoring function. The main purpose of XP docking is to weed out false positives and to provide a better protein ligand poses and ligand binding score. Therefore, it consumes considerably longer CPU time than SP.

The best binding pose were analyzed based on the GlideScore. Each protein-ligand complexes were inspected visually in PyMOL molecular visualization software to get an insight into their binding mode of interactions. In each protein-ligand complex hydrogen bond, van der Waal interaction, Hydrogen bond donor and Hydrogen bond acceptor were inspected carefully.

2.5 Drug metabolism and drug-drug interaction

Drug-drug interaction has become an important issue in health care (Bibi, Z, 2008). To avoid the adverse effect of any potent or drug candidate it is important that during drug development process drug-drug interaction study has to be performed (Lin, JH et al., 1997). Therefore, it is important for any drug to avoid the accumulation of drug in our body or any toxic effect, drug should metabolize properly in our body (Akula, R et al., 2003; Pandey, V et al., 2011), and if it is co-administrative drug, it should not have any drug-drug interaction. Since, in this study we are recommending co-administration of analogues of Clorsulon and DEC. Therefore, it is important here to examine that these drugs should not have any drug-drug interaction and it should metabolized properly in human body system. Most of the hepatic drug-metabolizing enzymes including Cytochrome (CYP) are found in smooth endoplasmic reticulum. Cytochrome P450 enzymes are heme containing protein. CYP450 have seventy four CYP gene families out of which mainly CYP1, CYP2 and CYP3 actively take part in the drug metabolism. The important isoforms of P450 enzymes are CYP1A2, CYP2B6, CYP2C8, CYP2C19, CYP2D6, CYP2E1 and CYP3A4, 5, 7. Since, It is known that Clorsulon is being metabolized with P4503A Cytochrome (Sibille, P et al., 2000; Siegers, CP et al., 1981) (PDB ID: 3NXU) and DEC with the iso-enzyme of CYP450 (CYP2E1) (Siegers, CP et al., 1981) (PDB ID: 3E4E). We have done the molecular docking study of (1) CYP2E1 with DEC and Clorslulon and, (2) CYP4503A with the DEC and Clorslulon to get an insight into the drug interactions with the metabolizing enzyme and to examine whether co-administation of DEC could be prescribed with analogues of Clorsulon are safe and effective against the filarial treatment.

3 Results and Discussion.

3.1 Docking analysis.

Molecular docking analyses have been performed to investigate the inhibitory mechanism of *Wb*-iPGM with Clorsulon and similar compounds. Docking result has been summarized in Table 1 and shown in Figure 2 and Figure 3. The results are briefly described in the following sections

Clorsulon is an anthelmintics drug. It is being used for the treatment of helminthiasis. Several studies suggest it's effectiveness against adult flukes in sheep and cattle (Foreyt, WJ, 1988). In blood plasma, Clorsulon binds to the blood protein and when it is being ingested by any parasitic worm or flukes, it inhibits there glycolytic pathways. The docking analysis of Clorsulon with Wb-iPGM shows that it has satisfactory binding affinity. It is exhibiting G_score of -9.946 kcal/mol and -10.357 kcal/mol G_energy. Eight hydrogen bond sharing has been found during the docking experiment which is providing a stable and stronger binding conformation.

Compound 14 is exhibiting better docking result than the Clorsulon. Our detail investigation of docking analysis reveals that Compound14 could be a high potent drug for the treatment of bancroftian filariasis. It can successfully inhibit the active site of Wb-iPGM and interrupt their glycolytic and gluconeogenic pathway so that the nematodes would not be able to survive more. The current docking study suggests that it has a stronger binding affinity towards the target protein with the significant G_Score of -13.398 kcal/mol and -38.398 kcal/mol G_energy. PyMOL visualization of this protein-ligand complex has shown that it is strongly bound to the characteristics residues of Wb-iPGM (Glu64) with the bond length of the 1.64 Å which provides a stable conformation to the complex.

