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3D QSAR, molecular docking studies and virtual screening of Tg DHFR inhibitors

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Abstract
The protozoan parasite Toxoplasma gondii (Tg) is a ubiquitous organism capable of infecting a wide range of vertebrate hosts including man. Toxoplasmosis is the disease caused by Tg. The parasite infects most genera of humans and warm blooded animals. Toxoplasma gondii Dihydrofolate reductase (Tg DHFR) are attractive drug target for treating Toxoplasmosis. Molecular modeling techniques are now widely used in medicinal chemistry for design and optimization of inhibitors for a specific target. A pharmacophore model was generated using reported Tg DHFR inhibitors in PHASE module of Schrodinger suite, the pharmacophore model was able to accurately predict Tg DHFR activity. Further the molecules were subjected to molecular docking to understand the binding mode, the docking results also provide additional confidence in the proposed Pharmacophore model. Results suggested that the proposed 3D QSAR model and docking analysis can be useful to rationally design new inhibitors. A combined pharmacophore based and docking based virtual screening was performed to obtain a possible lead molecule for further optimization.

Keywords: 3D-QSAR molecular docking and virtual screening of Tg DHFR, schrodinger suite

Introduction

Toxoplasmosis

It is a parasitic disease caused by *Toxoplasma gondii*. Infections with toxoplasmosis usually cause no symptoms in adult humans. Occasionally there may be a few weeks or months of mild flu-like illness such as muscle aches and tender lymph nodes. In a small number of people, eye problems may develop. In those with a weak immune system, severe symptoms such as seizures and poor coordination may occur. If infected during pregnancy, a condition known as congenital toxoplasmosis may affect the child.

Toxoplasmosis is usually spread by eating poorly cooked food that contains cysts, exposure to infected cat feces, and from a mother to a child during pregnancy if the mother becomes infected. Rarely the disease may be spread by a blood transfusion. It is not otherwise spread between people.[1] The parasite is only known to reproduce sexually in the cat family. However, it can infect most types of warm-blooded animals, including humans. Diagnosis is typically by testing the blood for antibodies or by testing the amniotic for the parasite's DNA.

Prevention is by properly preparing and cooking food. It is also recommended that pregnant women not clean cat litter boxes. Treatment of otherwise healthy people is usually not needed. During pregnancy spiramycin or pyrimethamine /sulfadiazine and folinic acid may be used for treatment.

Up to half of the world's population is infected by toxoplasmosis but have no symptoms. In the United States about 23% are affected and in some areas of the world this is up to 95%. About 200,000 cases of congenital toxoplasmosis occur a year. Charles Nicolle and Louis Manteaux first described the organism in 1908. In 1941 transmission during pregnancy from a mother to a child was confirmed.

Methodology

Development of new drugs is undoubtedly one of the most challenging tasks of today's science and several new technologies have been developed, Applied in drug research to shorten research processes and to reduce expenses. In post genomic area, Computer-Aided Drug Design (CADD) has considerably extended its range of applications, spanning almost all stages in drug discovery pipeline, from target identification to lead discovery, from lead optimization to pre-clinical or clinical trials. In early 1960s, Quantitative Structure–Activity Relationship (QSAR) analysis emerged as the first computer aided drug design technique. In recent decade the concept of CADD has evolved very quickly, with an unprecedented development of structural biology and computer capabilities. However, despite all these advances, revolutionary era of drug design has not yet arrived. There is no unique solution to a drug design problem, appropriate experimental techniques or computational methods to use will depend on characteristics of the system itself and information available. A variety of computational approaches can be applied at different stages of drug-design process: in an early stage, these focuses on reducing number of possible ligands, while at the end, during lead-optimization stages, emphasis is on decreasing experimental costs and reducing time. CADD now plays a critical role in search for new molecular entities. Current focus includes improved design and management of data sources, creation of computer programs to generate huge libraries of pharmacologically interesting compounds, development of new algorithms to assess the potency and selectivity of lead candidates and design of predictive tools to identify potential ADME/Tox liabilities.

