Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [6] May 2023 : 200-208

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Identification of Potent Lead to Inhibit DHFR for the Treatment Of Cancer: A Computational Approach

Bandral Sunil Kumar[1], Genne Soujanya[1], Golla Sireesha[1], Nayana Ravindra Jawale[1], Agasa Ramu Mahesh[1], Selvaraj Kunjiappan[2], Theivendren Panneerselvam[3], S. Kanimozhi[4] Parasuraman Pavadai[1]*

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy,
M.S. Ramaiah University of Applied Sciences, Bangalore-560054, Karnataka, India
[2]Department of Biotechnology, Kalasalingam Academy of Research and Education, Krishnankoil-626126, Tamilnadu, India
[3]Department of Pharmaceutical Chemistry, Swamy Vivekanandha College of Pharmacy, Elayampalayam, Tiruchengode- 637205, Tamilnadu, India
[4]Department of Anatomy, Sri SaiRam Siddha Medical College and Research Center, Chennai – 600 047, Tamil Nadu, India
E-Mail: pvpram@gmail.com

ABSTRACT

For researchers in the field of drug discovery the developing moiety for cancer treatment that is effective and has minimal side effects is a significant problem. Due to the enzyme's critical function in de novo purine synthesis, dihydrofolate reductase is an essential target for anticancer medicines belonging to the antimetabolite class. Here, we present the results of in silico screening using ligand-based computational methods to identify the most suitable compounds as DHFR inhibitors. We used the known DHFR inhibitors methotrexate, pralatrexae, piritrexin, talotrexin, and nalotrexin in our pharmacophore modelling using Pharmagist webserver to uncover the novel entities. Through the ZINCPharmer web server, 1000 most comparable pharmacophoric ligands that are feasible were identified. By the application of data warrior tool, the compounds were further filtered and used for molecular docking using Autodock Vina. Based on the binding energy and amino acid interaction top 8 ligands are subsequently evaluated for ADMET properties using PKCSM webserver. ZINC59491741 was found to be the best moiety with good binding affinity and having good interaction with key amino acids of DHFR.

Key words: *Cancer, DHFR, Pharmacophore Modelling, Molecular Docking*

Received 20.02.2023 Revised 17.03.2023 Accepted 23.05.2023

INTRODUCTION

Since the middle of the previous century, it has been established that the dihydrofolate reductase [DHFR] enzyme holds promise as a therapeutic target for the treatment of infections [1]. By using NADPH to catalyse the conversion of dihydrofolate to tetrahydrofolate, DHFR is involved in producing the building blocks for cell proliferation in both prokaryotic and eukaryotic cells. Infections caused by fungi, bacteria, and mycobacteria, as well as malaria and other protozoal diseases, are routinely treated with DHFR inhibitors [2]. Over time, numerous compounds have been discovered, and numerous new drugs have been introduced. We must notably highlight proguanil, pyrimethamine, and trimethoprim as antimalarial drugs, as well as methotrexate, a first-in-class anti-cancer drug that inhibits DHFR, and trimethoprim, an antibacterial drug that is usually used with sulfonamides like sulfamethoxazole. As a result of methotrexate's strong affinity for inhibiting DHFR, fewer tetrahydrofolates are required for the synthesis of pyrimidine and purines. The cancer cells die because of the disruption of RNA and DNA synthesis [3-5]. Numerous drawbacks of methotrexate exist, such as poor solubility and considerable harmful side effects [6,7]. Finding novel medications with a favourable pharmacological profile continues to be one of the main challenges for medicinal chemists. According to the literature trend of the past 20 years, DHFR inhibitors are one of the most often used families of anticancer drugs. In this review, we focus on DHFR inhibitors for cancer therapy after briefly describing how DHFR functions physiologically in general in cancer cells. We explicitly include commercially available compounds as well as brand-new scaffolds that may be advantageous for anticancer therapy. A water-soluble vitamin necessary for biological systems is

folic acid [FA]. It isn't physiologically active in and of itself, but it is the building block for the biologically active form of folate known as tetrahydrofolate [THF], which is necessary for the de novo production of purines, amino acids, and thymidylate [TMP]. It has been proven that without it, cell development and proliferation are inhibited. The synthetic process described in that article that enables the conversion of FA into THF as shown in fig 1 [8].

