International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 5, 2016

Original Article

VIRTUAL SCREENING OF PHYTOCHEMICALS FOR METHOTREXATE LIKE DIHYDROFOLATE REDUCTASE AND AMINOIMIDAZOLE-4-CARBOXAMIDE RIBONUCLEOTIDE (AICAR) TRANSFORMYLASE INHIBITORY PROPERTY USING MOLEGRO VIRTUAL DOCKER

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Received: 25 Dec 2015 Revised and Accepted: 15 Mar 2016

ABSTRACT

Objective: Methotrexate is an effective treatment for autoimmune diseases like Rheumatoid arthritis, vasculitis, and Psoriasis. Our aim is to do *in silico* screening of various phytochemicals present in common medicinal plants used in India for arthritis and fever for possible Dihydrofolate reductase (DHFR) and 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) Transformylase inhibitory property similar to methotrexate (MTX) using molecular docking method.

Methods: We did docking of 142 phytochemicals against DHFR and AICAR Transformylase domain of ATIC (AICAR Transformylase/Inosine monophosphate cyclohydrolase) enzyme structures extracted from Protein Data Bank (4M6K, 1P4R respectively), by utilizing the Molegro virtual docker Software. The docking scores of phytochemicals were compared with the scores of respective native reference ligands present in the crystal structures.

Results: Compounds, dicrocin, melianone, calotropin, uscharidin and mauritine A showed more negative moldoc and rerank scores compared to folic acid and MTX when docked in the folate-binding pocket of DHFR. Dicrocin, melianone and hecogenin showed more negative moldoc and rerank scores compared to scores of AICAR and MTX, when docked in the cavity in AICAR Transformylase, which binds AICAR.

Conclusion: The protein-ligand interaction plays a significant role in structural based drug designing. The present analysis shows that Melianone and dicrocin could be the potential lead molecules for the inhibition of both DHFR and AICAR transformylase like MTX. Uscharidin, calotropin, and mauritine A may be the potential DHFR inhibitor lead molecules and Hecogenin could be the potential lead molecule for inhibition of AICAR transformylase.

Keywords: Dihydrofolate reductase, AICAR Transformylase, Melianone, Hecogenin, Mauritine A

INTRODUCTION

Mechanism of action of methotrexate (MTX)

Folic acid is an important cofactor for various enzymes involved in DNA synthesis. Various drugs which antagonise folic acid and thereby interfere with DNA synthesis and cell division have been tried for treatment of cancers and some autoimmune diseases. Prototype among these drugs is methotrexate (MTX). MTX is a folate analogue originally synthesised in the 1940s and designed to inhibit dihydrofolate reductase [1]. MTX binds dihydrofolate reductase (DHFR) with high affinity and has a fairly high affinity for enzymes that require folate cofactors, including thymidylate synthetase (TS). MTX inhibits dihydrofolate reductase (DHFR), an enzyme responsible for the conversion of dihydrofolate (DHF) to tetrahydrofolate (THF) which acts as the proximal single carbon donor in several reactions involved in the de novo synthetic pathways for pyrimidines and purines, including thymidylate synthesis by TS [2, 3]. Consequently, there is a reduction in thymidylate and purines in dividing cells. DNA synthesis eventually halts, and cells can no longer replicate. In 1951 the rationale for the introduction of MTX for the treatment of rheumatoid arthritis (RA) was that it inhibited cell division and proliferation of the lymphocytes and other cells responsible for inflammation in the joint [4, 5].

MTX action on adenosine pathway

MTX also works on the adenosine pathway by inhibiting enzyme Amino-imidazole-4-carboxamide ribonucleotide (AICAR) transformylase/ IMP cyclohydrolase (ATIC). ATIC is a bifunctional enzyme with folate-dependent AICAR transformylase and IMP cyclohydrolase activities that catalyzes the last two steps of purine synthesis. AICAR transformylase inhibition by MTX leads to intracellular accumulation of AICAR [6] and ultimately leads to increased levels of adenosine. Adenosine is a potent inhibitor of inflammation and induces vasodilation. Adenosine's anti-inflammatory effects include regulation of endothelial cell functions; including cell trafficking resulting in decreased leukocyte accumulation in inflamed sites [6-8]. This effect of MTX does not seem to be affected by folate supplementation.