There are two major types of drug design, first is referred to as ligand-based drug design and second, structure-based drug design. These are two distinct approaches used in the area of computer-aided drug design. When only lead is a set of known active compounds or knowledge of a biochemical

transformation which is to be interrupted, then the path is less direct, this approach is referred as Ligand (analogue) Based Drug Design (LBDD). Currently favored tactics include use of molecular similarity methods and employment of neural networks. Recent advances include the prediction of relative potency of different chiral forms of drugs. If molecular structure of the target macromolecule is known methods are direct and can give a high level of sophistication, this approach is referred as Structure Based Drug Design (SBDD).

Ligand-Based Drug Design (LBDD)

Relies on knowledge of analogue molecules that bind to biological target of a particular interest. These analogues are used to derive a pharmacophore model which defines the minimum necessary structural characteristics required for a molecule to bind to the target. Another approach in which a correlation between calculated properties of molecules and their experimentally determined biological activity is derived, it is referred as Quantitative Structure-Activity Relationship (QSAR). These QSAR relationships in turn may be used to predict activity of new analogs.

Results and Discussion

The importance of Di Hydro Folate Reductase (DHFR) (EC: 1.5.1.3) in parasitic chemotherapy arises from its function in DNA biosynthesis and cell replication. Inhibitors of DHFR block the function resulting in inhibition of DNA synthesis. 3D-QSAR model was performed on 167 previously reported Tg DHFR inhibitors. There structures are shown in figure 1.

3D QSAR Pharmacophore Generation: The present QSAR model was developed using the 'pharmacophore based' option of PHASE. To find the common pharmacophore hypothesis, the dataset was divided into actives and inactive set. Molecules with pIC_{50} values higher than 6.80 were considered to be active, and those with pIC_{50} values less than 5.00 were considered to be inactive, whereas those in-between were considered to be moderately active. One hypothesis was identified from the set of 12 actives (Table 1 and 2).

Table 1: Best pharmacophore hypotheses according to scoring values.

Hypotheses	Survival	Surv-inactive	Post-hoc	# matches
DDHRR.340	2.610	1.296	2.910	12

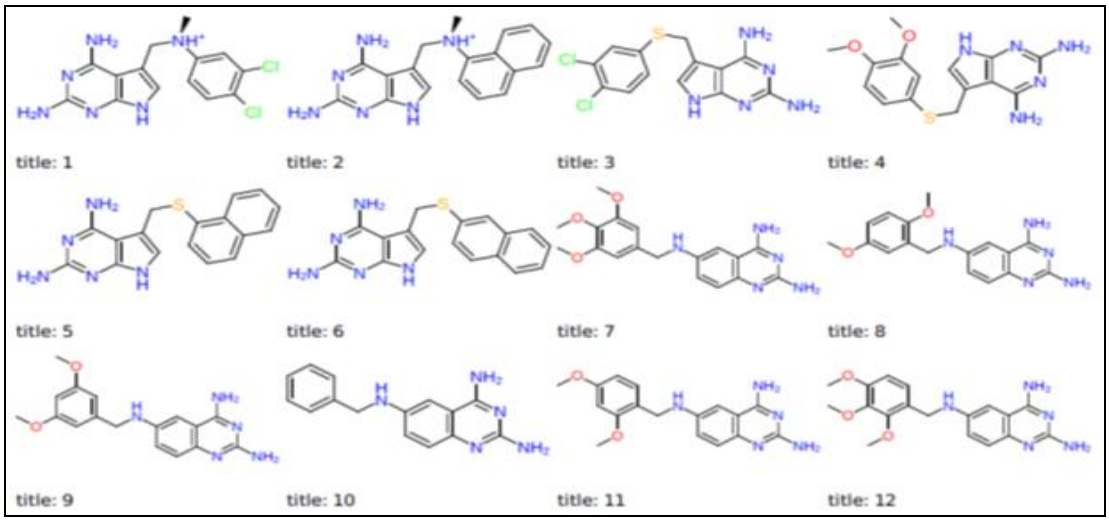

Fig 1: Structure of T*g* DHFR inhibitors

Table 2: Statistic parameters for the best pharmacophore hypotheses DDRRR

PLS factor	SD	R^2	Q^2	F	RMSE
1.	0.6737	0.4973	0.3789	87.1	0.7504
2.	0.5173	0.7071	0.4813	105	0.6858
3.	0.3026	0.9009	0.6156	260.5	0.5904