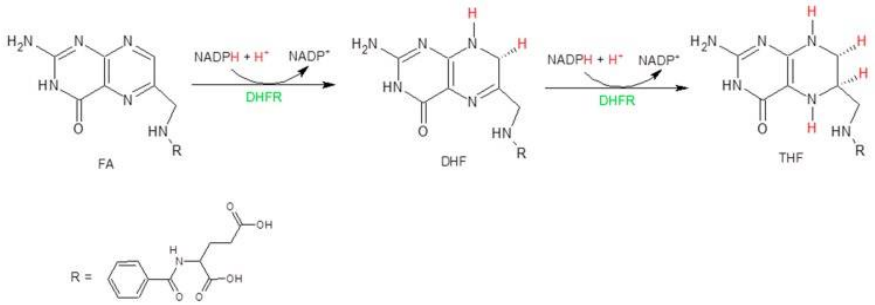


Fig1: Conversion of Folic acid to Tetrahydrofolate

Dihydrofolate synthase [DHFS] and DHFR, whose blockage causes cell death, are the only two enzymes that can synthesise folates in both eukaryotic and prokaryotic cells. The ubiquitous enzyme DHFR is of particular importance from a medicinal chemistry standpoint since it is crucial for cell proliferation, purine and thymidylate production, and folate metabolism. Tetrahydrofolate insufficiency and cell death result from insufficient DHFR activity [9]. DHFR is a relatively tiny, water-soluble protein having a molecular weight between 18,000 and 25,001 Da. The structure of DHFR isoforms has been attempted to be clarified numerous times throughout the course of years of intensive study of DHFR. The Protein Data Bank [PDB] currently contains more than 100 structures that were discovered in both eukaryotic and prokaryotic organisms, including humans, Lactobacillus casei, Pneumocystis carinii, Mycobacterium tuberculosis, and Escherichia coli. In a nutshell, DHFR is made up of eight sheets that together create a stiff skeleton. Seven of the sheets are parallel, and the other sheet is antiparallel. At least four -helices that intersect in the lengthy loops of the sheets are present in every enzyme isoform. In addition, one loop creates the substrate binding site, and the other two create the coenzyme NADPH binding site [10]. SubbaRow developed the folate inhibitor methotrexate in 1947. Sidney Farber, a physician, proposed a theory a year later that methotrexate might impede the spread of cancer because cancer cells require folate to support their rapid growth. He showed that methotrexate was useful in symptom management in pediatric acute lymphoblastic leukemia patients. A few years later, the medication was introduced into therapy for the management of psoriasis and rheumatoid arthritis. Currently, several autoimmune disorders and cancers are treated with methotrexate in humans. The cytotoxicity of methotrexate against oral mucosa, gastrointestinal [GI] tract epithelial cells, bone marrow cells, and testicular tissue is caused by its limited selectivity of action against different isoforms of DHFR [11-13]. The numerous endeavors undertaken over the past five decades to find more effective and selective medications have resulted in the discovery of the medications [14]. Currently, various substances are being used in therapeutic settings. These include the "traditional antifolates" raltitrexed, pralatrexate, and pemetrexed as well as the "non-classical antifolates" piritrexim, trimetrexate, talotrexin, and nolatrexed. Classical antifolates are structurally like folate and have a glutamate tail, an aromatic ring, and a pterin ring [15]. They are actively transported by the decreased folate carrier system because they have a charged glutamate tail, which prevents them from passively diffusing across cell membranes. Non-classical antifolates are lipophilic compounds that passively diffuse into cells without the assistance of folate transport mechanisms. They are helpful for the creation of new anticancer, antibacterial, and antiparasitic drugs because they can limit the proliferation of tumor cells. Pralatrexate was the most recent DHFR inhibitor to receive FDA approval. Rapid internalization into the cell, a strong affinity for dihydrofolate reductase, and good intracellular retention are its distinguishing characteristics [16].

Hence the present work focuses to identify novel molecules to inhibit the DHFR enzyme for the treatment of Cancer disease through the application of computational tools.

MATERIAL AND METHODS

Identification of Drug Molecules: Based on the literature research of DHFR, the current identified medications were methothrexate, raltitrexed, pralatrexate, pemetrexed, piritrexim, trimetrexate, talotrexin, and nolatrexed. Methothrexate is the most often used therapy for the treatment of cancer and

is also a DHRF inhibitor. The compounds' structures were obtained in SDF format from NCBI Pubchem [17] or Chemdraw [18]. Using DISCOVERY STUDIO, create a cluster that contains all the ligands and save it in Sybyl Mol2 format. The Protein Data Bank [PDB] has gathered more than 100 structures that were isolated either alone or in association with various ligands from both eukaryotic and prokaryotic organisms, including humans, Escherichia coli, Lactobacillus casei, Pneumocystis carinii, and Micobacterium tuberculosis [19]. The most recent version in PDB format has been downloaded to isolate the protein with the best resolution, which is 2.0-2.5. Utilize the SPDV programme to purify the protein [OPEN web server].

Pharmacophore Modelling: A pharmacophore is a three-dimensional framework created by mapping the physiologically powerful chemicals necessary for a ligand to bind to and interact with a certain target protein. A pharmacophore is a spatially mutually oriented atom or group of atoms that is thought to interact with and recognize a receptor or the active site of a receptor. Using an open web server called PharmaGist, the abovementioned compounds were put through pharmacophore modelling. An indirect technique for creating pharmacophoric characteristics for a group of compounds is called PharmaGist [20-21]. The service generates the pharmacophore in mol2 format by considering various methods to mix the given chemicals.