Our docking experiment suggests that compound 5 also have an excellent inhibition competence to the Wb-iPGM enzyme. Here, it is noticeable that Glu64 is involved in making two hydrogen bonds with a receptor protein with the bond length of 1.57Å and 2.79Å. Moreover, it is exhibiting excellent G_score and Glide energy of -12.932 kcal/mol and -35.807 kcal/mol respectively.

Other Clorsulon analogues such as Compound 13 (4-Amino-6-methyl-1,3-benzenedisulfonamide; 4-amino-6-methylbenzene-1,3-disulfonamide), 16 (4-amino-6-(1,2,2-trifluoroethenyl)benzene-1,3-disulfonamide), 10 (4-amino-6-(1,1-dichloro-2,2,2-trifluoroethyl)benzene-1,3-disulfonamide), 8 (4-amino-6-(1,2,2-trichloroethenyl)benzene-1,3-disulfonyl chloride) and 6 (4-amino-6-(1,2,3-trichloro-1,2-difluroethyl)benzene-1,3-disulfonamide) are also showing the favorable G_score of -12.036 kcal/mol, -11.836 kcal/mol, -11.275 kcal/mol, -10.968

kcal/mol and -7.749 kcal/mol with the G_energy of -39.277 kcal/mol, -37.304 kcal/mol, -34.393 kcal/mol, -29.232 kcal/mol and -33.584 kcal/mol respectively. Here, it is noticeable that except compound 6, remaining similar compound of Clorsulon is exhibiting better and stable docking conformation. It is also noticeable that compound 8 and Clorsulon is not targeting the characteristic residues of Wb-iPGM but it has hydrogen bond interaction with the other active site residues (Sharma, OP et al., 2013). Other residues such as Asp79, Gln78 and Gln295 are common active site residues involved in the inhibition mechanism of Wb-iPGM enzyme. These hot spot residues are playing critical role for the inhibition of glycolytic and gluconeogenic pathways of Wb.

3.2 Pharmacokinetics prediction of the compounds.

Selected analogues of Clorsulons were further scrutinized based on their pharmacokinetics properties. Therefore, we used QuikProp module of (W Jorgensen – SchrÖdinger LLC, 2003) QuikPropTM to study the Absorption, Distribution, Metabolism and Excretion (ADME) properties of Clorsulon and their analogues. Currently ADME investigation becomes a mandatory process to enhance the drug discovery and optimization process. QuikProp also evaluates the acceptability of the analogs based on Li-pinski's rule of 5 (Lipinski, CA, 2000; Lipinski, CA et al., 2001). In this current study, we have concentrate to analyze the Solvent Accessible Surface Area (SASA), Hydrophobic component of the SASA (FOSA), Hydrophilic component of the SASA (FISA), ¼ component of the SASA (PISA), Total solvent accessible area (Vol), predicted polarizability (QPpolrz), predicted log of hexadecane/ gas partition co-efficient (QPlogPC16), predicted log of water/gas partition co-efficient (QPlogPw), Percent of human absorption (PHOS) and predicted log of aqueous solubility (QPlogS). Toxicity of drug molecules was estimated based on the Toxtree-v2.5.1 tool (Devillers, J et al., 2010; Enoch, SJ et al., 2008) (Table 2). Our QuikProp analysis reveals that analogues of Clorsulon are non-toxic and pharmacologically suitable for further use. However, all of the proposed drug molecule should be tested experimentally before use.

3.3 Drug metabolism and drug-drug interaction.

Docking study of CYP2E1 and CYP4503A with Clorsulon and DEC was performed to investigate whether these two drugs can be co-administrated to inhibit the function of Wb-iPGM. Therefore, their drug-drug interactions were investigated with their drug metabolizing enzyme to avoid the drug toxicity and its side effects.

DEC is the drug of choice in most of the filariasis endemic countries. It is effective against microfilaria and adult worms (Anitha, K et al., 2001). If DEC would be given with Clorsulon, it may reduce the microfilaria counts in blood more efficiently and effectively. Our docking results suggest that co-administration of Clorsulon and their analogues with DEC are safe and effective. There is no drug-drug interaction between DEC and Clorsulon. The docking of CYP2E1 with DEC exhibits a good docking result of -4.64 kcal/mol while, Clorsulon exhibits +44.76 kcal/mol docking energy, which suggests that Clorsulon is not binding to the CYP2E1 while DEC inhibits the target metabolizing Cytochrome P450 2E1 (Figure 4).