This hypothesis survived three different phases of PHASE scoring procedure (Survival, Surv-inactive, Post-hoc), and therefore this was used for the generation of QSAR models. For the QSAR models generation, non-modeled (inactive or moderately active) molecules in the dataset were then aligned, based on matching with at least three pharmacophore features. The dataset was randomly divided into training set of 90 compounds and 77 in the test set, in order to create, at least, the standard 2:1 training and test set ratio needed for QSAR study. The best pharmacophore model resulted DDHRR.340 (R^2 = 0.9009). The goodness of the model was validated by Q^2 for test set (Table 2). Plot of predicted vs. experimental pIC_{50} for training and test set is shown in Fig 2.

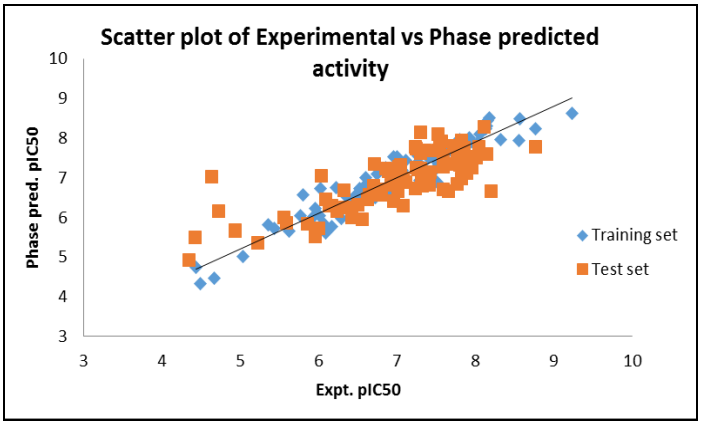

Fig 2: Scatter Plot of Experimental Phase predicted pIC_{50} for training and test sets

Pharmacophore sites spatial distribution of DDHRR.340 model (Figure) shows that two donor site (D6, D8), One hydrophobic (H12) and two aromatic ring (R17, R19) are found to be in the space of about 3.2 to 9.3 Å. In the pharmacophore mapping study, it was found that the major structural factors, affecting the potency of these compounds, are related to the basic skeleton. The pharmacophore hypothesis shows distance between pharmacophoric sites are depicted in figure 3 and values are given in table 3 and 4.