Screening of zinc database from ZincPharma by pharmacophore model: When mol2 were put to an open webserver like ZINCPharmer, the pharmacophoric characteristic was obtained. An online tool called ZINCPharmer allows users to search the ZINC database using pharmacophoric information gleaned from a collection of compounds. It aids in locating compounds that possess the necessary pharmacophoric characteristics. further investigation and measurement of the pharmacophoric characteristics using Discovery Studio version 20.1 Depending on the need, the input parameters for RMSD, molecular mass, and rotatable bond can be changed. The ZINCPharmer website was used to process the pharmacophore from PharmaGist, and a total of 100 tiny compounds with RMSDs of 0.1–0.2 were generated. All these compounds were obtained as SDF download files for molecular docking experiments [22-23].

Molecular Docking protocol: Docking studies were carried out to analyze the different types of biomolecular interactions and ligand receptor binding affinities. The docking studies were carried out by means of Autodock vina, Biovia Discovery Studio 2020, PyRX, and PyMOL. Protocol for docking as shown in fig 2.

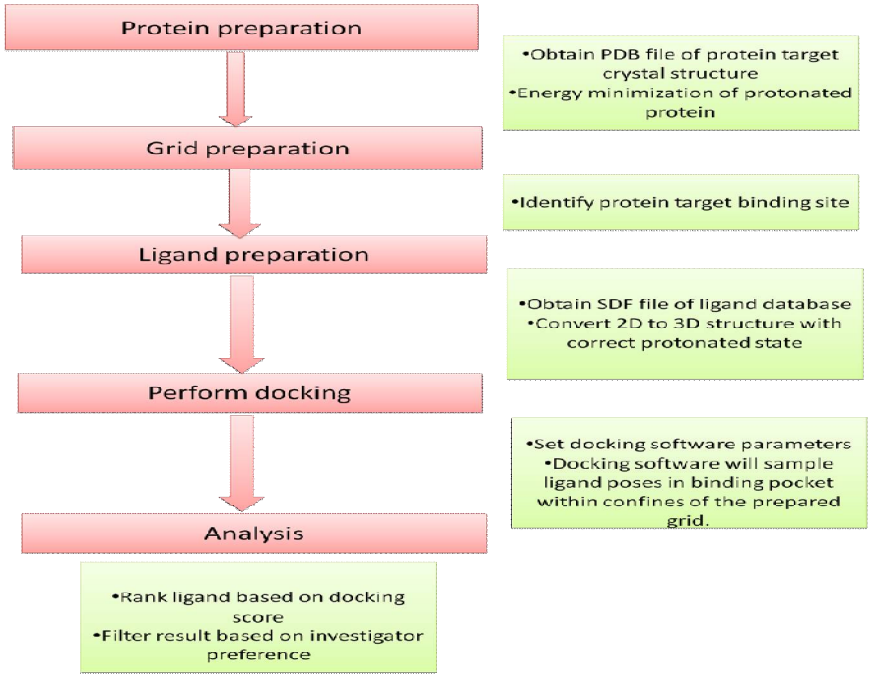


Fig 2: Protocol for docking study

Then, on substances that had been received by the ZINC database, molecular docking experiments were carried out. To ascertain the relationships between residues and the ligand's potential to attach to the chosen target protein, molecular docking studies were carried out. The ligand and protein were generated using Autodock Tools, a component of MGL Tools 1.5.6, and docking research was completed using Autodock Vina. A molecular visualization programme called PyMol version 2.4 was used to further confirm the docking process. The docking process' validity was checked by calculating the RMSD value.

The command line was used to calculate the RMSD value with default settings while superimposing the docked output structure and co-crystallized posture. Using Autodock Vina, all the ligands were produced, minimized, and optimized. The protein must first be prepared before docking investigations can be carried out. The target protein DHFR was then further prepared using the Protein Data Bank [PDB] and its PDB ID of 4p68 [24-26]. Docking result of standard drugs as shown in table.1

Table 1: Docking Score and Type of Interaction of Standard Drugs with DHFR [4P68]

compound	Docking score	Interaction residue	Type of interaction
Methothrexate	-9.9	ASP27 GLY97 MET20	Hydrogen bonding interaction Π-Sulfur
Pralatrexate	-9.9	ASP27 ILE5 MET20	H-bonding interaction Π-Sulfur
Pemethrexate	-9.8	ASP20 ALA7 MET20	H-bonding interaction Π-Sulfur
Ralititrexed	-9.6	PHE MET20	Π- Π Stacked interaction Alkyl interaction
Talotrexin	-9.3	THR46 MET20	H-bonding interaction H-bonding interaction Π-Sulfur
Piritrexim	-8.6	THR123 ILE14 MET20 HIS45	H-bonding interaction
Trimetrexate	-8.6	ILE14 ARG98 MET20	H-bonding interaction Π-Alkyl
Nalotrexed	-7.9	ASP46 ALA20 MET20	H-bonding interaction Π-Sulfur