Docking of Clorsulon and DEC with CYP4503A has been performed to investigate the any drug-drug interaction with the Clorsulon drug metabolizing enzyme (CYP4503A). Our docking result suggests that both the drugs are having good binding affinity toward CYP4503A enzyme with the docking score of -6.27 kcal/mol and -6.63 kcal/mol respectively (Table 3). Here, it is noticeable that in both the CYP enzyme drugs are sharing different binding pockets (Figure 5). Hence, we can suggest that there is no drug-drug interaction between DEC and Clorsulon and these drugs can be co-administrated for the treatment of bancroftian filariasis for the destruction of microfilaria more efficiently.

4 Conclusions

In this present study, we have investigated the pharmacokinetics and inhibitory activity of Clorsulon and their analogues with web-iPGM. Our docking study suggests that Glu64 and Asp79 are the most critical residues for protein-ligand interactions. The best Glide score -13.398 kcal/mol was shown by Compound 14 while other analogues of Clorsulon are also showing stronger binding affinity toward the target protein. The Glide score of docking of C5, C13, C16, C10, C8, Clorsulon and C6 are -12.932, -12.036, -11.836, -11.275, -10.968, 9.946 and -7.749 respectively. Since, DEC is a primary agent for the treatment of bancroftian filariasis (Duke, BO, 1981; Rivas-Alcala, AR et al., 1981) and does not contain any toxic metallic elements we are recommending co-administration of Clorsulon with DEC. Drug-drug interaction study was performed to avoid the drug-drug interaction. Our study reveals that there is no drug-drug interaction between Clorsulon and DEC, Hence, co-administration of Clorsulon and DEC could be co-administrated safely. All these findings provide a new insight into the interacting mechanism of iPGM with anti-filarial drugs and it could be a promising ground work for new drug discovery in the hunt of improved therapies against Lymphatic Filariasis.

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Table 1.

Docking result of Glide XP for the analogues of Clorsulon and their similar compounds with *Wb-iPGM* using SchrÖdinger 9.2 has been shown. Here, bold letters refer to the active site residues of the *Wb-iPGM* and Hydrogen bond distance have been shown in bracket (Å).

Compound ID	G Score	G_energy	Hydrogen Bond Donors and Hydrogen Bond Acceptors
C14 (21393678)	-13.398	-38.740	GLU309/O::LIG1/O(2.71 Å), ASP79/OD2::LIG1/HO3(1.60 Å), ASP79/OD2:: LIG 1/H1(1.95 Å), GLU64/OE2::LIG1/H1(1.64 Å), GLN78/OE1::LIG1/HO1(1.86 Å), LIG1/HO2::GLY344/O(1.84 Å), TYR77/O::LIG1/H16(2.03 Å)
C5 (21487684)	-12.932	-35.807	GLU64/OE2::LIG1/HO1(1.57 Å), GLU64/OE2::LIG1/N2(2.79 Å), GLN78/OE1::LIG1/HO3(1.56 Å), GLN78/OE1::LIG1/H6 (2.48 Å), TYR258/O::LIG11/N2(2.88 Å), THR294/OG1::LIG1/O(2.63 Å), GLN295/O::LIG1/HO (2.15 Å), ASP79/OD2::LIG1/H3 (1.73 Å)
C13 (517781)	-12.036	-39.277	GLN295/O::LIG1/HO1(1.60 Å), GLN295/O::LIG1/N2(3.42 Å), ASP79/OD2::LIG1/HO3 (2.28 Å), LIG1/H2::ASP79/OD2 (1.80 Å), LIG1/HO2::ASP79/OD2(2.14 Å), THR294/OG1::LIG1/02 (2.80 Å), GLN295/N::LIG1/O1(3.21 Å), GLU64/OE2::LIG1/N (2.56 Å), LIG1/O::GLU309/N(2.84 Å), LIG1/O::GLU1/O(3.10 Å)
C16 (21392408)	-11.836	-37.304	ASP79/OD2::LIG1/H5 (1.52 Å), GLN295/O::LIG1/HO (2.17 Å), LIG1/HO2::GLY345/O (1.79 Å), GLY78/OE1::LIG1/H7(2.04 Å), GLN78/OE1::LIG1/O3(3.12 Å),