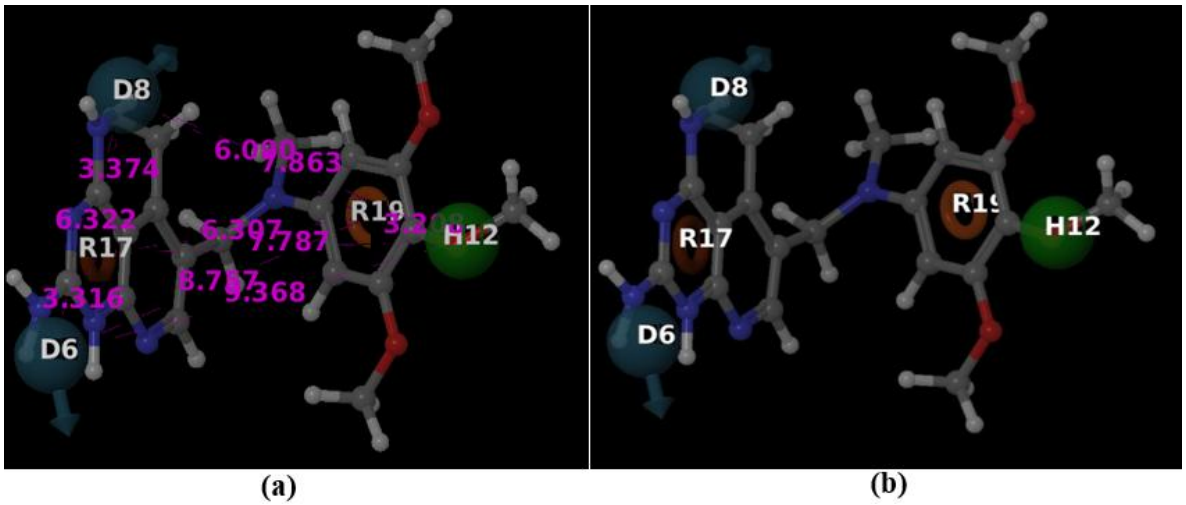

Fig 3(a): Pharmacophore model aligned with the best active compound 150 (b) Pharmacophore model DDHRR.340, all distances are expressed in Å.

Table 3: Distances between different sites of model DDHRR

Site 1	Site 2	Distance (A)
D6	D8	6.322
D6	H12	9.368
D6	R17	3.316
D6	R19	8.757
D8	H12	7.863
D8	R17	3.374
D8	R19	6.09
H12	R17	7.787
H12	R19	3.208
R17	R19	6.307

Table 4: Angles between different sites of model DDHRR

Site 1	Site 2	Site 3	Angle (A)
D8	D6	H12	56.2
D8	D6	R17	19.3
D8	D6	R19	44.1
H12	D6	R17	52.2
H12	D6	R19	20
R17	D6	R19	34.4
D6	D8	H12	81.9
D6	D8	R17	18.9
D6	D8	R19	89.7
H12	D8	R17	76.3
H12	D8	R19	22.3
R17	D8	R19	77.8
D6	H12	D8	41.9
D6	H12	R17	19.7
D6	H12	R19	69.2
D8	H12	R17	24.9
D8	H12	R19	46
R17	H12	R19	51.4
D6	R17	D8	141.8
D6	R17	H12	108.2
D6	R17	R19	128.3
D8	R17	H12	78.8

D8	R17	R19	70.7
H12	R17	R19	23.4
D6	R19	D8	46.2
D6	R19	H12	90.8
D6	R19	R17	17.3
D8	R19	H12	111.7
D8	R19	R17	31.5
H12	R19	R17	105.1

The pharmacophore map and QSAR contour maps can be used to design new and more active analogues. A descriptive representation of the contours generated in the QSAR is shown in figure 4. The major advantages of 3D-QSAR techniques are the cubes generated using PLS regression which could be visualized in 3D space.

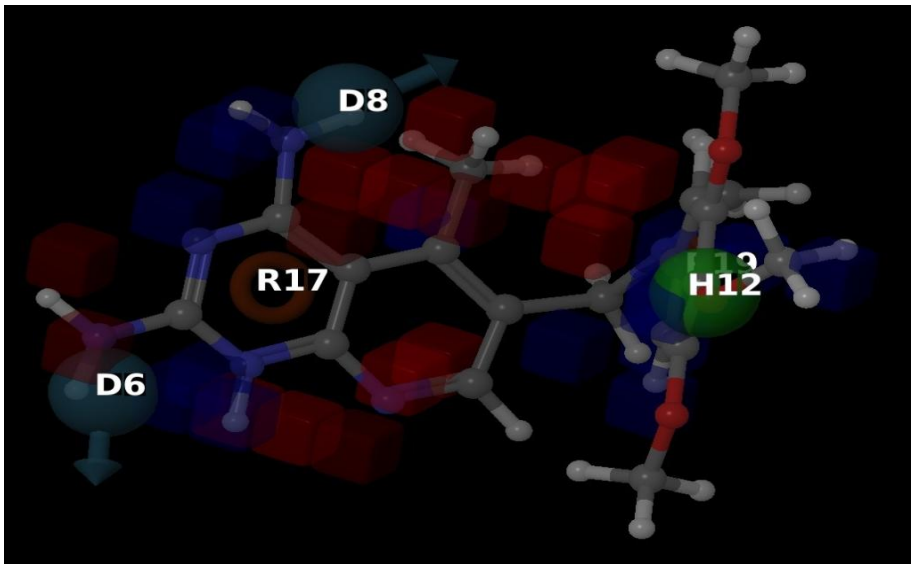

Fig 4: Pictorial representation of the cubes generated using the QSAR model of most active molecule. Blue cubes indicate favorable regions, while red cubes indicate unfavorable region for the activity.

The activity cubes can be generated for different properties such as hydrogen bond acceptor, hydrogen bond donor, hydrophobic, positive and negative ionic features, which define the non-covalent interactions with receptor. In these generated cubes, blue cubes indicate the favorable features and red cubes indicate the unfavorable features for the biological activity spectrum.

Table 5: QSAR set, Experimental activity, predicted activity, Phase fitness and Docking score of P*c* DHFR inhibitors

Molecule	QSAR Set	Activity	Predicted Activity	Fitness	Docking score in kcal/mol
1	Test	6	5.73	1.77	-3.594
2	Training	6.06	5.83	1.69	-4.127
3	Test	4.936	5.65	1.96	-6.956
4	Test	5.585	5.86	2.09	-6.954
5	Training	6.092	5.61	1.81	-7.819
6	Training	5.036	5.02	2.07	-7.623
7	Test	7.076	6.29	2.34	-7.143
8	Test	6.796	6.57	1.84	-6.805

Molecular Docking

For the docking analysis of 167 molecules, crystal structure of T*g* DHFR was collected from the RCSB protein data bank (PDB ID: 4KY4). The protein was prepared in 'protein preparation wizard' of Maestro. Protein preparation included addition of hydrogen atoms, deletion of solvent molecules except for active site waters, completion of bond order. Finally, the protein complex was prepared which was considered for docking analysis. Glide extra precision (XP) was utilized for docking.

Docking studies were carried out on all molecules and the most active molecule was analyzed in the receptor ligand binding region. Figure 5 shows docked model of best active compound within the active site of 4KY4. Best active molecule showed hydrogen bonding interactions with Val 8, and Asp31. Dock score of all the T*g* DHFR inhibitors is given in table 5.