Molecular Docking Study of ZINC Database: Molecular entities from the pharmacophore based ZINCpharmer zinc database. The chemical was next put through a molecular docking study with 26 compounds from the ZINC library as the target PDB ID: 4p68. To compare the binding interactions of the compounds from the existing medications, the docking was examined to find the compounds with good docking scores [mostly negative]. The hit compound's identification by docking the zinc database and this is useful in comparison for the DHFR inhibitor. Docking result of zinc database as shown in table.2

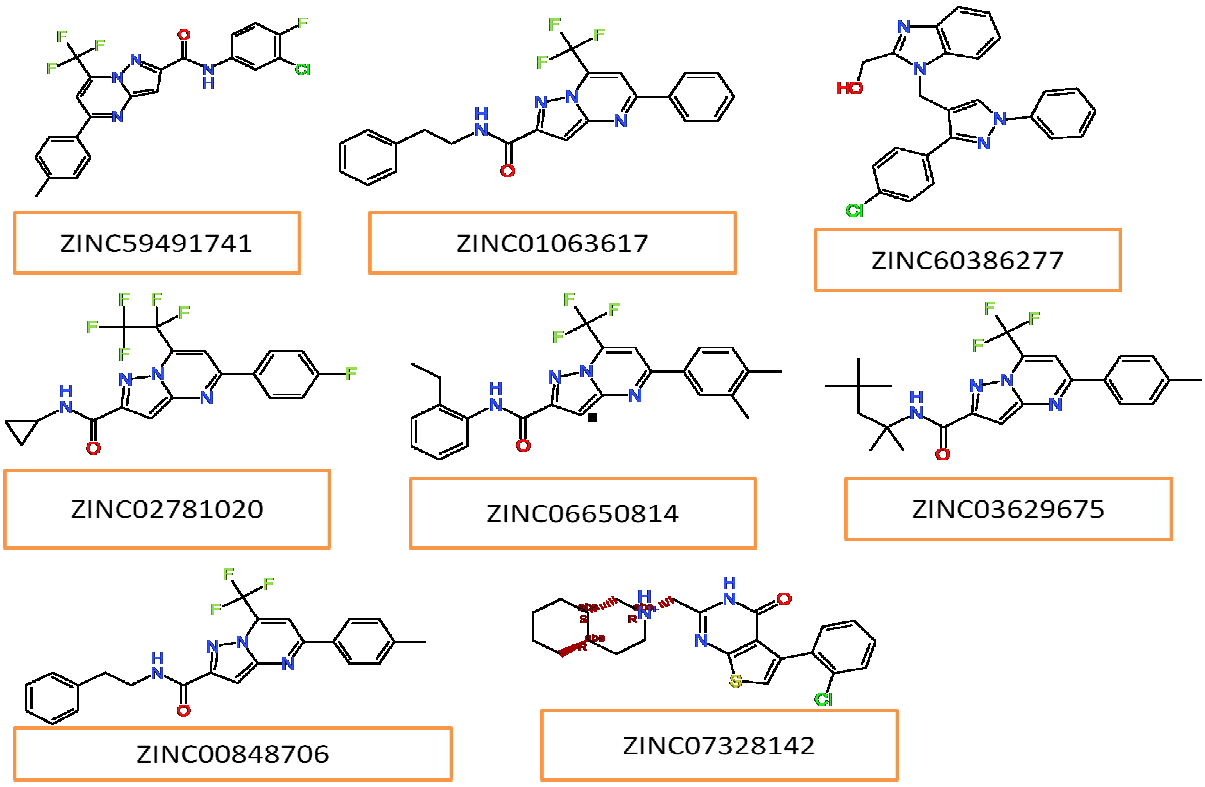


Table 2: Docking Score and Type of Interaction of Zinc Database with DHFR [4P68]

compound	Docking score	Interaction residue	Type of interaction
ZINC01062617	-10.3	ARG43, HIS44, THR45 HIS44 LEU50	H-bonding interaction Π- Π T-shaped Π-sigma interaction
ZINC02781020	-9.1	TYR92 MET19 PHE30 ILE49 THR45	H-bonding interaction Alkyl interaction Π- Π T-shaped Π-sigma interaction
ZINC60386227	-9.8	ALE6 MET19 ILE13 ALE6	H-bonding interaction Π-Sulfur Π-sigma interaction
ZINC07328142	-9.3	THR45 THR115 MET19	Π-sigma interaction Pi-donor H-bond interaction
ZINC00848706	-10.1	TYR92, SER48 ILE13, ILE88 PHE45	H-bonding interaction Halogen [fluorine] Π- Π -stacked
ZINC03629675	-9.1	ALA6, TYR92, THR45 MET19 ILE15 PHE30	H-bonding interaction Π-Sulfur Halogen [fluorine Π- Π -stacked
ZINC00650814	-10.1	HIS44 MET19 THR45	Π- Π T-shaped Alkyl interaction H-bonding interaction
ZINC59491741	-10.4	GLY89, THR45, ARG43 HIS44 ARG90	H-bonding interaction Π-Cation interaction