Side effects of MTX and need for alternative drugs

Polyglutamation of this drug prolongs its intracellular presence. An increase in polyglutamation results in an abnormal increase in the intracellular concentration of MTX on the prolonged use and thereby results in increased risk of toxicity as a result of direct, prolonged intracellular exposure [9, 10]. Myeloid lineage cells such as leukemic myeloblasts, megakaryocytes, synovial macrophages, lymphoblasts, and epithelia are more susceptible to this medication consequently patients may experience MTX toxicity in the form of liver damage mucositis and pancytopenia. Various plant compounds and preparations are being used in Indian system of medicine for treatment of autoimmune diseases like Rheumatoid arthritis, vasculitis, and Psoriasis. These diseases are chronic and have a tendency to relapse after treatment. Since MTX is an effective treatment of these autoimmune diseases, we postulated that some of the plant products may contain phytochemicals with MTX like action

and screened plant products for phytochemicals which have MTX like action so that people who have intolerance to MTX can be substituted with these products.

Currently, molecular docking approach has been used in modern drug design and to understand drug-receptor interactions. This paper reports screening of various phytochemicals present in common medicinal plants used in India for arthritis and fever for DHFR and AICAR Transformylase inhibitory property similar to MTX, by utilizing the Molegro Virtual Docker (MVD) Software [11].

MATERIALS AND METHODS

The structures of DHFR (PDB ID 4M6K) and AICAR Trans formylase in complex with Inosine Cyclohydrolase (ATIC-PDB ID 1P4R) were obtained from the Protein Data Bank (http//;www.rcsb.org). The 4M6K structure contains folic acid co-crystallised to DHFR. The 1P4R structure contains Aminoimidazole 4-carboxamide ribonucleotide and xanthosine-5'-monophosphate co-crystallised to ATIC. ATIC forms an intertwined dimer with an extensive interface of approximately 5,000 $Å^2$ per monomer. Each monomer is composed of two novels, separate functional domains. The N-terminal domain (up to residue 199) is responsible for the IMPCH activity, whereas the AICAR Trans formylase activity resides in the C-terminal domain (residues 200-593) [12-16]. MVD automatically identifies potential binding sites (also referred as cavities or active sites) by using its cavity detection algorithm. The cavities within a 30 x 30 x 30 $Å^3$centered at the experimentally known ligand position were used for docking. In the crystal structure of DHFR (4M6K), the program identified three different binding sites. From these three predicted cavities, Cavity where folic acid binds were identified and selected for docking. In the case of the crystal structure of dimer ATIC (1P4R), the program identified five different binding sites. From these five predicted cavities, two Cavities one in each monomer in the C-terminal domain with AICAR Trans formylase activity where AICAR molecules bind were selected for docking. Native ligands were extracted and re-docked into appropriate active sites. The results yielded control parameters for comparing with Phytochemicals. Docked native ligands were viewed in workspace and were found to have optimal orientation. Literature search was done, and List of plants used in native medicine in the region of Tamilnadu in India for arthritis was compiled. 34 plants were principally used to treat arthritis either as single or polyherbal formulations. A further literature search was done and individual phytochemicals constituents reported to be present in each of the plants were identified. A final list of 142 phytochemicals molecules which satisfy at least 4 conditions of the Lipinsky 5 rule (Molecular weight<=500 g/mol, Oil/water distribution coefficient (Log P)<= 5,Hydrogen bond donors<= 5, Hydrogen bond acceptor<=10, Number of rotatable bonds<=10) was prepared for docking studies [17-20]. The structure of these compounds was downloaded as sdf files from PubChem compounds database. In order to make accurate predictions, it is important that the imported structures have been properly prepared, that is, the atom connectivity and bond orders are correct and partial atomic charges are assigned. SDF files often have poor or missing assignment of explicit hydrogens.