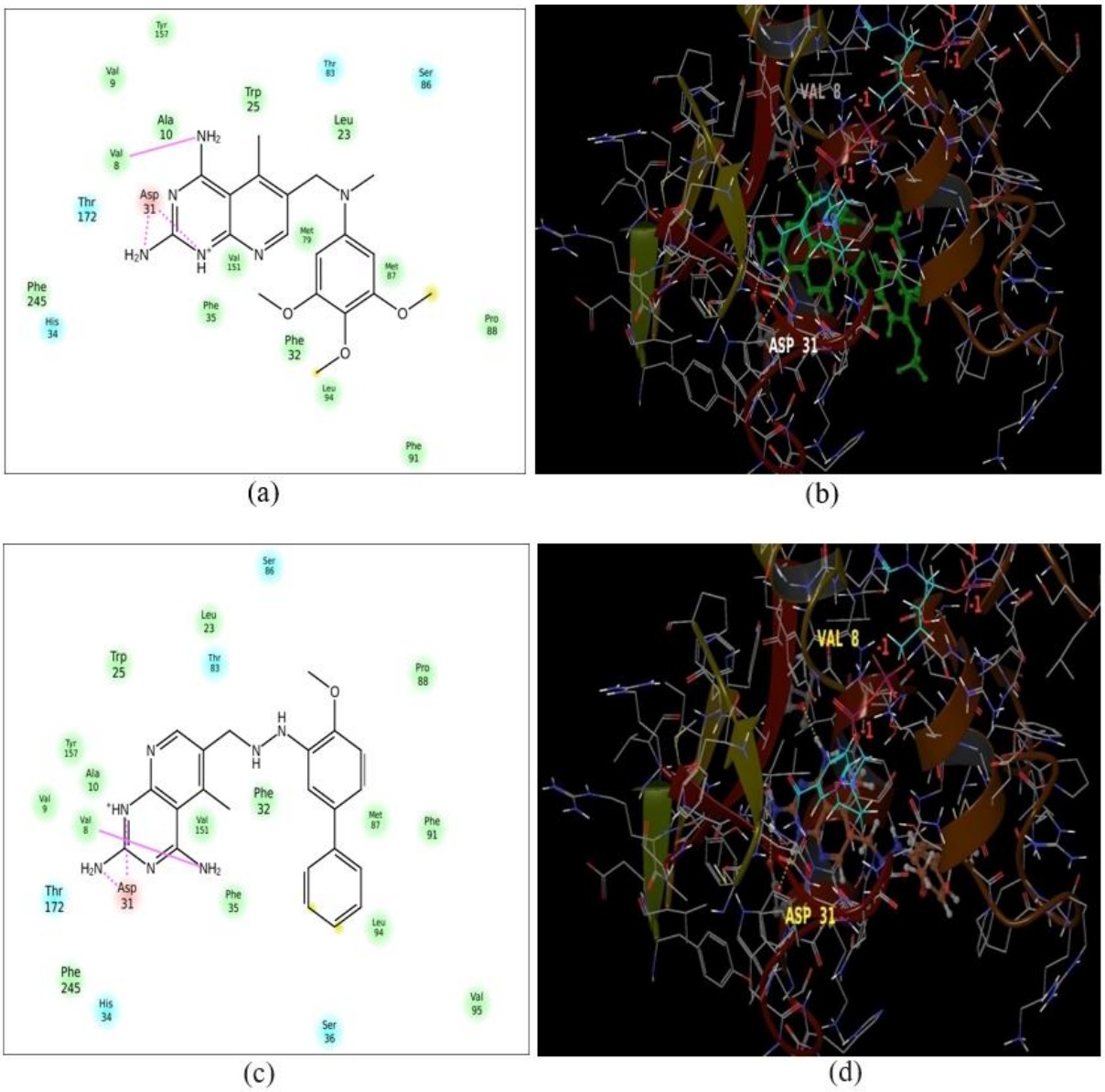

Fig 5: Dock pose and ligand interaction diagram of (a) and (b) most active molecule 43 and (c) and (d) best docked molecules 135 with hydrogen bond interactions.

Virtual Screening

The pharmacophore model has given an idea about how to get the necessary and sufficient molecular required for a new candidate. Thus the well validated pharmacophore DDHRR. 340 was used as a 3D query for retrieving potential new scaffolds against T*g* DHFR in the chemical databases. To Slab containing 7,054 molecules were used for screening process by the Find matches for hypothesis method. Initial screening and retrieved hits were filtered by using Lipinski's rule of five for the refinement of drug likeness and applying ADME properties, a total of 131 molecules were passed out these filtration. These hit molecules were taken for molecular docking studies, Different docking studies have been applied to identify suitable orientation of a ligand, ability to interact with the active site of the protein and also to check the retrieved molecules from the database whether the pharmacophore chemical features are mapped with structure-based interaction mode or not.

One hit was obtained from the virtual screen that carried common structural features for behaving as pharmacophoric features i.e the donor groups being NH group which are forming the hydrogen bond interactions with the essential active site amino acid residues Asp 31. From the above analysis, we conclude that the small molecule, which was retrieved from TO Slab databases have satisfied all necessary conditions such as binding affinity, calculated drug-like properties (figure 6) and thus could be treated as good leads in the design of potent inhibitors of T*g* DHFR. Hence, these filtering results have given a confidence of the validity and robustness of the quantitative pharmacophore model DDHRR.340. The dock pose and ligand interaction diagram of the virtual hit is shown in the.