RESULT AND DISCUSSION

AutoDock Tools, a component of MGL Tools 1.5.6, was used to prepare the ligand and protein, while AutoDock Vina was used to continue the docking investigations. PyMol version 2.4, a molecular visualisation programme, was used to validate the docking methodology further. Calculating the RMSD value served to validate the docking process. by the docking study the methothrexate and pralatrexate having the best binging affinity with the target PDB ID 4P68 and the docking score will be the -9.9 for both the ligands and having interactions i.e., for methothrexate ASP27, GLY97 have H-Bond interaction and MET20 have pi-sulfur interaction for the pralatrexate ASP27, ILE5 have H-Bond interaction, MET20 have pi-sulpur interaction and 2D and 3D structure of methothrexate and pralatrexate as shown in fig 3.

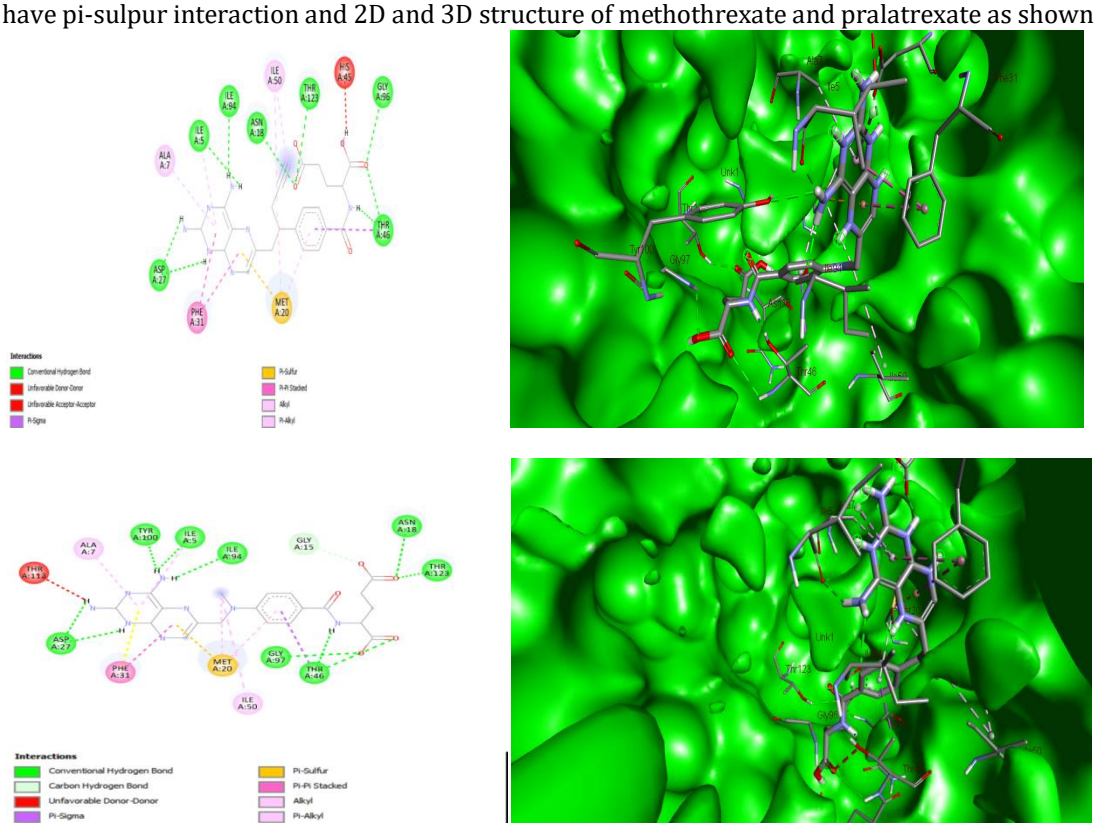
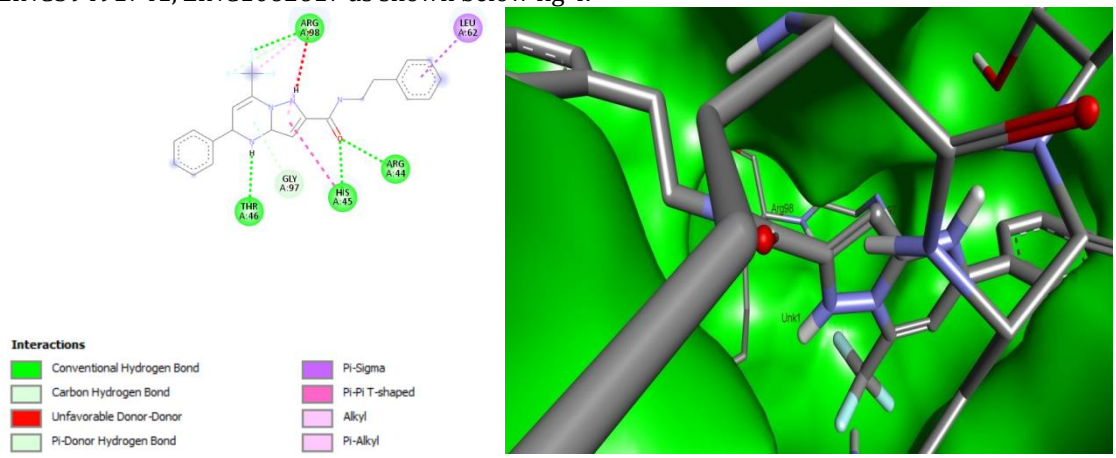