			GLU64/OE2::LIG1/HO2 (1.52 Å), GLU64/OE2::LIG1/H6 (2.18 Å), GLN295/O::LIG1/H2(2.20 Å)
C10 (21487686)	-11.275	-34.393	TYR258/O::LIG1/H1(2.44 Å), GLU64/OE2::LIG1/HO3 (1.57 Å), GLN78/OE1::LIG1/H6 (2.20 Å), ASP79/OD2::LIG1/H1(2.06 Å), ASP79/OD2::LIG1/N1(2.80 Å), GLY345/O:: LIG1/HO (2.22 Å)
C8 (108916)	-10.968	-29.232	GLN295/O::LIG1/O1(2.79 Å), ALA260/N:: LIG1/O(3.0 Å), ASP79/OD2::LIG1/HO3 (1.77 Å), ASP79/OD2::LIG1/HO2 (1.83 Å), ASP79/N::LIG 1/02 (2.89 Å)
Clorsulon (43231)	-9.946	-10.357	GLN295/O::LIG1/H (1.67 Å), TYR258/O::LIG1/O(2.77 Å), ASP79/OD2::LIG1/H (1.69 Å), ASP79/OD2::LIG1/HO(1.94 Å), THR294/OG1::LIG1/O(3.03 Å), ALA260/N::LIG1/O (3.42 Å), GLN78/OE1::LIG1/HO(1.72 Å), ASP79/N::LIG1/O (2.48 Å)
C6 (21487680)	-7.749	-33.584	GLY121/O::LIG1/HS (1.67 Å), ASP120/OD2::LIG1/H7(1.68 Å), THR156/OG1::LIG1/N(3.21 Å), ASP155/O::LIG1/HO3 (1.77 Å).

Table 2.

Table 2. ADMET score of the selected compound. SASA: Solvent Accessible Surface Area (300-1000 A2), FOSA: Hydrophobic component of the SASA (0-750 A2), FISA: Hydrophilic component of the SASA (7-330 A2), PISA: ¼ component of the SASA (0-400 A2), Vol: Total solvent accessible area (500-2000 A2), QPpolrz: predicted polarizability (range 13-70 A3), QPlogPC16: predicted log of hexadecane/ gas partition co-eficient (range-4-18), QPlogPw: predicted log of water/gas partition co-efficient (5-48), PHOS: Percent of human absorbtion, QPlogS: prdected log of aqueous solubility (range -6-0.5 M)Toxicity.

Compound ID	SASA	FOSA	FISA	PISA	Vol	QPpolrz	QPlogPC16	QPlogPw	PHOS	QPlogS	Toxicity
C14(21393678)	519.749	175.97	295.073	47.518	883.750	24.531	9.952	17.724	29.064	-2.362	No
C5(21487684)	513.421	0	287.732	33.516	890.684	25.959	10.736	18.027	35.166	-3.005	No
C13(517781)	427.402	67.71	300.869	57.685	707.757	19.511	8.556	18.272	23.364	-1.723	No
C16(21392408	461.598	0	294.459	54.531	792.467	22.229	8.590	18.108	29.510	-2.754	No
C10(21487686)	476.256	3.132	290.195	35.834	828.305	23.484	9.353	17.999	32.069	-3.380	No
C8 (108916)	515.746	0	182.178	43.725	900.066	27.716	10.500	11.060	76.651	-3.563	No
Clorsulone (43231)	497.217	0.00	284.250	44.988	860.078	24.843	10.918	18.078	34.599	-2.720	No
C6(21487680)	502.481	0	280.399	28.078	894.511	26.060	10.856	17.959	37.079	-2.891	No

Table 3.

