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DESIGN OF NOVEL 1, 3, 5 TRIAZINES AS ANTI CANCER AGENTS TARGETING AS DIHYDROFOLATE REDUCTASE ENZYMES (HUMAN DHFR): IN SILICO STUDIES

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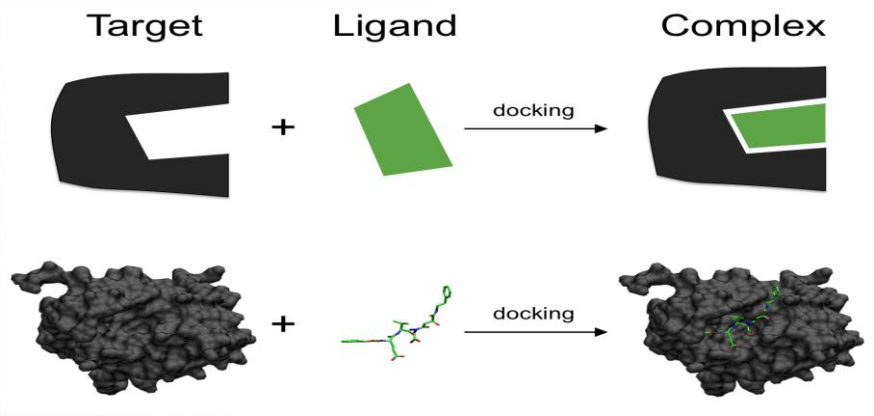
ABSTRACT

Docking is routinely used for understanding drug-receptor interaction in modern drug design. We focused on the docking of 1, 3, 5 Triazines derivatives as inhibitors to human dihydrofolate reductase (DHFR). We docked 15 derivatives collected from literature to DHFR and studied their specific interactions with DHFR. A new shape-based method, Ligand Fit, was used for docking 1, 3, 5 triazines derivatives into DHFR active sites. The result indicates that the docking approach is reliable and produces a good correlation coefficient (r= 0.156) for the 15 compounds between docking score and IC_{50} values (Inhibitory Activity). The nitrogen substituted triazine ring of compound 1 makes significant hydrophobic contact with Glu 30, Phe 31 and Phe 34 of the DHFR active site leading to enhanced inhibition of the enzyme. The docked complexes provide better insights to design more potent DHFR inhibitors prior to their synthesis.

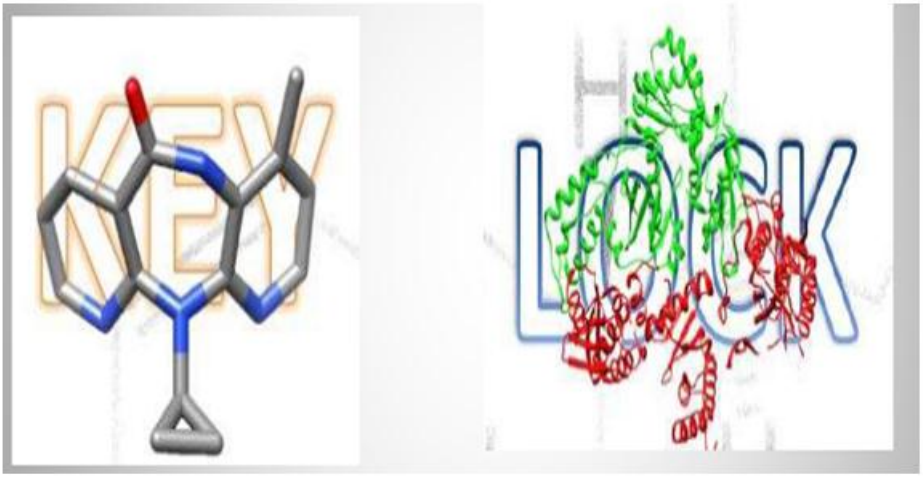
KEYWORDS: DHFR inhibitors, IC_{50}, docking, drug, receptor.

INTRODUCTION

Docking is an attempt to find the best matching between two molecules. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to form a stable complex with overall minimum energy. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, Scoring function.