Fig 3: 2D and 3D structure of methothrexate and pralatrexate

Zinc database: The top 8 compounds were determined and listed based on docking score, and the results fall between 11 and 8 kcal/mol. ZINC59491741 and ZINC01062617 are the two best compounds with the best interactions according to molecular docking for the zinc database. ZINC59491741 has a docking score of -10.4 and the interactions are GLY89, THR45, ARG43, HIS44 H-bond interaction, ARG90 -Cation interaction, and the nest compound has a docking score of -10.3 and the interactions are ARG43, HIS44, THR45 H-bon interaction, HIS44 Π- Π T-shaped, LEU50 Π-sigma interaction and 2D and 3D structures of ZINC59491741, ZINC1062617 as shown below fig 4.



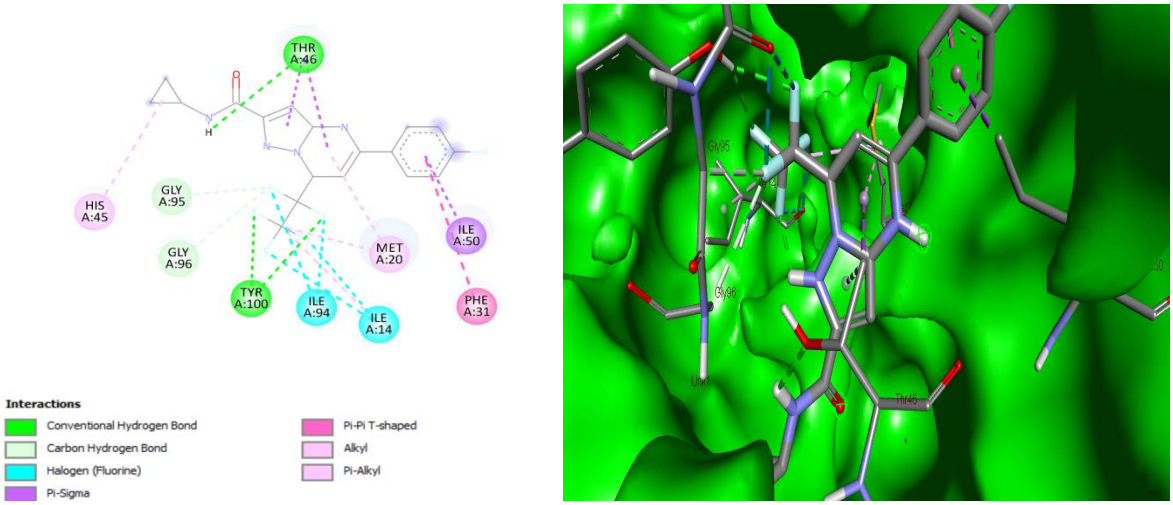


Fig 4: 2D and 3D structure of ZINC59491741 and ZINC01062617

ADMET study: A molecule's fate inside the body is largely determined by the ADMET characteristics. When a molecule is administered orally, a good absorption is sought. The distribution and absorption of a molecule throughout the system will be governed by the balance of lipophilic and hydrophilic groups in the structure. From a therapeutic standpoint, effectiveness and toxicity are important. The liver contains a variety of cytochrome enzymes that are responsible for the molecules' metabolism. As a result, after metabolization, the activity of the metabolite should be known and removed from the body when the intended therapeutic impact has been achieved. using the PKCSM software to determine whether a molecule cluster has lickness and to forecast the ADMET attribute. drug ability studies of the ligands viz., parameters of lipinski's rule of 5 as shown in table.3.

