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Research article

Discovery of novel drug candidates for inhibition of soluble epoxide hydrolase of arachidonic acid cascade pathway implicated in atherosclerosis

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ABSTRACT

Soluble epoxide hydrolase (sEH), a key enzyme belonging to cytochrome P450 pathway of arachidonic acid cascade is a novel therapeutic drug target against atherosclerosis. The enzyme breaks down epoxyeicosatrienoic acid (EETs) to dihydroxy-eicosatrienoic acids (DHETs) and reduces beneficial cardiovascular properties of EETs. Thus, the present work is aimed at identification of potential leads as sEH inhibitors which will sustain the beneficial properties of EETs in vivo. PubChem and ZINC databases were screened for drug-like compounds based on Lipinski's rule of five and in silico toxicity filters. The binding potential of the drug-like compounds with sEH was explored using molecular docking. The top ranked lead (ZINC23099069) showed higher GOLD score compared with that of the control, 12-(3-adamantan-1-yl-ureido)-dodecanoic acid butyl ester (AUDA-BE) and displayed two hydrogen bonds with Tyr383 and His420 and eleven residues involved in hydrophobic interactions with sEH. The apo_sEH and sEH_ZINC23099069 complex showed stable trajectories during 20 ns time scale of molecular dynamics (MD) simulation. Molecular Mechanics Poisson-Boltzmann Surface Area (MM/PBSA) binding free energy analysis showed that electrostatic energy is the driving energy component for interaction of the lead with sEH. These results demonstrate ZINC23099069 to be a promising drug candidate as sEH inhibitor against atherosclerosis instead of the present urea-based inhibitors.

1. Introduction

Atherosclerosis is a chronic inflammatory disease characterized by formation of macrophage-derived foam cells and plaque accumulation in the arterial wall (Yu et al., 2013). It is a leading cause of most of the cardiovascular diseases (CVDs) including myocardial infarction and stroke (Wang et al., 2010). Over 17.7 million people died from CVDs in 2015 representing 31% of all global deaths. Of these deaths, around 6.7 million were due to stroke and approximately 7.4 million were due to coronary heart disease (WHO CVDs report, 2017). The current pharmaceuticals used for the intervention of atherosclerosis include lipid lowering drugs such as statins (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor), anti-inflammatory drugs such as low-dose aspirin (cycloxygenase (COX) inhibitor) and antihypertensive drugs-angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) (Wang et al., 2010).

Of many important pharmaceutical drug targets for atherosclerosis, soluble epoxide hydrolase (sEH) emerged as a promising therapeutic drug target for CVDs from its initial findings that administration of sEH inhibitor N, *N*'-dicyclohexylurea (DCU) lowered blood pressure in spontaneously hypertensive rats (SHR) (Yu et al., 2000). Furthermore, it was demonstrated that apolipoprotein E (apoE) deficient mice treated with orally bioavailable, selective and potent sEH inhibitor, 1-(1-nicotinoylpiperidin-4-yl)-3-(4-(trifluoromethoxy)phenyl)urea (AR9276) showed diminished abdominal aortic aneurysm formation and atherosclerosis

Abbreviations: CVDs, cardiovascular diseases; sEH, soluble epoxide hydrolase; MD, molecular dynamics; EETs, epoxyeicosatrienoic acids; DHETs, dihydroxy-eicosatrienoic acids; MM/PBSA, molecular mechanics poisson-boltzmann surface area; ROF, rule of five; NVT, number of particles, volume and temperature; NPT, number of particles, pressure and temperature; RMSD, root mean square deviation; RMSF, root mean square fluctuation; SASA, solvent accessible surface area; Rg, radius of gyration; PCA, principal component analysis; ED, essential dynamics; NHBs, number of hydrogen bonds; AUDA-BE, 12-(3-adamantan-1-yl-ureido)-dodecanoic acid butyl ester.

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development (Zhang et al., 2009). It was further demonstrated that administration of urea-based small molecule inhibitors of sEH to C57BL/6 mice decreased lipolysaccharide induced mortality and systemic hypotension and plasma levels of pro-inflammatory cytokines and nitric oxide metabolites (Schmelzer et al., 2005). Thus, it can be a key pharmacological target for treating acute systemic inflammation. In humans, a single copy gene (EPXH-2) consisting of 19 exons located on chromosome 8, encodes soluble epoxide hydrolase (sEH) enzyme of 555 amino acids (Larsson et al., 1995; Sandberg and Meijer, 1996). The human sEH (EC 3.3.2.3) belongs to the α/β hydrolase fold family of proteins and the subcellularaly localized in cytosol and peroxisomes (Arand et al., 1994). It is a 62 kDa bifunctional homodimeric enzyme, consisting of N- and C-terminal domains connected by a short proline-rich linker (Fig. 1) (Newman et al., 2005). The N-terminal domain (~25 kDa) exhibits phosphatase activity which hydrolyses lipid phosphates and the C-terminal domain (~35 kDa) possesses an epoxide hydrolase activity which catalyzes conversion of epoxides to their corresponding diols (Morisseau and Hammock, 2013). The X-ray crystal structure of human sEH complexed with an inhibitor (PDB ID: 3I28) revealed a catalytic triad (Asp335, Asp496 and His524) of sEH epoxide hydrolase domain. The epoxide hydrolase catalytic pocket consists of two tyrosine residues (Tyr383 and Tyr466) which activate the epoxide ring-opening by Asp335 (Shen and Hammock, 2012). The enzyme is distributed across various tissues types with its highest activity in the liver and kidney (Draper and Hammock, 1999; Harris and Hammock, 2013).

