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In silico investigation of medicinal spectrum of imidazo-azines from the perspective of multitarget screening against malaria, tuberculosis and Chagas disease

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ABSTRACT

A chemical database of 30 representative imidazo-azines was built and screened against important tropical disease targets by computational docking. After three rounds of screening, an interaction profile was generated and analyzed. On the basis of binding energy and ligand efficiency, it was concluded that in general, imidazo-azine scaffold has a potential of being selective and simultaneous inhibitor against the five receptors *Pf*-dihydrofolate reductase, *Pf*-enoyl acyl carrier protein reductase, *Pf*-protein kinase 7, *Mt*-pantothenate synthetase and *Mt*-thymidine monophosphate kinase. Interestingly, two compounds 2-(4-chlorophenyl)-*N*-cyclohexyl-6-methyl*H*-imidazo[1,2-*a*]pyridine-3-amine (MCL011) and *N*-cyclohexyl-2-(4-methoxyphenyl)-6-methyl*H*-imidazo[1,2-*a*]pyridine-3-amine (MCL017) showed highest binding energy against four targets *namely Pf*-dihydrofolate reductase, *Pf*-enoyl acyl carrier protein reductase, *Pf*-protein kinase 7 and *Mt*-pantothenate synthetase. Eventually, in order to improve the decision making and success rate in actual efficacy evaluations other criteria such as lead-likeness were envisaged.

1. Introduction

Reported as "global burden" malaria, tuberculosis and Chagas disease are amongst the major contributors to mortality and morbidity in most of the tropic and subtropics [1]. Chagas disease has been recognized as a neglected tropical disease (NTD) by WHO while malaria and tuberculosis are placed in "the big three diseases" along with AIDS [1]. These devastating diseases alone claim millions of death annually.

The current antimalarial arsenal relies on quinolines, antifolates and related substructures but because of the genesis and spread of the resistant malarial strain, most of the drugs except artemisinin have lost their effectiveness [2]. Likewise, isoniazids and related drugs like rifampicin, pyrazinamide, ethambutol, *etc.* form the mainstay of tuberculosis treatment. However, the emergence of extreme drug resistant (XDR), multi-drug resistant (MDR) and total drug resistant (TDR) forms of pathogen has seriously compromised the clinical utility of the current therapy [3]. Moreover, co-infection of HIV with tuberculosis and/or malaria has worsened the situation further. Chagas disease is usually treated by benznidazole (a nitroimidazole derivative) or nifurtimox (a nitrofuran derivative, Nfx). However, this therapy suffers from severe side effects and poor clinical efficacy [4]. The prevailing danger of drug-resistance and severe limitations of current chemotherapies has created a large gap between demand and supply of effective therapeutics. In order to bridge this gap effectively and timely; it is important to find newer and more effective molecules continuously.

In this context, we decided to investigate the medicinal spectrum of imidazo-azines against some important therapeutical targets from the perspective of computational multitarget screening. There were primarily three reasons for selection of amino-azines: (i) a wide range of therapeutic activities exhibited by this class of heterocycles like analgesic [5], anti-inflammatory [5], anti-proliferative [6], allosteric modulator of $GABA_A$ receptor [7], anti-viral [8], anti-bacterial and anti-microbial [9]. This scaffold is represented by marketed drugs such as zolimidine (an antiulcer drug), zolpidem (a hypnotic drug), alpidem (a non sedative anxiolytic), olprinone, and divaplon (Fig. 1); (ii) secondly, easy accessibility of N-fused bicyclic imidazo-azines through Groebke-Blackburn-Bienymé multi component reactions [10]; (iii) last but not the least, our ongoing interest in the synthesis of this class [11] and an understanding that these heterocycles have not been explored for the titled disease targets, especially at the molecular level.

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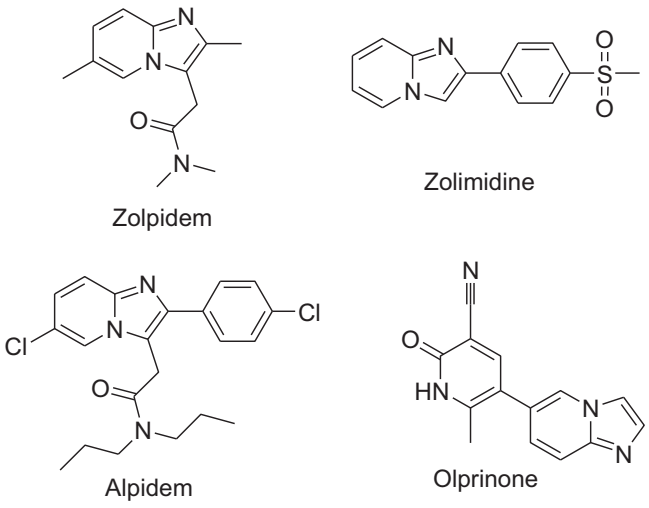

Fig. 1. Marketed drug having N-fused bicyclic imidazo-azines scaffold.

Above-mentioned multitarget screening is a relatively newer paradigm of drug discovery ventures. The aim of this approach is to identify a "key compound or scaffold" that can favorably interact with more than one receptor. The basic benefit of this screening is that inhibitor resistance can be greatly overcome by the decreased probability of simultaneous mutations of all the target proteins [13].