Table 3: Drug ability studies of the ligands viz., parameters of Lipinski's rule of 5

COMPOUND CODE	Physical Properties						Lipinski Rule
	Mol_Weight	Logp	#Rotatable Bonds	#Acceptors	#Donors	Surface_ Area	
ZINC6650814	438.453	5.84664	4	4	1	182.469	0
ZINC3629675	432.49	5.66812	4	4	1	179.237	0
ZINC0848706	424.426	4.69602	5	4	1	176.104	0
ZINC59491741	448.807	5.76832	3	4	1	177.843	0
ZINC01063617	410.399	4.3876	5	4	1	169.739	0
ZINC60386227	414.896	5.083	5	5	1	178.358	0
ZINC02781020	414.309	4.0817	4	4	1	158.903	0
ZINC07328142	414.982	3.9	3	3	2	173.112	0
PIRITREXIM	325.372	2.10562	4	7	2	139.196	0
PRALATREXATE	477.481	0.9816	10	9	5	199.365	0
RALITREXED	458.496	1.97722	9	7	4	186.079	0
TALOTREXIN	573.57	1.2877	12	11	7	237.954	4
TRIMETREXATE	369.425	2.74052	6	8	3	157.004	0
METHOTREXATE	454.447	0.2684	9	10	5	187.031	0
NALOTREXED	284.344	2.35992	2	5	2	118.819	0
PEMETREXE	427.417	0.6664	9	6	6	174.862	1

The idea of drug-likeness serves as a helpful guide during the early stages of drug research. Molecular weight [MW] 500, octanol/water partition coefficient [A log P] 5, number of hydrogen bond donors [HBDs] 5, and number of hydrogen bond acceptors [HBAs] 10 are the original and most well-known rule-based filters of drug-likeness that Lipinski presented in 1997.

A molecule would not be orally active if it broke two or more of the four conditions, according to the Rule of Five. The "Rule of Five" and other drug-likeness rules/filters were subsequently proposed. For instance, the study found that more than 80% of the compounds matched the following criteria: 3.8 A log P 5.9, 400 MW 450, 40 MR [molar refractivity] 130, and the total atom count between 20 and 70, based on 8 molecules in the zinc database from pharmacophore modelling. The drug development process is sped

up by the drug-likeness filters based on physicochemical characteristics. The drug-likeness rules/filters based on physicochemical features, however, have drawbacks, as demonstrated by several research.

CONCLUSION:

The molecules which are used to treat DHFR inhibitor include, methotrexate, pralatrexate, piritrexim, talotrexin, and nalotrexin used to get ligand-based pharmacophore model. The model gives molecules with similar pharmacophoric feature, which is obtained from zincdata base, these molecules were subjected to docking by using Autodock vina and based on docking score and binding interaction, top 8 molecules are chosen which shows potent activity on dhfr inhibitor. Next further admet studies were done by using PKCSM and Swiss ADME online software, which predicts the drug-likeness feature and oral rat chronic toxicity and hepatotoxicity. The molecules zinc59491741 and zinc01062617 are the two best compounds with the best interactions according to molecular docking for the zinc database were predicted as the DHFR inhibitor. Further dynamic study is done to obtain stability, safety and efficacy of the drug and additional properties with potential DHFR inhibitor activity.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, M S Ramaiah University of Applied Sciences, Bengaluru for providing computational facility for research work.

CONFLICT OF INTEREST

The authors declare that no conflict of interest.

