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Molecular docking of amphetamine, cathine and cathinone with dihydrofolate reductase: a computational analysis of inhibition of dihydrofolate reductase by khat alkaloids

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Competing interests
The authors declare no conflicts of interest.

Abbreviations
CATHI, cathine; CATHO, cathinone; DHFR, dihydrofolate reductase; MTX, methotrexate; ADTR, AutoDock Tools; DHFRA, A of dihydrofolate reductase; AMPH, amphetamine; LGA, Lamarckian Genetic Algorithm.

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Abstract
Interaction of amphetamine, cathine and cathinone with the enzyme dihydrofolate reductase was studied by molecular docking using AutoDock 4.2 as the docking software application. AutoDock 4.2 software serves as a valid and acceptable docking application to study the interactions of small compounds with proteins. Interactions of amphetamine, cathine and cathinone with dihydrofolate reductase were compared to those of methotrexate, a known inhibitor of the enzyme. The calculated free energy of binding (ΔG binding) shows that the three ligands (ΔG = –6.87 to –7.21 kcal/mol; K_i = 9.15 to 5.18 μM) bind with affinity slightly lower than methotrexate (ΔG = –8.78 kcal/mol; K_i = 363 nM). Binding interactions of the three ligands with active site residues of the enzyme are also predicted. All the ligands appear to bind in a similar conformation making extensive VDW contacts in the active site of the enzyme. Hydrogen bonding and pi-pi interaction with key active site residues is also observed. Thus, a probable inhibition of dihydrofolate reductase by khat alkaloids can be explained on the basis of this *in silico* binding and khat alkaloids can be considered as potential lead compounds in the development of new inhibitors of dihydrofolate reductase which is a potential target of anti-cancer drugs. The results of these studies can serve as a starting point for further computational and experimental studies.

Keywords: amphetamine; cathine; cathinone; methotrexate; docking; AutoDock; DHFR; khat alkaloids

Introduction

Khat (*Catha edulis* Forsk.) is a psychopharmacological active shrub, that grown in Arabian Peninsula and some African countries such as Somalia, Kenya and Ethiopia [1, 17]. The chewing of khat is a deep-rooted sociocultural practice and the fresh leaves possess euphoric properties. It's also used as a traditional medicine by some indigenous people of these regions [4, 5]. The biological activities of the polar and non-polar extracts of khat are reported. Various phytochemicals with biological and stimulant effects were isolated from khat [33]. Mainly, cathinone (CATHO) and cathine (CATHI) are the major psychotropic compounds which are structurally related to amphetamine and norephedrine [7]. Moreover, some biological activities are also reported to be associated with these monoamine alkaloids, which include anticancer, anti-inflammatory, analgesic, CNS stimulant and antimicrobial [18, 31]. Analgesic activity of CATHO (S-(−)-α-aminopropiophenone) is associated with monoaminergic pathways [21]. CATHO is also found to modulate the release of acetylcholine and cause muscular contraction [9]. Previous studies also showed that CATHO is responsible for the increase of the levels of dopamine and the euphoric activities of khat are dopaminergically mediated [10]. CATHI (S, S-(+)-norpseudoephedrine) is less potent stimulant [3].

Interference of biologically active phytochemicals with the normal and pathological conditions of human body is of a great research interest to biomedical scientists [13]. Researchers use various *in vivo*, *in vitro* and *in silico* approaches to understand the biopharmacological effects of synthetic and natural compounds [19]. Mechanisms of the targeted compounds were studied using molecular and cellular targets. One of these targets is dihydrofolate reductase (DHFR). DHFR is responsible for the conversion of dihydrofolate into tetrahydrofolate. The converted substrate is used for the synthesis of pyrimidines and thymidylic acid. *In silico* molecular docking study of some phytochemicls and their interaction with DHFR was previously done in compared to methotrexate [11]. DHFR antagonists are a significant class of new drug targets, as evidenced by their use as antimicrobial, anticancer, and anti-inflammatory drugs [16, 32]. Methotrexate is the DHFR inhibitor used most often in a clinical setting as an anticancer drug and as an anti-inflammatory and immunosuppressive agent [8]. Trimethoprim and pyrimethamine are potent inhibitors of bacterial and protozoal DHFRs. New generation of DHFR inhibitors that is now in clinical trials [30]. DHFR was the first enzyme to be targeted for cancer chemotherapy [27].