All necessary valence checks and H-atom addition were thus performed using the utilities provided in MVD. Ten independent runs were performed for each phytochemicals with the guided differential evolution algorithm, with each of these docking runs returning one solution (pose). The Moldock scoring function used by MVD is derived from the scoring functions originally proposed by Gehlhaar *et al.* and extended later by Yang *et al.* [21]. The 10 solutions obtained from the 10 independent docking runs were re-ranked, in order to further increase the docking accuracy, by using a more complex scoring function. The docking scores of phytochemicals in DHF were validated by comparing them with the docking scores of folic acid and MTX. The docking scores of phytochemicals in ATIC were validated by comparing them with the docking scores of AICAR and MTX respectively. In the studies reported here, MVD was used, because it showed higher docking accuracy when benchmarked against other available docking programs (MD: 87%, Glide: 82%, Surflex: 75%, FlexX: 58%) [11].

RESULTS AND DISCUSSION

Table 1 shows the mean Moldoc scores and the re-rank scores for each of the docked ligand in DHF. Negative values indicate that the bond is more stable.

Table 1: Moldoc scores and Re-rank scores and binding amino acids of docked ligands of DHFR

S. No.	Ligands	Moldoc score; kcal/mol	Rerank score kcal/mol	H-bonds	Amino acids
1	Folic acid	-153.299±8.00	-119.943±7.811	9	Lys54,Lys55,Ser119,Ser118,Thr146,Asn64, Arg70
2	MTX	-156.825±7.38	-126.633±4.653	7	Glu30, Thr136,Ile7,Val8,Arg70
3.	calotropin	-173.365±11.99	-126.475±7.74	4	Thr56,Ser59,Leu22,Arg70
4.	Dicrocin	-203.298±5.550	-145.607 ±14.564`	8	Lys54,Lys55,Ser119,Ser118,Thr146,Asn64, Arg70
5.	Mauritine a	-191.263±5.780	-128.517±9.90	4	Ser59,Arg28,Asp21
6.	Melianone	-187.761±4.942	-133.927±4.172	2	Thr56,Ser59
7.	Uscharidin	-179.90±10.45	-126.468±7.752	4	Thr56,Ser59,Arg70,Leu22

Data are means±SD, n = 10. Aminoacids binding to docked ligand–lysine (Lys), Serine (Ser), Threonine (Thr), Arginine (Arg), Asparagine (Asn), Isoleucine (Ile), Valine (Val), Glutamate (Glu), Leucine (Leu),