REFERENCES

1. Schweitzer, B.I., Dicker, A.P. and Bertino, J.R., (1990). Dihydrofolate reductase as a therapeutic target. *The FASEB journal*, *4*[8], pp.2441-2452.
2. Srinivasan*, B., Tonddast-Navaei, S., Roy §, A., Zhou, H. and Skolnick, J., (2019). Chemical space of Escherichia coli dihydrofolate reductase inhibitors: new approaches for discovering novel drugs for old bugs. *Medicinal research reviews*, *39*[2], pp.684-705.
3. Baird, J.K., (2005). Effectiveness of antimalarial drugs. *New England Journal of Medicine*, *352*[15], pp.1565-1577.
4. Alsaad, N., van Altena, R., Pranger, A.D., van Soolingen, D., de Lange, W.C., van der Werf, T.S., Kosterink, J.G. and Alffenaar, J.W.C., (2013). Evaluation of co-trimoxazole in the treatment of multidrug-resistant tuberculosis. *European Respiratory Journal*, *42*[2], pp.504-512.
5. Huennekens, F.M., (1994). The methotrexate story: a paradigm for development of cancer chemotherapeutic agents. *Advances in enzyme regulation*, *34*, pp.397-419.
6. Banerjee, R., Dey, M., Maity, S., Bagchi, S., Vora, A., Shakil, U. and Goswami, R., (2016). Preventive role of Curcumin against hepatotoxic effects of Methotrexate and Cyclophosphamide. *J. Chem. Pharm. Sci*, *4*, pp.38-42.
7. Padmanabhan, S., Tripathi, D.N., Vikram, A., Ramarao, P. and Jena, G.B., (2009). Methotrexate-induced cytotoxicity and genotoxicity in germ cells of mice: intervention of folic and folinic acid. Mutation research/genetic toxicology and environmental mutagenesis, *673*[1], pp.43-52.
8. Hao, M., Zhao, W., Zhang, L., Wang, H. and Yang, X., (2016). Low folate levels are associated with methylation-mediated transcriptional repression of miR-203 and miR-375 during cervical carcinogenesis. *Oncology letters*, *11*[6], pp.3863-3869.
9. Blount, B.C., Mack, M.M., Wehr, C.M., MacGregor, J.T., Hiatt, R.A., Wang, G., Wickramasinghe, S.N., Everson, R.B. and Ames, B.N., (1997). Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *Proceedings of the National Academy of Sciences*, *94*[7], pp.3290-3295.
10. Mhashal, A.R., Pshetitsky, Y., Cheatum, C.M., Kohen, A. and Major, D.T., (2018). Evolutionary Effects on Bound Substrate p K a in Dihydrofolate Reductase. *Journal of the American Chemical Society*, *140*[48], pp.16650-16660.
11. Hutchings, B.L., Mowat, J.H., Oleson, J.J., Stokstad, E.L.R., Boothe, J.H., Waller, C.W., Angier, R.B., Semb, J. and Subbabow, Y., (1947). Pteroylaspartic acid, an antagonist for pteroylglutamic acid. *Journal of Biological Chemistry*, *170*, pp.323-328.
12. Kremer, J.M., (2004). Toward a better understanding of methotrexate. *Arthritis & Rheumatism*, *50*[5], pp.1370-1382.
13. Chabner, B.A. and Longo, D.L., (2011). Cancer chemotherapy and biotherapy: principles and practice. Lippincott Williams & Wilkins.
14. Kaye, S.B., (1998). New antimetabolites in cancer chemotherapy and their clinical impact. *British journal of cancer*, *78*[3], pp.1-7.
15. Al-Rashood, S.T., Hassan, G.S., El-Messery, S.M., Nagi, M.N., Habib, E.S.E., Al-Omary, F.A. and El-Subbagh, H.I., (2014). Synthesis, biological evaluation and molecular modeling study of 2-[1, 3, 4-thiadiazolyl-thio and 4-methyl-thiazolyl-thio]-quinazolin-4-ones as a new class of DHFR inhibitors. *Bioorganic & Medicinal Chemistry Letters*, *24*[18], pp.4557-4567.

16. Hagner, N. and Joerger, M., (2010). Cancer chemotherapy: targeting folic acid synthesis. *Cancer management and research*, pp.293-301.
17. Available from: https://pubchem.ncbi.nlm.nih.gov/
18. Available from: http://glab.cchem.berkeley.edu/glab/chemdraw.html
19. Available from: http://www.rcsb.org/pdb/home/home.do
20. Chandrasekaran, J., Elumalai, S., Murugesan, V., Kunjiappan, S., Pavadai, P., & Theivendren, P. [2022]. Computational design of PD-L1 small molecule inhibitors for cancer therapy. *Molecular Diversity*.
21. Chaitra, R., Gandhi, R., Jayanna, N., Satyanath, S., Pavadai, P., & Murahari, M. [2022]. Computational design of MmpL3 inhibitors for tuberculosis therapy. *Molecular Diversity*.
22. Koes, D. R. and Camacho, C. J. [2012] "ZINCPharmer: pharmacophore search of the ZINC database," *Nucleic acids research*, 40[Web Server issue], pp. W409-14. doi: 10.1093/nar/gks378.
23. Irwin, J. J. *et al.* [2012] "ZINC: a free tool to discover chemistry for biology," *Journal of chemical information and modeling*, 52[7], pp. 1757–1768. doi: 10.1021/ci3001277.
24. Kalimuthu, A. K., Panneerselvam, T., Pavadai, P., Pandian, S. R. K., Sundar, K., Murugesan, S., Ammunje, D. N., Kumar, S., Arunachalam, S., & Kunjiappan, S. [2021]. Pharmacoinformatics-based investigation of bioactive compounds of Rasam [South Indian recipe] against human cancer. *Scientific Reports*, *11*[1], 21488.
25. Theivendren, P., Kunjiappan, S., Govindraj, S., Chandrasekarn, J., Pavadai, P., Saraswathy, G. R., & Murugan, I. [2019]. Graph Theoretical Analysis, Insilico Modeling and Formulation of Pyrimidine Nanoparticles as p38α MAP Kinases inhibitors: A Quantitative Proteomics Approach. *Drug research*, *69*[2], 100–110.
26. Pires, D. E. V., Blundell, T. L. and Ascher, D. B. [2015] "PkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures," *Journal of medicinal chemistry*, 58[9], pp. 4066–4072. doi: 10.1021/acs.jmedchem.5b00104.

CITATION OF THIS ARTICLE

Bandral S K, Genne S, Golla S, Nayana R J, Agasa R M, Selvaraj K, Theivendren P, S. Kanimozhi, Parasuraman P. Identification of Potent Lead to Inhibit DHFR for the Treatment of Cancer: A Computational Approach. Bull. Env. Pharmacol. Life Sci., Vol 12[6] May 2023: 200-208