Plant-originated alkaloids such as pergularinine, tylophorinidine, deoxytubulosine and sanguinarine, have been reported earlier to inhibit both DHFR activity and cell growth using various *in vitro* experimental techniques [12, 15, 26]. Versatile biological activities of Khat alkaloids (CATHO and CATHI) as antibacterial and anticancer, the potential of DHFR as a novel drug target and the practicability of *in silico* computational method encourage us to persist our continuous research on the understanding of the versatile pharmacological properties of Khat. Therefore, the current study was designed to use molecular docking program (AutoDock 4.2) to dock khat principles with DHFR. This is a first study of its kind involving active principles of khat, CATHI, and CATHO.

Materials and methods

Computational Software

Molecular docking was performed by using the molecular docking software AutoDockR Version 4.2 [20]. For preparation of the protein and the ligands for docking, AutoDock Tools (ADTR), which is a graphic user interface for AutoDockR 4.2, was used [20, 28]. The source of both the software was The Scripps Research Institutes, San Diego, CA, USA.

Preparation of protein and ligand

The docking procedure has been described earlier [11, 14]. It is outlined as follows. PDB file ID 1DRE was downloaded from the Protein Data Bank for the three-dimensional structure of subunit A of dihydrofolate reductase (DHFRA). The three-dimensional structure of DHFRA with secondary structural details is shown in Figure 1. Structures of ligands, Amphetamine (AMPH), CATHI and CATHO were obtained as sdf files from the PubChem database (respective PubChem CIDs-3007, 441457 and 62258) and converted to pdb formats using Open Babel software. The chemical structures and systematic names of AMPH, CATHI and CATHO are listed in Table 1. In the preparation of the protein and ligands for docking nonpolar hydrogens were merged and Gestgeiger partial charges were assigned to all atoms. In applying torsions in ligands all rotatable bonds were rotated. Ligands were made flexible and protein was kept rigid. PDBQT files (file format that contains partial charges and torsion records along with atom coordinates) were written for protein and each ligand and were used as input files for docking experiments in next step.

Docking

Standard docking procedures for a rigid protein and a flexible ligand were used as per user guide for AutoDockR 4.2. Briefly, using AutoGrid (a component of the software), a grid of 60 × 60 × 60 points in x, y, and z directions was built with grid points spaced at 0.375 Å. Electrostatic maps were calculated by using a distance dependent function of dielectric constant. All other parameters were set as default as per user guide. Docking simulations were performed by employing Lamarckian Genetic Algorithm (LGA) (as per user guide). The implementation of LGA included creation of an initial population of 150 individuals. Random torsions were applied to each of 150 individuals. In each docking run, a maximum of 2500, 000 energy evaluations was performed. For each of three ligands, at least 20 such runs were performed. The best binding modes for each ligand obtained from docking were analyzed by using LigPlot+, ADTR and RasMolR (Roger Sayle) programs [29].

Results and discussion

Interference with the normal and abnormal conditions of the human body by biologically active phytochemicals is of great scientific interest to biomedical scientists [13]. Compared to methotrexate, an *in silico* molecular docking analysis of certain phytochemicals and their association with DHFR was previously performed [23]. Therefore, the current study was designed to understand the molecular interaction of AMPH, CATHI and CATHO with DHFRA using computational analysis. There are no previous studies that have addressed this important biological interaction between the components of khat and this human and bacterial enzyme. The importance of this study is underlined by the extensive use of khat in African and Middle Eastern societies [1].

All binding parameters of AMPH, CATHI and CATHO obtained after docking with DHFRA are listed in Table 2. Estimated total free energy of binding of the three ligands was –6.93, –7.21 and –6.87 kcal/mol, respectively. The estimated K_i values were 8.34 μM, 5.18 μM and 9.15 μM, respectively. The total free energy of binding (and hence the K_i) estimated for MTX (15) is comparable (slightly lower) than that of these ligands which suggests a good affinity of binding. Binding affinities of AMPH, CATHI and CATHO are almost equal to each other. Inhibitor constants in the micromolar range for AMPH, CATHI and CATHO indicate weak inhibition of the enzyme but it is very similar to MTX, a drug used in cancer therapy, which is a known inhibitor of DHFR.

An analysis of docked complexes of all inhibitors was done using LigPlot+ software. All LigPlots are shown in Figures 2–4. Table 3 lists all significant interactions of ligands with active site residues of protein. Binding of MTX to DHFRA has been described in detail earlier [22]. Several interactions of AMPH, CATHI and CATHO with DHFRA are comparable to interactions of MTX. LigPlot analysis of the docked complexes of the three ligands with DHFRA reveals several significant interactions of the ligands within the active site of DHFRA. The ligands appear to bind in the active site in extended conformations

and make extensive van der Waals contacts on either side with the active site residues of the enzyme (Figures 2–4). The benzene rings and side chains of the ligands make extensive hydrophobic contacts with active site residues notably, Ile-7, Val-8, Ala-9, Phe-34, Val-115, Tyr-121 and Thr-136 (except CATHO). In MTX binding also hydrophobic contacts are made with the bulky side chains of Phe-31 and Phe-34 which cover one face of the pteridine rings and nonpolar interactions occur with the side chains of Ile-7, Val-8, Ala-9 and Val-115 and with some main chain atoms of Val-8 and Ala-9. Hydrogen bonding interactions are observed between residue side chain CO of Glu-30 and ligand amino of both AMPH and CATHO. Hydrogen bonding interactions seen in case of CATHI are different from the other two ligands. In case of CATHI, two hydrogen bonds are seen, one H-bond between residue backbone CO of Glu-30 and ligand amino and another H-bond between residue side chain CO of Glu-30 and ligand CO. This additional H-bond explains the lower binding energy of –7.21 kcal/mol. Notably, H bonds with Glu-30 are also seen in MTX binding. Glu-30 carboxylate makes an H bond with 2-amino and N1 in MTX. Additionally, in MTX binding, H bonds are observed between 4-amino of MTX and the main-chain carbonyls of Ile-7 and Val-115 [22, 24]. These additional H-bonds may be responsible for the higher affinity of MTX (–8.78 kcal/mol) compared to AMPH, CATHI and CATHO. All the ligands appear to be stabilized in the active site predominantly by the hydrophobic interactions. These interactions orient the ligands for adequate H-bonding (Figures 2–4).

Considering the similarities of binding modes of AMPH, CATHI and CATHO to MTX, a known inhibitor of DHFR, it is very much probable that these alkaloids may inhibit DHFR *in vivo* and hence potentially serve as lead compounds to target DHFR activity [24]. Phenanthroindolizidine plant alkaloids pergularinine and tylophorinidine inhibit the activity of dihydrofolate reductase, thereby inhibiting nucleic acid synthesis [6]. The pergularinine and tylophorinidine plant alkaloids were reported to inhibit DHFR [25].

Conclusion

Khat alkaloids have been shown to modulate biological activities of a number of target molecules. In some instances, such binding has been studied using computational methods like molecular docking. In an earlier molecular docking study, binding of khat alkaloids to monoamine oxidase was detailed [2]. In the present docking study, it is seen that amphetamine, cathine and cathinone bind to DHFRA with an affinity comparable to that of methotrexate, which is an established anticancer drug targeting DHFR. Several of the interactions of these ligands with in the active site of DHFR are similar to those of methotrexate. The structures of the ligands allow these to bind in an extended conformation in the active site of DHFR thus optimizing interactions on either side of the active site pocket. These docking analyses suggest that khat alkaloids and its derivatives may have similar modes of action as those of known inhibitors of the enzyme like MTX. Khat alkaloids can be considered as potential starting molecules for the design of inhibitors targeting DHFR.

Table 1 Chemical structures and IUPAC names of ligands, AMPH, CATHI and CATHO

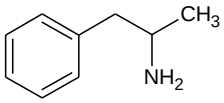
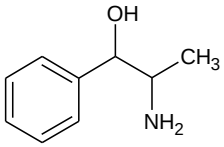
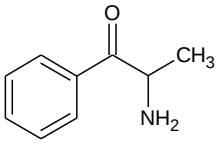
Ligand	Acronym	Structure	Systematic Name
Amphetamine	AMPH		1-phenylpropan-2-amine
Cathine	CATHI		2-amino-1-phenyl-1-propanol
Cathinone	CATHO		2-amino-1-phenyl-1-propanone

Table 2 Interaction energies and inhibitor constants (K_i) for the binding of amphetamine, cathine, and cathinone with dihydrofolate reductase

Parameters	AMPH	CATHI	CATHO
(1) vdW + Hbond + desolvation energy (kcal/mol)	–5.65	–6.04	–5.67
(2) Electrostatic energy (kcal/mol)	–2.18	–2.36	–2.10
(3) Final intermolecular energy (kcal/mol) [a]	–7.82	–8.40	–7.77
(4) Final total internal energy (kcal/mol)	–0.16	–0.93	–0.89
(5) Torsional free energy (kcal/mol)	+0.89	+1.19	+0.89
(6) Unbound system's energy (kcal/mol)	–0.16	–0.93	–0.89
(7) Estimated free energy of binding (kcal/mol) [b]	–6.93	–7.21	–6.87
(8) Estimated inhibition constant (298.15 K), K_i (μM)	8.34	5.18	9.15

a = (1) + (2), b = (3) + (4) + (5) - (6)

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Table 3 Interactions of different active site residues of DHFR-A with ligand groups of AMPH, CATHI and CATHO

Amino acid residue	AMPH	CATHI	CATHO
Ile-7	Hydrophobic	Hydrophobic	Hydrophobic
Val-8	Hydrophobic	Hydrophobic	Hydrophobic
Ala-9	Hydrophobic	Hydrophobic	Hydrophobic
Glu-30	H-bond between residue side chain CO and ligand amino	H-bond between residue backbone CO and ligand amino H-bond between residue side chain CO and ligand CO	H-bond between residue side chain CO and ligand amino
Phe-34	Hydrophobic	Hydrophobic	Hydrophobic
Val-115	Hydrophobic	Hydrophobic	Hydrophobic
Tyr-121	Hydrophobic	Hydrophobic	Hydrophobic
Thr-136	Hydrophobic	Hydrophobic	--

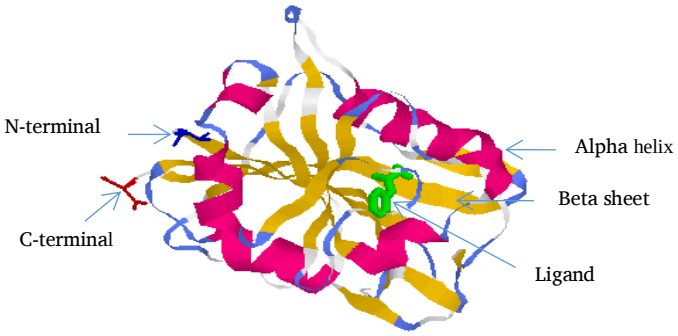

Figure 1 3-D structure of DHFRA showing secondary structural details along with amphetamine (ligand) bound in the active site. Alpha chains are in red, beta strands are in yellow and ligand is shown in green.

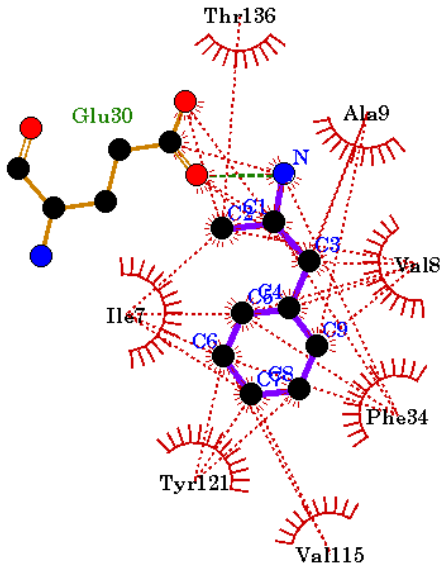

Figure 2 LigPlot of binding interaction of amphetamine in the active site of DHFR-A. Atoms are shown in CPK colors, H-bonds are shown as green dotted lines, Hydrophobic interactions are shown as red dotted lines and rays from residues towards ligand atoms.

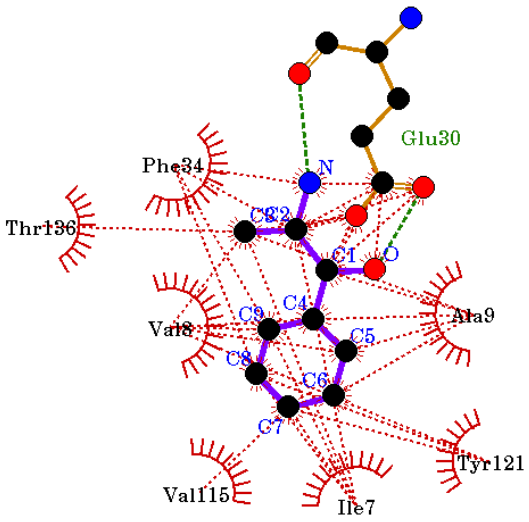

Figure 3 LigPlot of binding interaction of cathine in the active site of DHFR-A. Atoms are shown in CPK colors, H-bonds are shown as green dotted lines, Hydrophobic interactions are shown as red dotted lines and rays from residues towards ligand atoms.

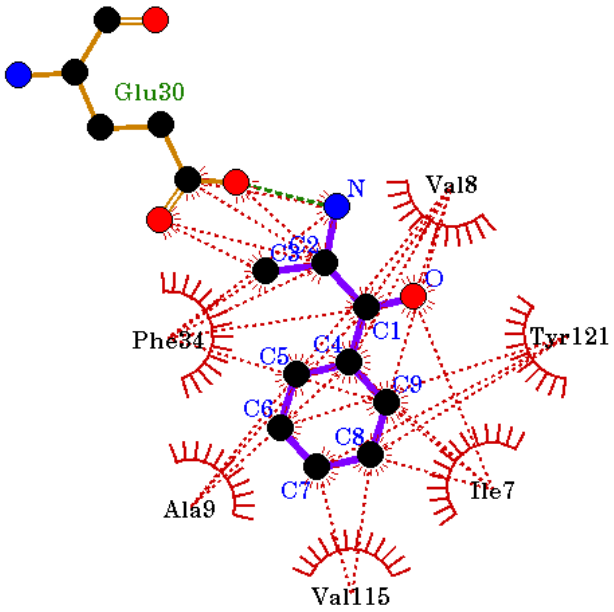

Figure 4 LigPlot of binding interaction of cathinone in the active site of DHFR-A. Atoms are shown in CPK colors, H-bonds are shown as green dotted lines, Hydrophobic interactions are shown as red dotted lines and rays from residues towards ligand atoms.

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