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Plerixafor and related macrocyclic amines are potential drug candidates in treatment of malaria by "filling the flap" region of plasmepsin enzymes

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ABSTRACT

Death by *Plasmodium falsiparum*, the leading cause of malaria, is going to remain a major obstacle among the infectious diseases. Plasmepsin aspartic proteases are key proteins in the pathogenesis of plasmodium species which break down the hemoglobin and exploit it as a source of amino acids. These enzymes are one of the favorite targeting agents for medicinal chemists to design new drugs. Plasmepsin proteins show a "flap" region in their N-terminal domain, predisposing them to a good "filler" drug with an exceptional affinity to this enzyme. Plerixafor (Mozobil®, AMD3100), a CXCR4 antagonist with a bicyclam ring, historically discovered as an impurity in a mixture which had anti-HIV properties, is now a FDA approved drug for mobilizing haematopoietic stem cells in cancer patients. In this hypothesis, we focused on the similarity of the structure of plerixafor and its analogues with heme functional group of hemoglobin, the main substrate of plasmepsin, and also with some other recent azamacrocyclic compounds demonstrating antimalarial activity, to test whether these compounds are capable of exhibiting antimalarial activity by inhibiting plasmepsin or not. A preliminary *in silico* docking study was used to evaluate this hypothesis and docking results indicated that macrocyclic cyclams and cyclens can reliably act as potent lead drug or central pharmacophore in developing new plasmepsin inhibitors as compared with previously designed plasmepsin II inhibitors.

Introduction

Scientists in the late 20th century were trying to find an active compound as an inhibitor of HIV replication. Earlier it was found that polyoxometalates are capable of reducing the serum levels of HIV virus. So it was hypothesized that metal complexes of macrocyclic organic compounds which are similar to natural ligand complexes may also impede the growth of HIV virus. The attention brought to a newly synthesized mixture which possessed remarkable antiviral activity and its biological activity was attributed to cyclam rings. Further studies showed that all except one mixture are not capable of antagonizing HIV replication. The sample was suspected for an impurity which surprisingly had a modest potency against HIV in about 0.5 μM concentration. As it was impossible to synthesize this impurity scientists attempt to synthesize a bicyclam macrocycle tethered by a propyl spacer. Despite having the efficacy of its mother impurity, the product didn't provide enough anti-HIV activity. A remarkable increase in the potency of these bicyclam compounds was achieved by replacement of the aliphatic linker with an aromatic group. This new bicyclam molecule fused together by a phenyl ring and carbon spaced in either side with a potency of 3 nM, is the so called plerixafor molecule; which is now used as a FDA approved drug for promoting hematopoietic stem cell mobilization in cancer patients [1–3].

Malaria, one of the world's biggest challenges in medical society, results in about one million deaths annually and nowadays resistance to different therapeutic regimens is considered to be a critical issue and discovering new chemicals to overcome this resistance is of paramount importance [4]. The four major plasmodium species which infect human are; *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*. About a half number of malaria cases are caused by *Plasmodium falciparum* [5].

Plerixafor (Mozobil®, AMD3100) specifically binds to CXCR4 receptor, blocking the binding of its native ligand (CXCL12) and expediting the mobilization of haematopoietic stem cells [6]. Plerixafor has a great selectivity index in inhibiting the CXCR4 receptor (about 100'000 times) and is not toxic to host cells to a concentration of 500 μM [7]. CXCL12 activates the CXCR4 receptor, triggering angiogenesis, proliferation and cell growth in tumor cells and plerixafor is regarded as an anti-cancer drug which mitigates the chemoresistance and metastatic tumor cell survival, in cancer cells [8]. CXCR4 inhibitors are being heralded as a regulating module with antimetastatic, anti-inflammatory and tissue-repairing properties [9]. Recent findings by

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Hubin et al. suggest that metal complexes of cyclam and cyclen rings (tetraazamacrocyclic compounds) are effective in inhibiting the growth of Plasmodium spp. *in vitro* but no specific protein target for these molecules was documented [10].

Plasmepsin II is an aspartic protease which is one the central targeting agents for researchers to develop new drugs because it is a necessary enzyme which breaks down hemoglobin to provide essential amino acids for the parasite [11]. Plasmepsin enzymes are classified into ten subtypes with high genomic similarity. Knock-out studies indicated that inhibiting at least several kinds of these subtypes (not one of them) simultaneously would suffice to suppress the growth of the parasite [12]. Inhibition of only one of these enzymes may only lead to a slow growth and explains the evolutionary benefit of having several subtypes of this enzyme [13].These enzymes besiege the hemoglobin molecule and act as a proteolytic catalyst, contributing to its rupture. Also, a number of recent researches are focusing on various subtypes of plasmepsin proteins like plasmepsin IV, V, IX and X as drug targets [14–16].

It is noteworthy that plasmepsin II has a β-hairpin structure near the N-terminal of the protein, also known as “flap”, which is the main interest in designing new drugs. Asp 34, Asp 214, Val 78 and Leu 292 are among the major amino acids contributing to the structure of this flap and a majority of known plasmepsin inhibitors are considered to be well-suited molecules for this pocket [17,18]. Previous molecular dynamic studies have shown that the flap region twists and the active site of the enzyme will be exposed, highlighting the flap’s necessary rule in the activity of plasmepsin proteins [19]. Plerixafor which was firstly identified for its high anti-HIV activity, and then approved as a strong inducer of stem cell mobilization is now a candidate for a new target; plasmepsin. In this study, we described the logic behind this hypothesis by reviewing the literature and also we set-up a preliminary docking study to evaluate our hypothesis in comparison with other plasmepsin II inhibitors *in silico*.

Hypothesis

We hypothesized that plerixafor can actually bind and inhibit the plasmepsin proteins of plasmodium parasites. It is assumed that the binding of the plasmepsin enzymes to the heme group (one of the best-known porphyrin complexes) of hemoglobin is necessary in the proper interaction of plasmepsin and hemoglobin and macrocyclic amine analogues of heme can essentially bind to these regions and inhibit the enzyme’s activity. According to the structural similarity of the heme moiety of hemoglobin and cyclam ring of plerixafor we can postulate that plerixafor and related macrocycles can act as a potential inhibitiory molecule against plasmepsin proteins (Fig. 1). As mentioned in the introduction, recent studies proposed that metal complexes of azamacrocyclic rings display impressive activity in inhibiting the growth of plasmodium parasites (Fig. 2). So plerixafor (compound 4), its meta- and ortho- analogues (compound 2 and 8), different aromatic spacer analogues of plerixafor (compound 3, 7 and 10), cyclen analogue of plerixafor (compound 1), analogues with different carbon or nitrogen spacers in the cyclam ring (compound 5 and 6) and a tricyclam with two benzene ring (compound 9) were chosen for a preliminary docking study (Fig. 3). Also three plasmepsin II inhibitors characterized with nanomolar inhibitory activity in three different studies were chosen as reference compounds (Fig. 4). All of the selected compounds had been synthesized and characterized in previous studies and their structures were identified in PubChem.

Evaluating hypothesis

To evaluate this hypothesis, we conduct a docking study to assess whether plerixafor and some related compounds show noticeable interaction with plasmepsin II *in silico* or not. To perform the docking study the structures were prepared via LigPrep using OPLS3 force field [20]. LigPrep generated all possible 3D-structures in a target PH range of 7 ± 2 and in cases where for a single structure there was more than one 3D structure, all structures were subjected to the docking study for a preliminary “blind” docking in molecular operating environment (MOE; 2015.10) [21].The first score in MOE was calculated with triangle matcher method using London dG scores and the final score was obtained by London dG refinement method using induced fit orientation (to provide a more accurate docking in comparison with rigid receptor docking). After that, the potential binding pocket evaluated by MOE was used as a grid for generation of a docking study in Glide V7.7 (Schrödinger Inc. 2017.4 suite) application and the results are shown (Table 1).Though the blind docking was used in the first docking procedure, all target structures in MOE occupied the flap region of enzyme as expected. The structure of the plasmepsin II protein from *Plasmodium falciparum* was obtained from RCSB data bank [22] and the docking procedure was performed according to Glide Extra-precision docking method [23–25]. Ligand sampling was set to flexible, Epik state penalties were generated and rewarding intramolecular hydrogen bond was enabled. Also strain correction terms are applied and a post-docking minimization was performed. All rotatable hydroxyl groups from 3 Å of flap region were considered flexible and the number of poses was set to 100. The results were used in comparison with MOE docking results to provide a better viewpoint.

Discussion

The result of this preliminary docking study with both softwares obviously depicts that plerixafor analogues are very robust inhibitors of plasmepsin (Table 1). All target compounds startlingly show higher affinity than all reference compounds. The 3D simulated structure of plasmepsin-plerixafor analogue (compound 1) shows that plerixafor is just in accurate size to fill this flap, acting as a “filler” and disrupting the enzyme’s normal activity (Fig. 5). Each cyclam ring of plerixafor analogues monopolize a cavity in each side of enzyme and the benzene ring is a good linker for these two rings (compound 9 is not very active in comparison with compound 4, because it is too large to fit in this cavity). The results also indicate that meta- and para- substitutions of benzene ring of plerixafor are more suited to inhibit the enzyme’s

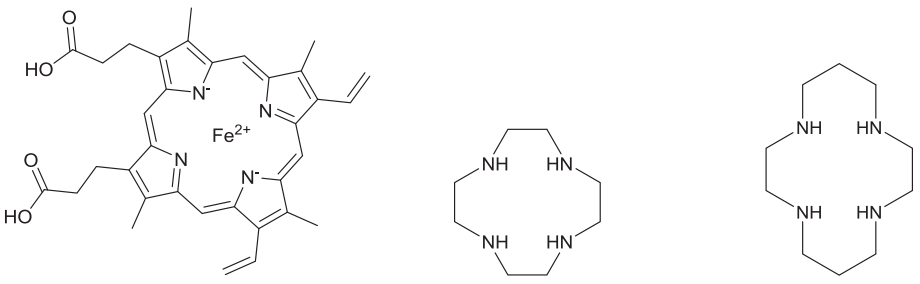

Fig. 1. Structure of protoheme (left), the basic core which is focused in this hypothesis for suggesting new potent inhibitors of plasmepsin enzymes, is similar to the cyclam ring of plerixafor (right) and also cyclen ring (middle).