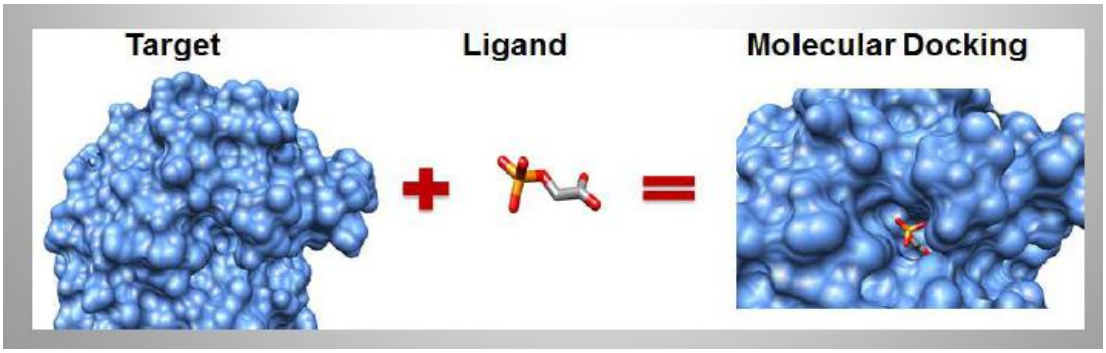
Lock and key finding the correct relative of the "key" which will open up the "lock". On the surface of the lock is the key in which direction to turn the key after it is inserted. The protein can be throught of the "lock" and the ligand can be throught of as a "key".

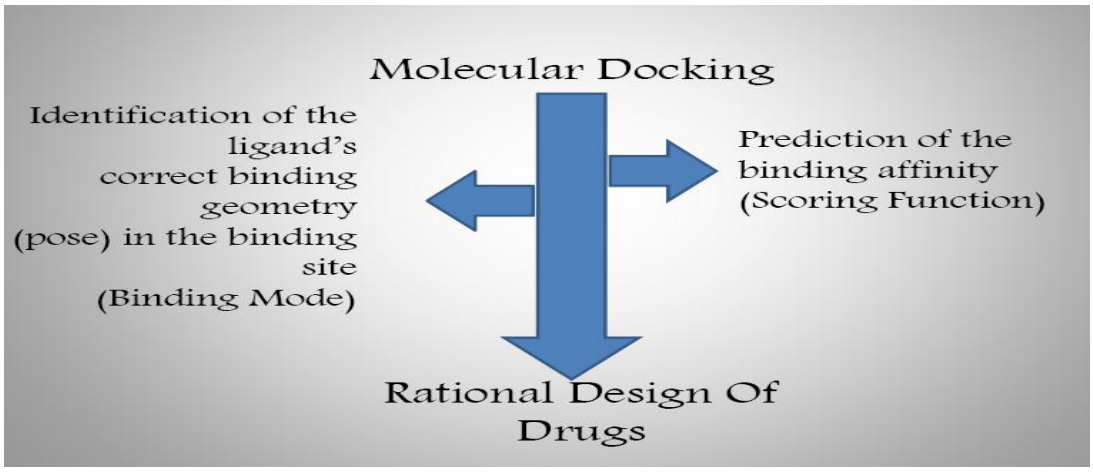


Nervirapine

Crystallographic structure of HIV -1
Reverse transcriptase Green coloured P 51
Red coloured P66 Subunit

To achieve an optimized conformation for both receptor and ligand& the relative orientation between protein and ligand such that the free energy of the overall system is minimized. Successful docking method search high –dimensional spaces effectively and use a scoring function that correctly rank candidate dockings.





Docking has initially – receptor(protein) and ligand rigid most current approaches receptor rigid, ligand flexible and advance approaches –receptor (to a degree) and ligand flexible fast simple to slow complex.