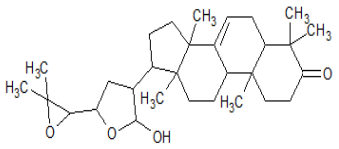

Fig. 1: Melianone

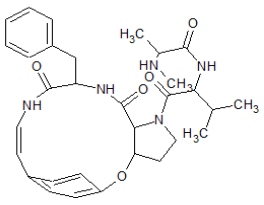

Fig. 2: Mauritine A

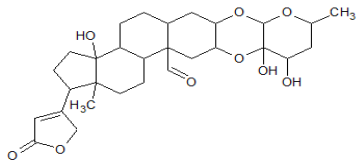

Fig. 3: Calotropin

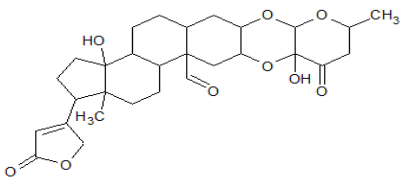

Fig. 4: Uscharidin

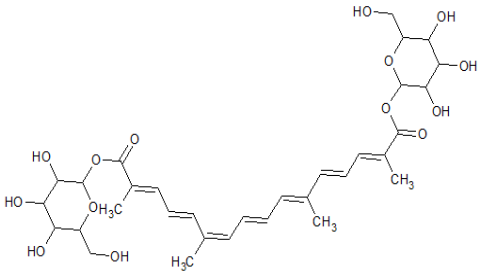

Fig. 5: Dicrocin

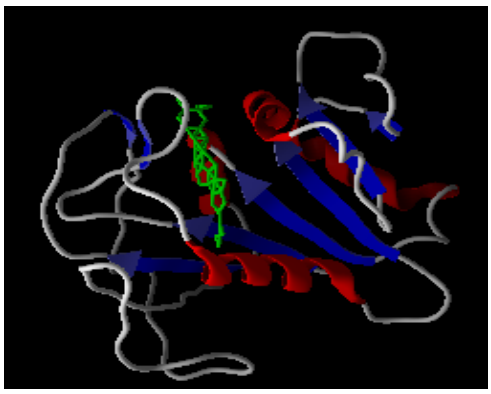

Fig. 6: Melianone in DHFR, secondary structure view

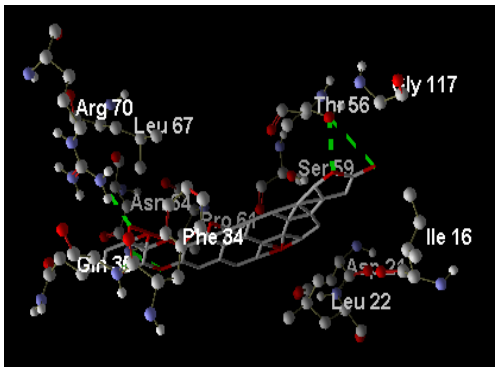

Fig. 7: Calotropin, H-bonds with Threonine 56, Arginine 70

Higher negativity means the bond is more stable and the interaction may hinder the performance of the enzyme. Among 142 compounds docked compounds, dicrocin, melianone, calotropin, uscharidin and mauritine A showed more negative moldoc score and rerank score compared to folic acid and MTX. Residues Ile7, Leu22, Phe31, Phe34, Arg70, and Val115 are present in the folate-binding pocket of DHFR [22]. Calotropin and uscharidin bind to Leu22 and Arg70, dicrocin binds to Arg70 of the folate binding pocket. Residues 9–24 are termed "Met20" or "loop 1" which along with other loops forms the major subdomain that surround the active site.

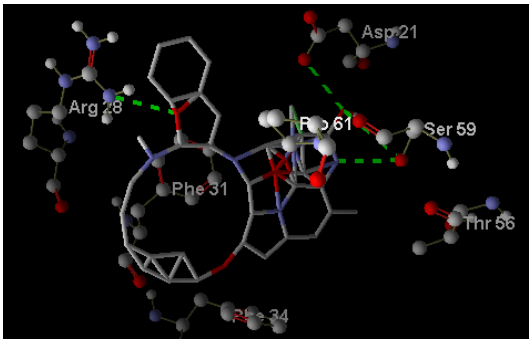

Fig. 8: Mauritine A, H-bonds with Serine59, Arginine 28, Aspartic acid 21

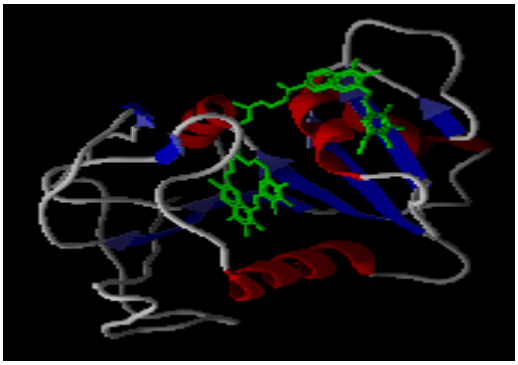

Fig. 9: Dicrocin in DHFR, secondary structure view

The high flexibility of Met20 and other loops near the active site play a role in promoting the release of the product, tetrahydrofolate. In particular, the Met20 loop helps stabilize the nicotinamide ring of the NADPH to promote the transfer of the hydride from NADPH to dihydrofolate [23]. Mauritine A binds to Asp21 of the Met20 loop. Leu 22 to which calotropin and uscharidin bind is also a part of Met20 loop. Ser-59, Ser-118, Asp-145, and Thr-146 are the main residues that form the NADPH binding site [24]. Dicrocin binds to Thr146 and Ser118, whereas melianone, calotropin and uscharidin bind to Ser59 of the NADPH binding site.

Table 2: Mean Moldoc scores and Re-rank scores of docked ligands of AICAR Trans formylase Domain of ATIC

S. No.	Ligands	Moldoc score Kcal/mol	Rerank score Kcal/mol	H-bonds	Amino acids
1	AMZ	-150.697±6.564	-121.055±1.441	9	Asp546,Phe315,Asn489,Lys266,Arg451, Met312, Phe541
	MTX	-158.386±8.342	-124.048±1.416	7	Asp242,His267,Asn431,Arg451,Asn547, Phe544, Lys266
2.	melianone	-195.050±0.713	-143.053±5.301	3	Arg207,Asn239,Ser313
3.	Dicrocin	-195.342±16.338	-134.751±1.527	7	Gly564,Ser563,Gly564,Arg588,Tyr208, Lys358
4.	Hecogenin	-175.508±2.197	-130.873±4.071	5	Arg588,Arg451,Asn431,Asn239,Ile238

Data are means±SD, n = 10. Aminoacids binding to docked ligand–lysine (Lys), Serine (Ser), Arginine (Arg), Isoleucine (Ile), Asparagine (Asn), Glycine (glycine), Phenylalanine (Phe), Methionine (Met), Tyrisine (Tyr).

Table 2 shows the Moldoc scores and the re-rank scores for the docked ligands in a cavity which binds AICAR present in AICAR Trans formylase domain in a monomer of ATIC. The scores were identical when docked to the cavity in another monomer. Of 142 phytochemicals docked Dicrocin, melianone and hecogenin showed more negative moldoc score and reranked score compared to scores AICAR and MTX for both the cavities. Asn431, Lys266 and His 267 are key catalytic residues involved in the transformylation reaction

by AICAR transformylase. The main chain carbonyl oxygen of Phe541 and the side chain guanido nitrogen atoms of Arg451 hydrogen bond to the 4-carboxamide amino and oxygen groups, respectively stabilising AICAR. The side chains of Tyr208, Arg207, and Arg588 anchor and neutralize the negatively charged AICAR Phosphate facilitating transformylation [25]. Melianone binds to Arg-207, Hecogenin binds to Asn431, Arg451 and Arg588. Dicrocin binds to Arg588.

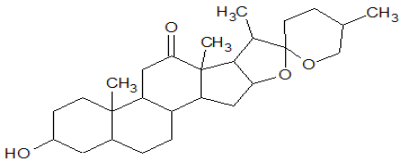

Fig. 10: Hecogenin

Fig. 11: Melianone in ATIC, Secondary structure view

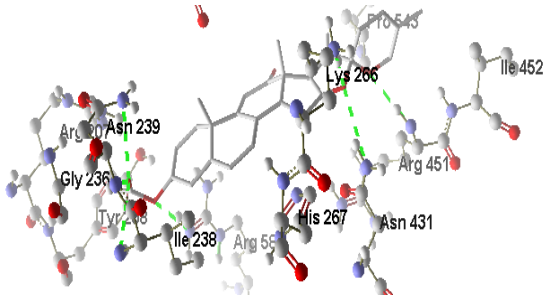

Fig. 12: Hecogenin, H-bonds with Arginine 451 Asn 431, Asn 239, Isoleucine 238

Melianone is present in of *Azadirachta indica* (neem) and *Melia azedarach* (chinaberry). Caboni *et al.* showed that among components of Azadirachta indica, melianone exhibited the highest cytotoxic activity and antiproliferative activity on the tumorigenic cell line A549 [26]. Shailima *et al.* showed that the cytotoxic potential of melianone could be due to binding of DHFR Using CHARMM (Chemistry at Harvard Macromolecular Mechanics) docking program [27]. We also got similar results by using Molegro virtual docker and additionally binding property of melianone to ATIC is also elicited in our study. Calotropin and uscharidin are phytochemicals present in *Pergularia Daemia* used in polyherbal preparations for arthritis in native medicine in Tamilnadu region in India. Mirunalini *et al.* showed that *Pergularia Daemia* extract showed significant cytotoxicity in oral KB cells in MTT assay experiment (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Increased level of intracellular reactive oxygen species, DNA damage (comet assay), apoptotic death and cell cycle arrest were seen in *Pergularia daemia* extract treated cells [28]. Calotropin and Uscharidin present in the extract may contribute to cytotoxicity through DHFR inhibition. This property may confer MTX like antiarthritic potential by the elimination of cytokine producing lymphocytes. However, calotropin at high doses has neurotoxicity profile like strychnine [29]. Mauritine A is present in of *Ziziphus jujube.* Huang *et al.* showed the anti-cancer activity of *Zizyphus jujuba* in human hepatoma cells (HepG2) [30]. *Ziziphus jujuba* leaves extract to possess significant anti-inflammatory activity against carrageenan induced rat paw edema [31]. Mauritine A may contribute to cytotoxicity and through DHFR inhibition along with other phytochemicals present in *Ziziphus.* Dicrocin (alpha-Crocetin diglucosyl ester) Isolated from saffron (*Crocus sativus*) is a water-soluble crocetin glycoside, a carotenoid pigment. Saffron has been used as a spice for flavouring and colouring food preparations, and in Chinese traditional medicine as an anodyne. Dicrocin is metabolised to crocetin in gut. Hence, cannot satisfactorily exert its binding property to DHFR and ATIC. Newer nano delivery systems may be used to overcome this disadvantage. Hecogenin is a component of *Agave sisalana* extract which shows anti-inflammatory properties when examined in three acute mouse models (xylene ear oedema, hind paw oedema, and pleurisy) and a chronic mouse model (granuloma cotton pellet). Hecogenin itself possesses the antinociceptive property and attenuates mechanical hyperalgesia by blocking the neural transmission of pain at the spinal cord levels and by cytokines-inhibition mechanisms [32]. These properties may be partly due to Hecogenin action on Adenosine pathway by AICAR inhibition.

CONCLUSION

The protein-ligand interaction plays a significant role in structural based drug designing. In the present work, potential phytochemicals with DHFR and AICAR transformylase inhibitory property like MTX have been identified. The present analysis shows that Melianone and dicrocin could be the potential lead molecules which can act as inhibitors of both DHFR and AICAR transformylase like MTX. Uscharidin, calotropin and mauritine A may be potential lead molecules for inhibition of DHFR and Hecogenin may be a AICAR transformylase inhibitor lead molecule. These natural compounds could be used as templates for designing therapeutic lead molecules which could result in massive reductions in therapeutics development time. This study may be the subject to experimental validation and clinical trials to establish these phytochemicals as more potent drugs for the treatment of different autoimmune diseases and cancers.

ACKNOWLEDGEMENT

The author is thankful to CLC Bio, Denmark for providing Molegro Virtual Docker 0.8.0.0 trial version, January 2014 with which the study has been done. The author is thankful to Abhinand Varman, Sri Ramachandra University for his help in the study.

CONFLICT OF INTERESTS

Declared none

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