Table 3. Docking results of Clorsulon and DEC with drug metabolizing enzyme of CYP4503A and CYPP4502E1 have been summarized in table. Images have been prepared using PyMOL, here hydrogen bond distance have been shown in bracket (Å).

Drug	Docking Score CYP4503A	Docking Score CYP2E1	HB_D and HB_A For CYP4503A	HB_D and HB_A CYP2E1
Clorsulon	-6.27	+44.76	ASP174/OD1:: LIG`1/H (2.03) PRO199/O:: LIG`1/O (2.58) LIG1/O::ASN`159/ND2 (2.99) SER195/O:: LIG`1/N (2.64) SER195/HG:: LIG`1/H (2.57) ASN198/O :: LIG`1/N (2.88)	PHE298/O:: LIG`1/H (3.02)
DEC	-6.63	-4.64	LIG1/O::LEU`483/N (2.72) LEU483/O:: LIG`1/O (3.40)	LIG1/O::LEU`442/N(3.13) LIG1/O::GLY`441/N(3.02)

Fig.1. Investigated analogues of clorsulon have been shown in figure in stick format. (a) C14, (b) C5, (c) C13, (d) C16, (e) C10, (f) C8, (g) Clorsulone and (h) C6. Ligands have been shown in stick format. Here, green color denotes to Clorine, yellow color for Sulphur, red color for (Oxygen), blue color for Nitrogen and White color denotes hydrogen molecule.

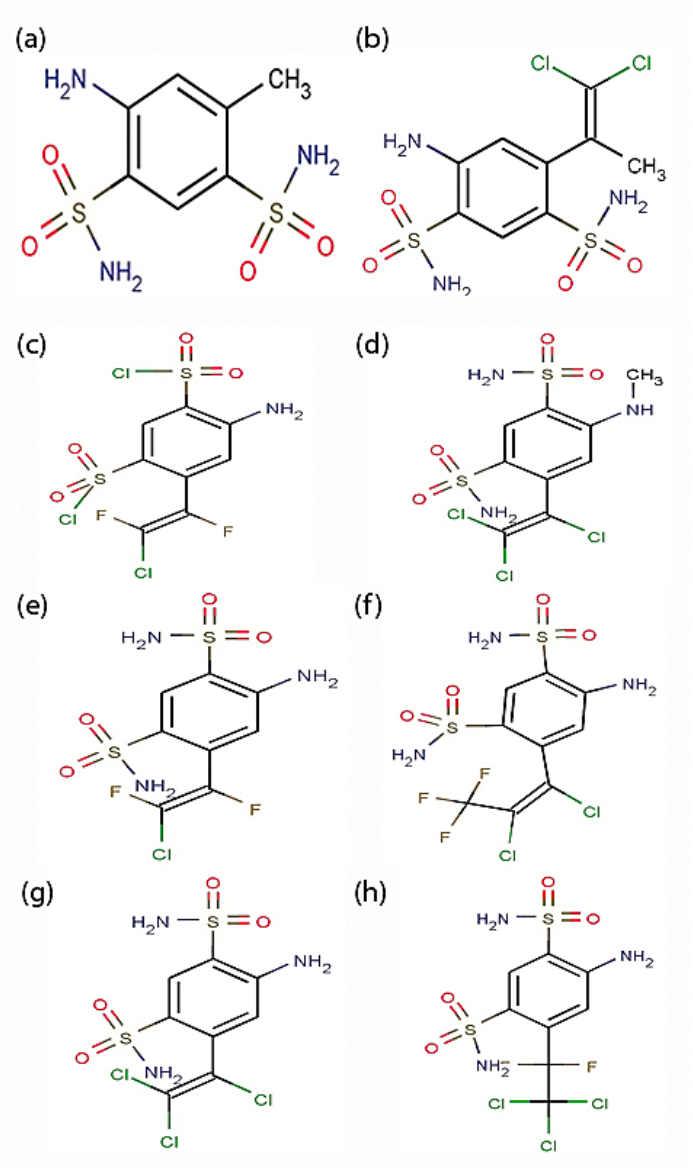

Fig. 2. Illustration of binding poses of (a) C14_ 21393678, (b) C5_21487684, (c) C13_517781 and (d) C16_21392408.