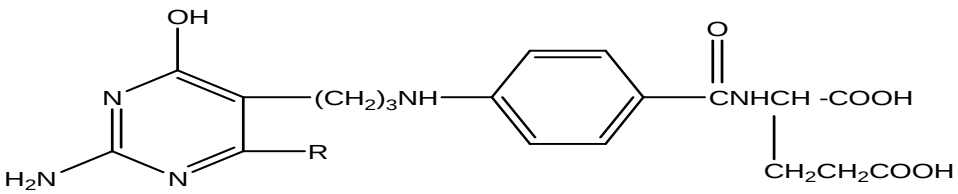
Two stages of docking

Pose generation: place the ligand in the binding site generally well solved .Rigid docking with a series of conformers most techniques use this approach and then techniques will generate the conformers internally rather than using conformers as inputs. Incremental Construction [Flexx]: Split ligand into base fragment and side-chains place base add side – chains to grow, Scoring as you grow. In general, uses a very basic vdw shape function often see variability with input conformers.

Pose Selection / Scoring: Where most of the current research focused more sophisticated scoring functions take longer. balance need for speed vs.need for accuracy virtual screening needs to be very fast studies on single compounds can be much slower.it can do multi-stage studies.

Dihydrofolate reductase (DHFR)

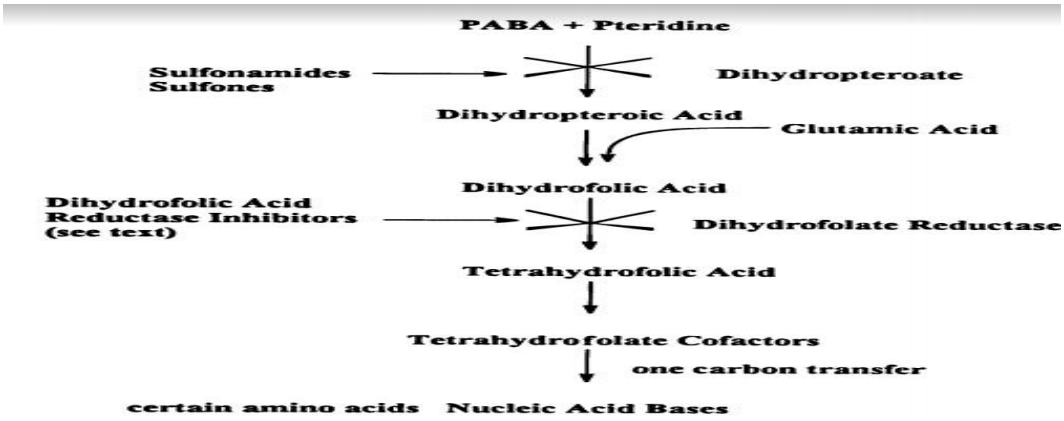
The chemistry and significance of dihydrofolate reductase (DHFR) was discussed in analogs of folic acid such ad methotrexate (MTX) were found to bind tenaciously to the enzyme's active site, exhibiting intense but nonselective cytotoxicity and there by becoming a useful anticancer drug. However, the inability of MTX to enter microbial cells (due to lack of a carrier mechanism that vertebrate cells have) and its considerable human toxicity made it useless as an antimicrobial agent. In a classic series of experiments to discover the molecule component of MTX responsible for the enzymes inhibition, Hitching's groups were added to this component to facilitate this property. Specially, 2, 4 diamino-6-ethyl-5-(P- chlorophenyl) pyrimidine resulted in the major anti malarial drug pyrimethamine (PM).The choice of a phenyl ring substituent at C-5 was particularly fortuitous. Over a decade later DHFR binding studies showed the phenyl substituent on the pyrimidinefolate analog Ia was a 12-20 times better inhibitor of the enzyme than the methyl group Ib. It was soon established that this improvement was primarily due to increased hydrophobic (Van der Waals) forces. Although highly effective as an antiplasmodial agent, pyrimethamine exhibiting inferior antibacterial activity. Further structural modifications that essentially provided the phenyl ring with some " peripheral" hydrophilicity(3,4,5 trimethoxy) resulted in the excellent broad –spectrum anti bacterial trimethoprim (TM).In order to understand the selective toxicity of drugs such as PM and TM and their significant differential activity between protozoa and bacteria, brief discussion of multiple forms of enzymes is in order.



Ia = C_6H_5
Ib = CH_3

Isoenzymes are multiple forms of an enzyme that differ from each other in such properties as substrate affinity, maximum activity, or regulatory properties.they may be found in different tissues or portions of the same cell. For example thymidine kinase catalyzing Phosphorlylation occurs as two isoenzymes- one in the cytoplasm and the other associated with the mitochondria of the same mammalian cells. Lactic dehydrogenase, which catalyzes the reduction of pyruvic acid, exists in five isozymic forms. These are tetramers formed by

the association of two polypeptides of equal size H (heart) and M (muscle).The impetus was the development of reversible inhibitors such as PM, TM, cycloguanil, and the carcinolytic drug MTX. It can be seen that inhibitory concentrations of MTX required to produce a 50% inhibition of DHFR from different source varies over a relativity narrow range, while those of the 2, 4 diamino –pyrimidine sometimes do so dramatically. Thus, in the case of PM one seen a, 3600-fold difference in inhibitory ability between the enzyme from a plasmodial species and the human liver. Thus 60.000 –fold difference for TM and E.coli is even more dramatic easily explaining the clinical effectiveness of TM for complicated UTIs caused ny this bacterium. The data also show that although TM would be active against the plasmodia species, PM is not likely to have antibacterial activity(50,000) – fold differential in binding site the plasmodial enzyme must have some significant difference from that of other source. certain variations of particular amino acids have been identified and even related to tertiary structural features of the "pockets" into which the pteridine nucleus fits. Stereoscopic representations generated from X-ray diffraction data have been obtained from DHFR co-crystallized with MTX and the co-enzymes NADPH has helped elucidate goodness of fit "or its absence, of the inhibitor another concept that should be considered at this point is sequential blockade as it relates to chemotherapy. Considering the outline of the folate biosynthesis scheme the likehood that the blockade with the selective agents of more than one reaction in sequence will increase the therapeutic value of treatment is apparent the two steps involved are the biosynthesis of dihydropteroic acid catalyzed by dihydropteroate synthetase and inhibited by sulfonamides and sulfones and the reduction of dihydrofolic acid by DHFR, which can be inhibited by MTX, PM, TM, and other DHFR inhibitors. The first synergism, which is a combined effect greater than the additive effects of the individual components of the drug product. In fact, a combination of TM and sulfadiazine at one-eight of their respective ED50s had an antimalarial effect equivalent to the ED5o of either drug singly.

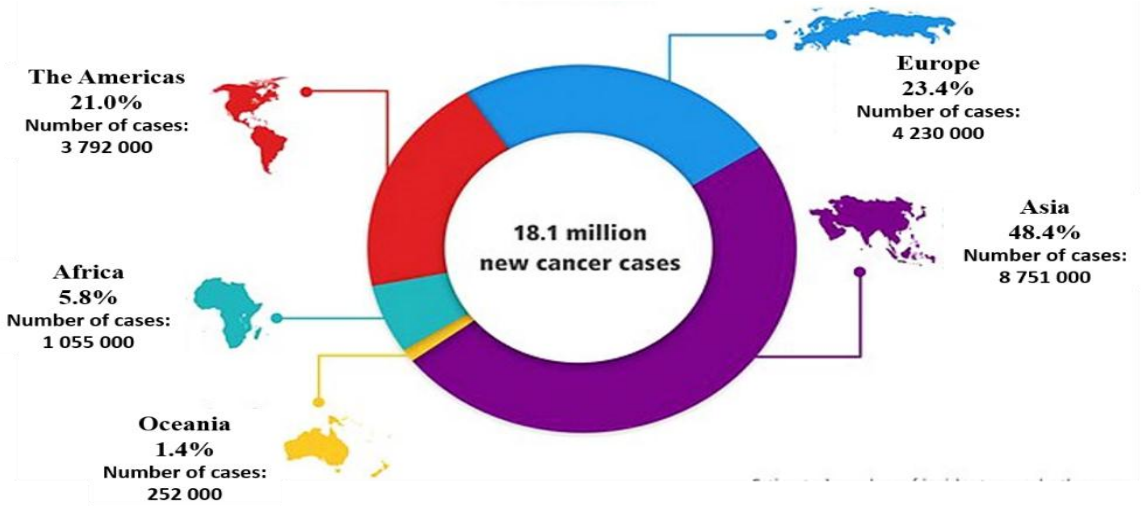


Further tests with similar combinations have corroborated this effect. The second potential advantage is to prevent, or at least delay, the emergence of resistance. The theory is that even though a single mutation is much less likely. This has in fact been found to be the case, even with some multi-drug resistant malarial organisms. Some of the sequential blocking combinations currently in use are listed in the long half life of PM (ca 4 days) requires it be combined with sulfone or sulfonamide of a comparable, or at least overlapping ,long excretion rate, so that drug's peak effectiveness would more or less coincide. The Dapsone-PM combination (Malprim^{R}, for example, has been useful chloroquin- resistant P. falciparum infections) the PM- Sulfadoxine product (Fansidar ^{R}) contains a 20:1 ratio of the two drugs and is also useful in similar situations. It is now considered to be effective against some strains of chloroquine – resistant falciparum yet toxic (P. Vivax does no respond).However, strains resistant to such combinations are being encountered. Even though the synergism observed with anti-folate –sulfonamide combinations appears real, whether or not the mechanism is truly a sequential blockade is questionable. For example it, was shown that a potent DHFR inhibitor, 2, 4 –diaminopteroyl aspartate, it not synergistic with sulfamethoxazole. In addition, it was found that DHFR isolated from E.coli could be inhibited by sulfonamides, suggesting a multiple simultaneous inhibition of DHFR by both drugs. It has also been called into question whether TM if used alone is more likely to induce resistant bacterial strains than in the cotrimoxazole product. A decade – long experience of testing UTIs with TM alone in Finland found no emergence of widespread resistance. Opposite results were apparently found in a British study with species of Enterobacteriaceae. Chinese herbal medicine may have made a contribution to the treatment of malaria. The herb Quinghao (Artemisia annua), in use over a millennium has finally yielded its active principle quinghaosu or artemisinine a sesquiterpene lactone containing a peroxide bridge clinical evaluation of it and several derivatives in the late 1970s was reported to have achieved successful treatment in over 2,000 patients, many with chloroquine resistant falciparum their activity is blood Schizonticidal. The mechanism does not appear to be antifolate or intercalation. The peroxide is essential, which raises a suspicion of free radical damage to the parasite.

Cancer

Cancer is one of the dreadful diseases in the world and mainly characterized by uncontrolled cell proliferation. Cancer burden rises to an approximate 18.1 million new cancer cases and 9.6 million cancer deaths in 2018. Worldwide, one in 6 women and one in 5 men develop

cancer during their lifetime, and one in 11 women and one in 8 men die from the disease. Global data clear shows that nearly half of the new cases and more than half of the cancer deaths worldwide in 2018 are estimated to occurs in Asian countries, because the region has nearly 60% of the global population and it is estimated to have a rise over 21.4 million new cases per year, with 13.2 million cancer deaths, by 2030.



Statistics of cancer

The top three cancer types *viz.* lung, breast and colorectal are responsible for one third of the cancer incidence and mortality burden worldwide.

MATERIALS AND METHODS

The molecular docking studies for the designed compounds were performed using Autodock Vina the three – dimensional crystal co-ordinates of the target protein PDB ID: 3GHC of resolution 1.30 Å, co-crystal (N-({4-[(2-amino-6-ethyl-4-oxo-3, 4-dihydrothieno [2, 3-d] pyrimidin-5-yl) sulfanyl] phenyl} carbonyl)-L-glutamic acid: IC_{50}-19nM) was retrieved from Protein Data Bank (PDB). The process of molecular docking included the following steps:

1. Protein and ligand preparation
2. Active site (grid) generation
3. Docking

First, the retrieved protein was prepared for docking by adding polar hydrogens, removing water, non –amino acid residue and adding Kolmann charges. Auto Dock atom types were defined using AUTODOCK Tools, graphical user interface of Auto dock supplied by MGL Tools.

2D structures of the designed compounds (C1-C4) used in the study were constructed using ChemDraw Ultra 8.0 Software [Cambridge soft corporation, USA (2003)] They were further converted to the Pdbqt format using OpenBabel 2.3.1. Further, the default root, rotatable bonds, and torsions of the ligand were set by TORSDOF utility in AutoDock Tools.

The grid was generated at the active site of co-crystal (GHC) with grid size of **40 x 40 x 40** while the grids centre having X, Y and Z Coordinates of **0.127, 14.665 and -1.005** respectively.

Finally docking was performed using genetic algorithm scoring function with population size 150, gene mutation rate 0.02, and crossover rate 0.8 .Total of 10 confirmations were generated for each compound. Ligand interactions are visualized and analyzed using the Discovery studio Visualizer.

Software used

Chemdraw ultra 8.0

AUTODOCK VINA

MGL TOOLS

Open Babel

Discovery Studio Visulizer

Docking Studies

To study in detail the binding mode and mechanism of action between designed compounds and DHFR molecular docking was performed .The docking results reveal that the inhibitors well fit into the active site pocket of DHFR molecular docking was preformed. The docking results that reveal that the inhibitors well fit into the active site pocket of DHFR. Docking score were displayed in Table

Table Docking scores of designed analogues.

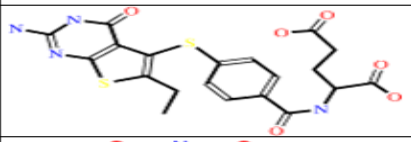
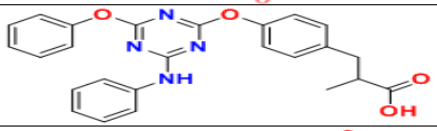
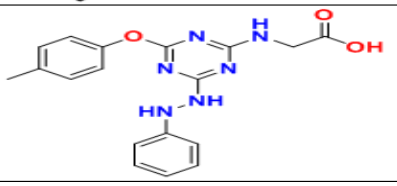
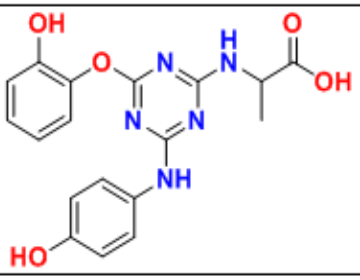
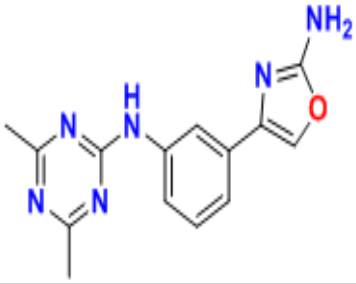
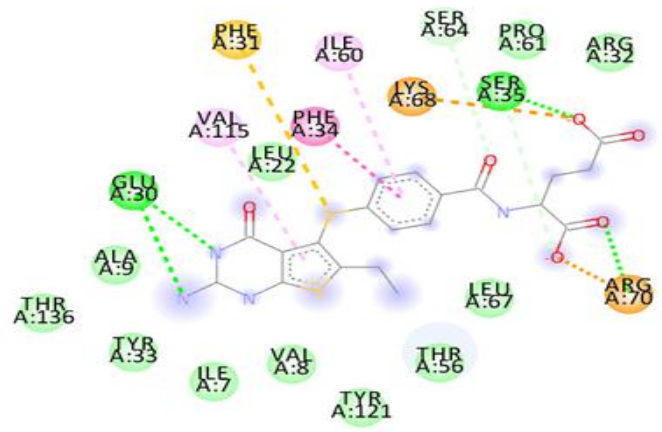
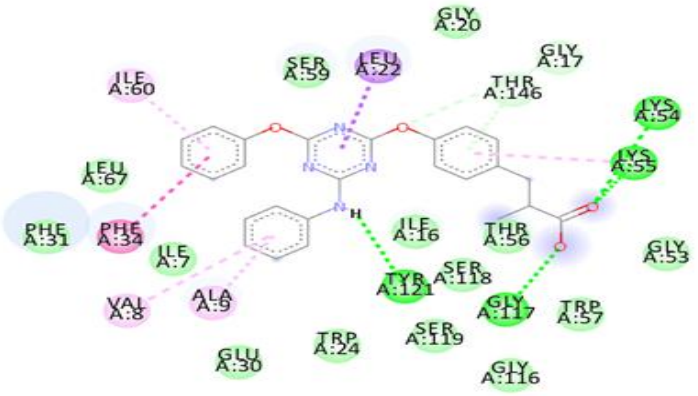
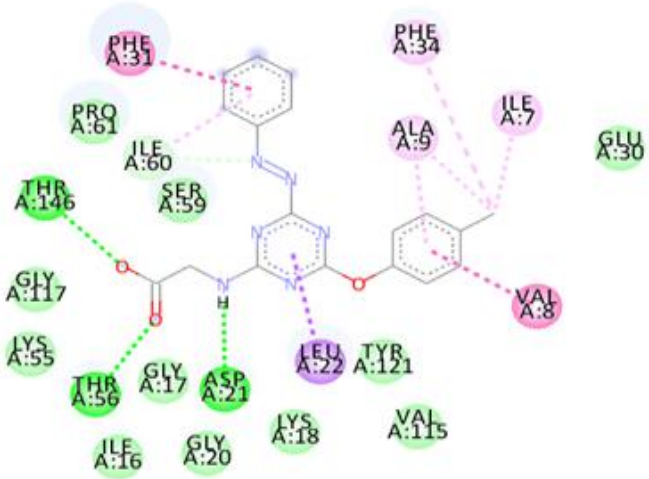
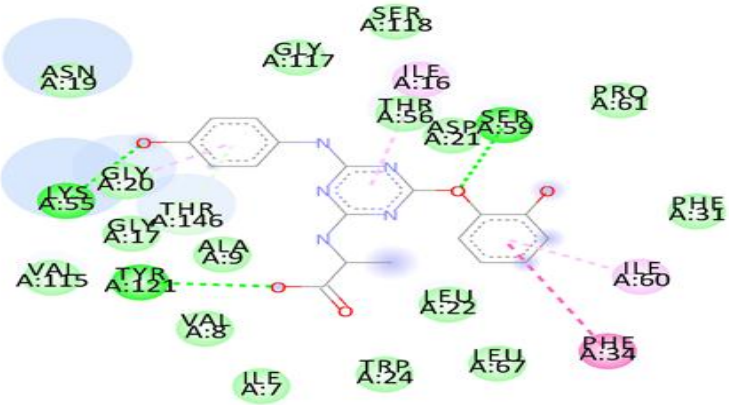
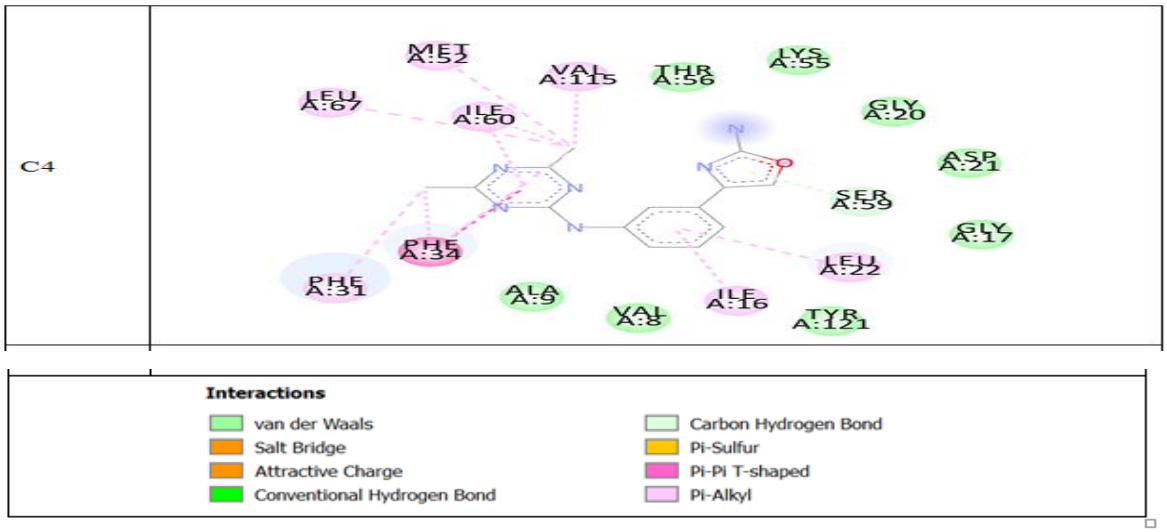
Ligand	Structure	Mol. wt.	Affinity (kcal/mol)
GHC(co-crystal/DHFR inhibitor)		476.54	-9.4sni
C1		442.47	-10.6
C2		366.38	-9.0
C3		383.36	-8.9
C4		282.30	-8.1