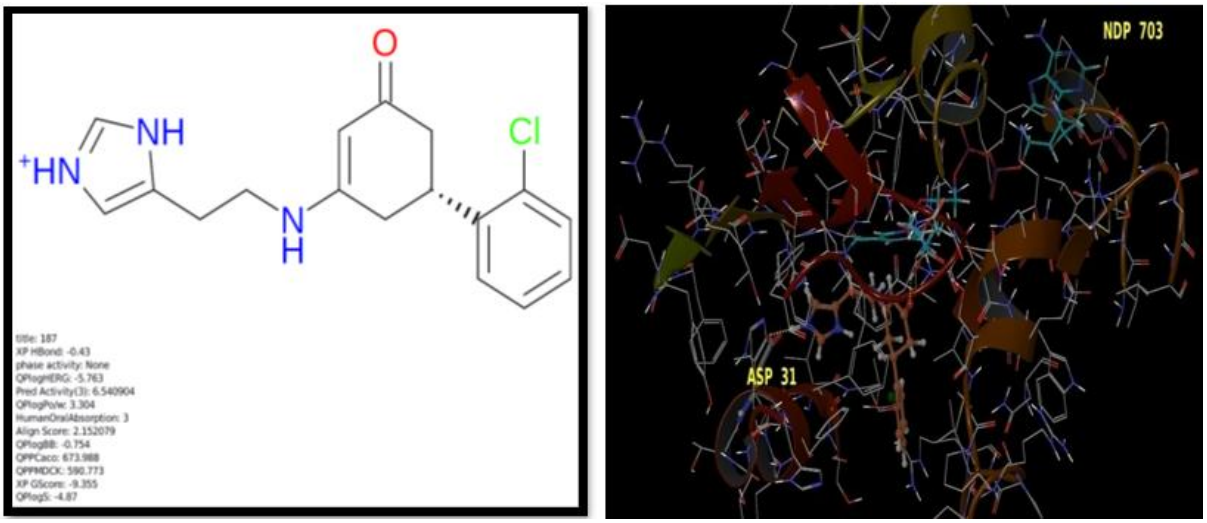

Fig 6: Structure of Virtual screening hit along with the calculated ADME and docking parameters.

Conclusion

This study shows the generation of a Pharmacophore model DDHRR for reported molecules as potent *Tg* DHFR inhibitors. Pharmacophore modeling correlates activities with the spatial arrangement of various chemical features. Hypothesis DDHRR represents the best Pharmacophore model for determining *Tg* DHFR activity. This Pharmacophore model was able to accurately predict *Tg* DHFR activity, the validation and the docking results also provide additional confidence in the proposed Pharmacophore model. Results suggested that the proposed 3D-QSAR model and docking analysis can be useful to rationally design new inhibitors. A combined pharmacophore based and docking based virtual screening was performed to obtain a possible lead molecule for further optimization.

References

1. Hunter CA, Sibley LD. Modulation of innate immunity by Toxoplasma gondii virulence effectors. Nature Reviews Microbiology. 2012; 10(11):766-78.
2. Parasites - Toxoplasmosis (Toxoplasma infection) Biology, 2015.
3. Flegr J, Prandota J, Sovičková M, Israili ZH. Toxoplasmosis--a global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. PLoS ONE. 2014; 9 (3):e90203. doi:10.1371/journal.pone.0090203.
4. Jones JL, Parise ME, Fiore AE. Neglected parasitic infections in the United States: toxoplasmosis. Am. J Trop. Med. Hyg. 2014; 90 (5):794-9. doi:10.4269/ajtmh.13-0722.
5. Torgerson PR, Mastroiacovo P. The global burden of con genital toxoplasmosis: a systematic review." Bulletin of the World Health Organization. 2013; 91(7):501-8. doi:10.2471/blt.12.111732.
6. Ferguson DJ. Toxoplasma gondii: 1908-2008. Homage to Nicolle, Manceaux and Splendore. Memórias Do Instituto Oswaldo Cruz. 2009; 104(2):133-48.
7. Dupont CD, Christian DA, Hunter CA. Immune response and immunopathology during toxoplasmosis. Seminars in Immunopathology. 2012; 34(6):793-813.
8. Dubey JP, Jones JL. Toxoplasma gondii infection in humans and animals in the United States. International Journal for Parasitology. 2008; 38(11):1257-78.