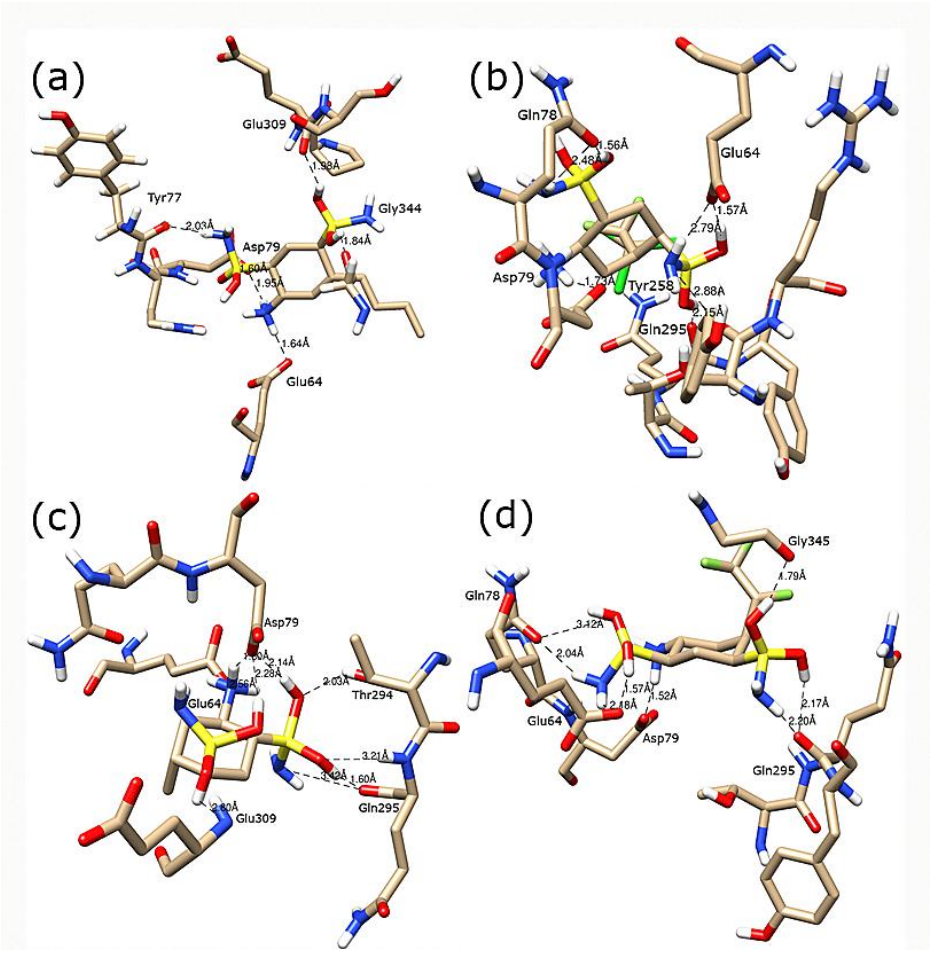

Fig. 3. Illustration of binding poses of (a) C10_21487686, (b) C8_108916, (c) Clorsulon_43231 and (d) C6_21487680.