Table: Molecular docking results representing docking sores and interaction diagrams

Molecule	2D Interaction Diagram
Co-crystal	

C1	
C2	
C3	



The docking protocol was validated by considering the RMSD <1A^0 between crystal and docked pose of co-crystal ligand. Ligand Interaction diagrams were displayed in the designed compounds (C1-C4) displayed molecular interactions like Hydrogen bond, pi-pi, pi-alkyl with Phe34, Phe31, Val 115, Glu30, Lys 68 and Arg 70. Compounds C1, C2, C3 and C4 displayed highest docking score (-10.6, -9.0, -8.9, -8.1) in comparison to co- crystal.

ADMET studies

ADMET properties were calculated using PreADMET server and results were displayed in (C1-C4).

Table: ADMET properties of designed molecules (C1-C4).

		C1	C2	C3	C4
ADME	BBB	1.19162	0.228486	0.273254	0.0204243
	Buffer_solubility_mg_L	44.9922	1.27756e-009	712.066	3577.98
	Caco2	22.7984	20.7298	19.1633	21.098
	CYP_2C19_inhibition	Non	Non	Non	Non
	CYP_2C9_inhibition	Inhibitor	Inhibitor	Inhibitor	Inhibitor
	CYP_2D6_inhibition	Non	Non	Non	Non
	CYP_2D6_substrate	Non	Non	Non	Non
	CYP_3A4_inhibition	Non	Non	Inhibitor	Non
	CYP_3A4_substrate	Non	Non	Non	Weakly
	HIA	97.855995	88.650501	69.405038	91.630778
	MDCK	0.0489217*	0.254599	0.0677044	19.2611
	Pgp_inhibition	Inhibitor	Inhibitor	Inhibitor	Non
	Plasma_Protein_Binding	90.826065	85.69138	85.378518	59.784003
	Pure_water_solubility_mg_L	0.004029	2.41808	2.13752	53.0173
	Skin_Permeability	-1.9751	-3.34481	-3.80092	-3.93072
	SKlogD_value	4.8883	3.32291	2.25087	1.95451
	SKlogP_value	6.1363	4.57091	3.49887	1.95451
	SKlogS_buffer	-3.99275	-14.45755	-2.73109	-1.89708
	SKlogS_pure	-8.04069	-5.18046	-5.2537	-3.7263

TOXICITY	**algae_at**	0.00120097	0.0058836	0.00460892	0.083956
	Ames_test	mutagen	mutagen	mutagen	mutagen
	Carcino_Mouse	positive	negative	negative	positive
	Carcino_Rat	negative	negative	positive	negative
	daphnia_at	0.00158498	0.022243	0.0186753	0.205657
	hERG_inhibition	low_risk	high_risk	high_risk	medium_

The designed compounds are satisfying the ADMET properties as per the standards but certain limitations with respect to mutagenicity. The BBB permeability is <0.1 hence, these compounds won't cross the BBB. All the compounds having weak HERG score showing no cardiotoxicity.

CONCLUSION

In conclusion, we have successfully designed 4 analogues of 1,3, 5 triazines and studies the interactions with hDHFR enzyme and found that all the designed compounds binding at the active site with significant docking scores and interacting with crucial AA like **Glu30, Phe31, and Phe34.**The ADMET properties also calculated.

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