The polyunsaturated fatty acids endogenously produced in the cells can be oxidatively modified by three pathways (Fig. 2). Out of which, Lipoxygenase (LOX) and Cycloxygenase (COX) pathways have been thoroughly investigated but the third relatively unexplored pathway is mediated by cytochrome P450 enzymes which convert arachidonic and linoleic acids to various biologically active metabolites such as hydroxyeicosatrienoic acids (HETEs), Epoxyeicosatrienoic acids (EETs) and Epoxyoctadecamonoenoic acids (EpOMEs) (Wang et al., 2010). EETs are hyperpolarizing factors derived from endothelium which possess anti-inflammatory properties and mediate vascular relaxation responses (Campbell et al., 1996; Falck et al., 2003; Node et al., 1999). In particular, 11,12-*E*ETs provide anti-inflammatory activity by downregulation of cytokine induced endothelial cell adhesion molecules such as E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (Node et al., 1999). EETs also impede leukocyte adhesion to the vascular wall presumably by inhibiting NF-kB and I kappa B (IkB) kinases (Kessler et al., 1999). EETs are further metabolized by sEH to their corresponding diols, dihydroxyeicosatetraenoic acids (DHETs) diminishing their biological properties. Thus, EETs serve as endogenous protective functions to ameliorate atherosclerosis and endothelial dysfunction (Roman, 2002). The enzymes of arachidonic acid cascade particularly soluble epoxide hydrolase (sEH) offer as a novel therapeutic drug target for atherosclerosis (Wang et al., 2010).

The aim of the present study is to identify lead compounds by virtual screening of chemical databases for drug-like compounds and further screening for inhibitor(s) of sEH by molecular docking studies. The apo_sEH and the top ranked compound complexed with sEH were also studied for their stabilities and conformational changes upon binding using molecular dynamics (MD) simulation.

2. Materials and methods

2.1. Screening of drug-like compounds

A total of 2500synthetic compounds were retrieved from PubChem (N = 1000) (https://pubchem.ncbi.nlm.nih.gov/) (Kim et al., 2016) and ZINC databases (N = 1500) (http://zinc.docking.org/) (Irwin and Shoichet, 2005). The compounds were first filtered based on Lipinski's rule of five (ROF) (Lipinski, 2004) according to which the physicochemical properties of an orally bioavailable drug falls within the following ranges-(a) Molecular weight (MW) ≤500 Da b) cLogP (partition coefficient between water and octanol) ≤5.0 c) Hydrogen bond acceptor (HBA) ≤10 and d) hydrogen bond donor (HBD) ≤5. The physiochemical properties of the compounds were determined using Molinspiration tool (http://www.molinspiration.com/). The compounds were further screened based on various physiochemical properties such as cLogS (aqueous solubility calculated at 25 °C, pH = 7.0) ≥−4, Topological polar surface area (TPSA) ≤ 140 Å^2, Rotatable bonds (RB) ≤10, Druglikeness ≥0 and in silico toxicity prediction such as mutagenicity, tumourigenicity, irritancy and effects on reproductive health were evaluated. These physicochemical properties of the compounds were calculated using OSIRIS DataWarrior (Sander et al., 2015).

2.2. Study of protein-ligand interactions

The three dimensional structure of target protein (sEH) was retrieved from Protein Data Bank (PDB) database (www.rcsb.org/)

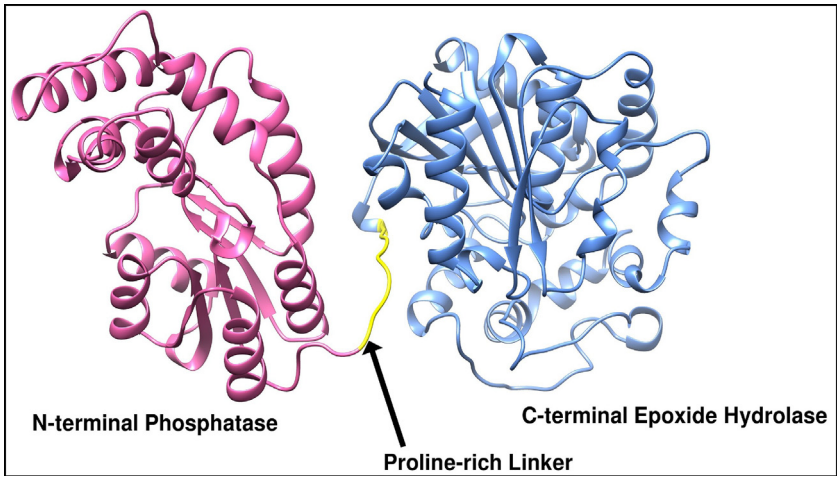

Fig. 1. The X-ray crystal structure of soluble epoxide hydrolase (sEH) (PDB ID:3I28). The N-terminal domain (positions 1–224) has phosphatase activity and the epoxide hydrolase function is contained in the C-terminal domain (positions 235–555). The N-terminal domain is connected to the C-terminal domain by a short Proline-rich Linker (positions 225–234).