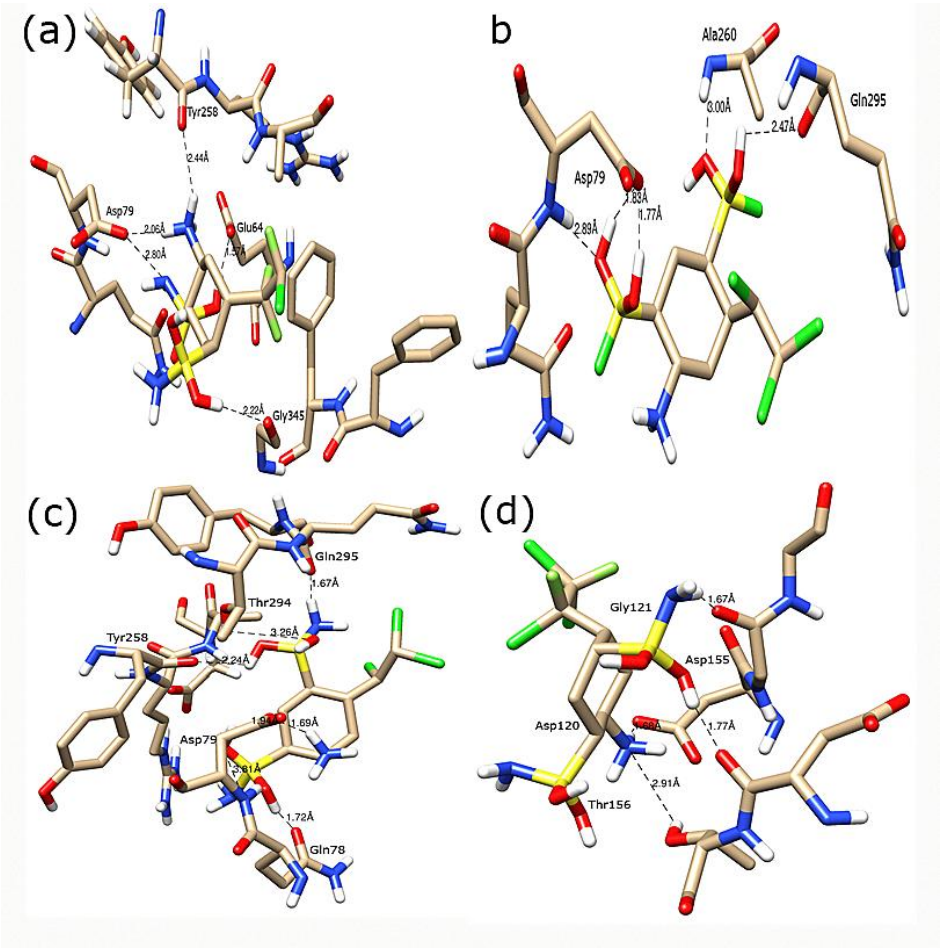

Fig. 4. Docking pose of DEC and Clorsulon with CYP2E1

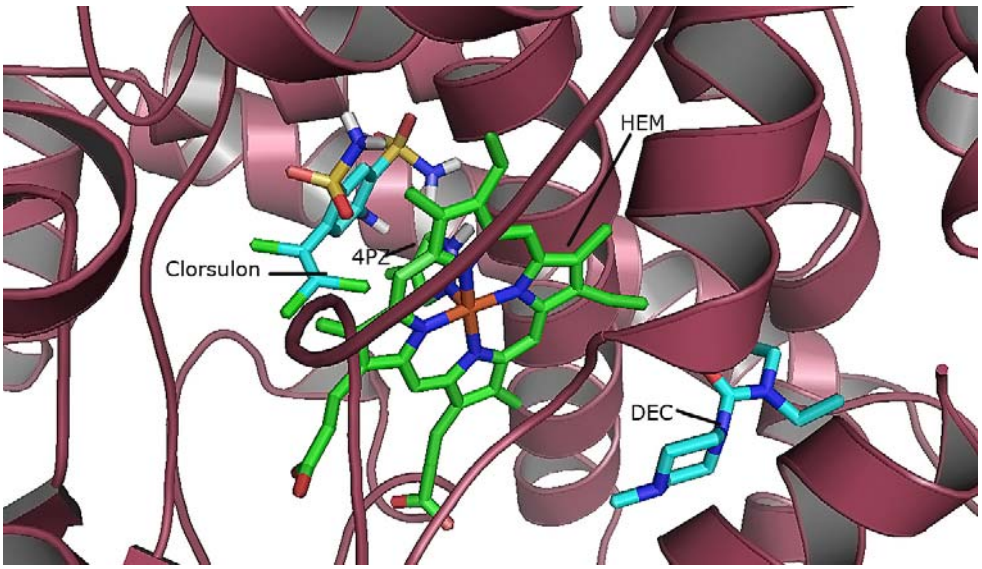

Fig.5. Docking pose of Clorsulon and DEC with P4503A