To achieve this aim, we selected a group of 10 well-validated drug targets from literature. The selection of targets was based on their biological role, selectivity and previous docking history.

The details of these targets are given below:

Malaria

Pf-dihydrofolate Reductase (DHFR) catalyzes the conversion of dihyrofolate to tetrahydrofolate that get converted into 5,10-methylenetetrahydrofolate which is necessary to provide a methyl group for the conversion of dUMP to dTMP in DNA synthesis pathway [12]. *Pf*-enoyl acyl carrier protein reductase (Enoyl ACP Reductase) catalyzes the reduction of *trans*-2 enoyl bond of enoyl-Acetyl CoA phosphate substrates of the malarial FAS II cycle [14]. *Pf*-protein kinase 7 (PK 7) can autophosphorylate as well as phosphorylate myelin basic protein and to a lesser extent, histone H2A as well as β-casein ultimately leading to the cell growth and proliferation [15].

Tuberculosis

Mt-shikimate kinase (SK) catalyzes the phosphorylation of shikimate to shikimate-3-phosphate, the fifth step of the shikimate pathway, leading to the biosynthesis of aromatic amino acids in pathogen [16]. *Mt*-pantothenate synthetase (PS) forms the amide bond formation between D-pantoate and β-alanine to form pantothenate (Vitamin B_5); required for the biosynthesis of Coenzyme A (CoA) and acyl carrier protein (ACP) which is necessary for many intracellular processes including fatty acid metabolism, cell signaling, synthesis of polyketides and non-ribosomal peptides [17]. *Mt*-thymidine monophosphate kinase (TMPK) promotes the conversion of deoxythymidine monophosphate (dTMP) to deoxythymidine diphosphate (dTDP) using ATP as a phosphoryl donor of the thymidine metabolism leading to the DNA synthesis [18]. *Mt*-MurE ligase preferentially adds meso-diaminopimelic acid (m-DAP) to the g-carboxyl groupof glutamic acid in uridine-5′-diphosphate-N-acetylmuramoyl-L-Alanine-D-Glutamate (UDPMurNAc-L-Ala-D-Glu) in peptidoglycan biosynthesis of the bacterial cell wall [19].

Chagas disease

Tc-trypanothione reductase (TR) reduces trypanothione disulfide (TS_2) to trypanothione dithiol [$T(SH)_2$] which scavenges the harmful oxygen species (ROS) such as superoxide anions (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH) formed as by-products of aerobic respiration in the parasite [20]. *Tc*-glyceraldehydes-3-phosphate dehydrogenase (G3PD) dehydrogenates glyceraldehyde-3-phosphate to 1,3-biphosphoglycerate, the sixth step of the glycolysis pathway [21]. The trypanosomal parasite *Trypanosoma cruzi* can evade host immune system. The parasite membrane-associated protein, *trans*-sialidase (TS), catalyzes the transfer of sialic acid molecules from host cell-surface glycoconjugates to its own surface mucin like glycoprotein, a very important step of its invasion stage [22].

Materials and methods

Ligand preparation

ChemDraw (Cambridgesoft Inc.) [23] was solely used for ligand preparation. 2D structure of the ligands (Table 1) were initially drawn which were converted into 3D. These 3D structures were then energy minimized and geometrically optimized (using AM1 force field).

Protein preparation

The X-ray crystal structures of wild type (WT) forms of *Pf*-DHFR–TS (1J3I.pdb) [24], *Pf*-Enoyl ACP Reductase (1NHG.pdb) [25], *Pf*-PK7 (2PMN.pdb) [15], *Mt*-SK (2DFN.pdb) [26], *Mt*-PS (1N2H.pdb) [27], *Mt*-TMPK (1G3U.pdb) [28], *Mt*-MurE ligase (2WTZ.pdb) [29], *Tc*-TR (1BZL.pdb) [20], *Tc*-G3PD (1QXS.pdb) [30] and *Tc*-TS (1S0I.pdb) [31] were used in this study. These structures contain the third-generation inhibitor WRA 609A, the inhibitor triclosan TCL 500A, the ATP-site inhibitor K51 344X, the substrate SKM 500A, the intermediate PAJ 1001A, the substrate TMP 217A, the substrate UAG 1498B, the substrates GCG 603A, S70 804A and SLT 923A respectively. Polar hydrogens were added and Gasteiger charges were computed in ADT. These structures were cleaned by removing crystallographic water, bound substrates and co-factors.

Docking Tool-AutoDock

AutoDock 4.2 (http://autodock.scripps.edu) [32] was employed for pose prediction and binding energy evaluation [33]. The default search function of AutoDock 4.2 is Lamarckian genetic algorithm (LGA), a hybrid of genetic algorithm and local search algorithm. The software accepts the ligand as well as the macromolecule coordinates as inputs and then seeks the help of LGA to generate the ligand positions and minimize binding energies using pre-calculated pair wise potential grid maps.

Grid parameter setting and docking calculation

Grids of suitable sizes (refer to supplementary information, Table S2) with default spacing were built and centered on the active site of the proteins under investigation with all the ligand atom types. Besides, an electrostatic and desolvation map was calculated. The parameters used in the docking study are mentioned below: