SCREENING AND IDENTIFICATION OF NOVEL INHIBITOR FOR IL-1B INVOLVED IN CORONARY ARTERY DISEASE

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ABSTRACT

The deadliest disease in the world is the coronary artery disease (CAD). World Health Organization (WHO) estimates that about 7.3 million people died of ischemic heart disease in 2012. In CAD, Inflammation also participates in all stages of local, myocardial and systemic complications of atherosclerosis. However, Interleukin (IL-1β) represents one of the most important mediators of inflammatory response that induces a cascade of proinflammatory effectors molecules. IL-1β may enhance atherogenesis and exacerbate left ventricular dysfunction is by contributing to endothelial dysfunction. In the present study, Curcumin (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione also known as diferuloylmethane, is the main ingredient of turmeric with regard to its anti-inflammatory action, curcumin was reported to downregulate the secretion of prominent cytokines, like TNFα , IL-1β and IL-6. Curcumin natural inhibitor was retrived from Pubchem database and designed new lead with its analogues by bioinformatics virtual screening methods. Further, drug lead molecules were evaluated for their drug likeness using "Lipinski rule of five" and pharmacokinetic and ADME Toxicity properties. In molecular docking studies curcumin derivative showed the better binding energy with the target protein. The In silico approach can be appropriate to develop new drug lead molecules against IL-1β in CAD.

KEYWORDS: *Coronary Artery Disease, IL-1β, Curcumin, Molecular Docking, Lipinski 5 Screening and In silico.*

INTRODUCTION

Coronary arteries are the blood vessels that carry blood to heart muscles. Coronary Artery Disease (also called CAD or coronary heart disease) is caused by thickening of inside walls of coronary arteries is also called as atherosclerosis[1]. According to the Centre for Disease Control and Prevention (CDC) in the United States about 600,000 people die of heart disease every year which makes it is the deadliest disease in the U.S. as well as in the world [2]. Heart disease is the leading cause of death for both in men and women. High blood pressure, high cholesterol and

smoking are the key risk factors for heart attack [3]. During the past decades, our understanding of pathophysiology of coronary artery disease (CAD) has undergone a remarkable evolution. Previously considered a cholesterol storage disease understands atherogenesis as complex interactions of risk factors including cells of artery wall and the blood and the molecular messages that they exchange. Inflammation also participates in all stages of local, myocardial and systemic complications of atherosclerosis [4]. Inflammatory cells, inflammatory proteins, and inflammatory responses from vascular cells play a pivotal role in the pathogenesis of various stages of atherosclerosis, including the initiation and progression of atheroma, plaque instability and rupture, and post-angioplasty and restenosis [5-7]. Elevated levels of IL-1 result in secretion of chemokines and other cytokines (eg, IL-6), increased expression of adhesion molecules, activation of endothelial and smooth muscle cell proliferation, macrophage activation, and increased vascular permeability. This cascade promotes atherosclerosis and plaque destabilization. IL-1 and other proinflammatory cytokines have also been implicated in the progression of heart failure, as a result of their negative inotropic effects and deleterious effects on left ventricular remodeling. Another important mechanism by which IL-1 may enhance atherogenesis and exacerbate left ventricular dysfunction is by contributing to endothelial dysfunction. IL-1 stimulates release of endothelin-1, a potent vasoconstrictor, and IL-1 stimulates inducible nitric oxide synthase, which increases the formation of reactive oxygen species and reactive nitrogen species (e.g. nitrotyrosine), which leads to oxidative and so-called nitrosative stress and endothelial dysfunction [8].

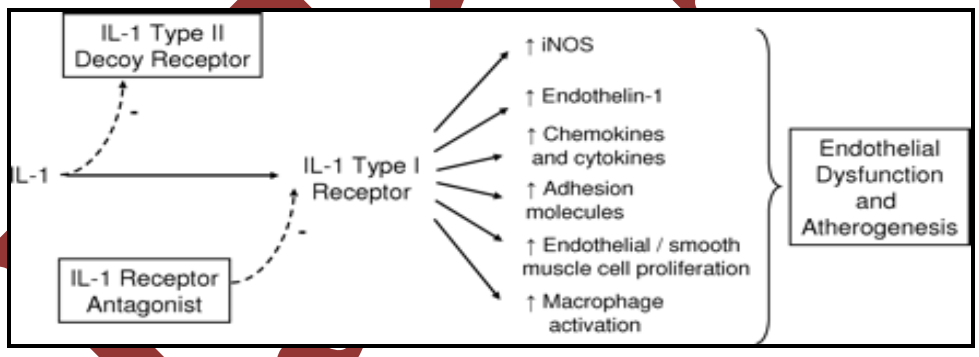


Figure 1. Potential roles of IL-1 and IL-1 receptor antagonist in atherogenesis.

In a clinically important additional level of regulation, innate immune cells including monocytes initially produce IL-1β as an inactive precursor (pro-IL-1β) that requires proteolytic cleavage to attain biological activity. This is typically mediated by a complex of intracellular protein known as NLRP3 inflammasomes, which in response to the presence of crystalline structures, leads to activation of caspase-1(also known as IL-1β converting enzyme). This recognition that cholesterol crystals can activate IL-1β production has provided a common hypothesis linking hyperlipidaemia to vascular inflammation[9]. Curcumin shows strong anti-oxidation and anti-inflammatory activities in various disease [10]. In the present study was focused on curcumin

(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione also known as diferuloylmethane, is the main ingredient of turmeric(*Curcuma longa,* Zingiberaceae) which play potential role in anti-inflammatory in CAD. Due to its chemical structure, curcumin may act as a natural free radical scavenger. Curcumin can decrease the release of different interleukins through NF-κB[11]. The study shows that curcumin-treated mice exhibited relative decreases in aortic tissue activator protein-1 and NF-κB DNA binding activities and significant lower concentrations of IL-1β, IL-6, MCP-1, and MMP-9 in experimental AAAs [12]. It exerts a great variety of actions and is amongst the most frequently investigated natural compounds [13]. With regard to its anti-inflammatory action, curcumin was reported to downregulate the secretion of prominent cytokines, like TNFα, IL-1β and IL-6[14].

MATERIALS AND METHODS

1. **Protein Preparation and Active site prediction:** The Interleukin (IL-1β) protein molecule (PDB ID: 9ILB) was downloaded from Protein Data Bank database. The Protein Data Bank (PDB) is a crystallographic database for the three dimensional structural data of large biological molecules [15]. The active site of IL-1β protein was predicted by using CASTp (Computed Atlas of Surface Topography of proteins) server. The identification of active sites is often the starting point for protein function, annotation and structure-based drug design [16].

2. **Designing Drug Library:** The natural inhibitor curcumin (CID: 969516) for IL-1β was downloaded from PubChem chemical database in Mol2 chemical file format. PubChem is a database of chemical molecules and their activities against biological assays [15]. The analogues of curcumin molecule were generated by using ZINC database. ZINC is a free public resource for ligand discovery [17]. The analogues were analyzed by MarvinView chemical viewer package. It allows for analysing easy scrolling of thousand of molecules either in a grid view or in a spreadsheet view [18].

3. **Virtual Screening by Lipinski rule of Five:** Molecular descriptors and drug likeliness properties of compounds were analyzed using the Molinspiration server with based on Lipinski's Rules of Five. Molinspiration server supports for calculation of important molecular properties such as LogP, polar surface area, number of hydrogen bond donors and acceptors, as well as prediction of bioactivity score for the most important drug targets GPCR ligands, kinase inhibitors, ion channel modulators, enzymes and nuclear receptors [19].

4. **Virtual Screening by ADME Toxicity:** The ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties of the target compounds were calculated using admetSAR which describes the deposition of pharmaceutical compounds within an organism. Blood-Brain Barrier (BBB) penetration, HIA (Human Intestinal Absorption), and AMES toxicity were

calculated. The cytochromeP450 super family plays an important role in drug metabolism and clearance in the liver [20].

5. **Molecular Docking:** The molecular docking analysis of between curcumin and its analogue with receptor IL-1β was carried by Hex 8.0 molecular docking software. Hex is an interactive protein docking and molecular superposition program. Hex package reads protein structures in PDB format and small-molecules in SDF files [21].It is an interactive molecular program for calculating and displaying feasible acids and small biomolecules with binding or docking score. The further docked complex was visualized by PyMOL visualizations tool for interaction studies.

RESULTS AND DISCUSSION

1. Protein Preparation and Active site prediction: The PDB structure file of the IL-1β was downloaded in PDB format. The structure file is visualized using Chimera 1.10.2 free open source computer programme. The resolution of IL-1β was 2.28 Å and 3D structural data of IL-1β was determined by X-RAY DIFFRACTION method which shown in Figure 2. Active site of IL-1β was predicted by using CASTp server. 22 pockets were identified in Interleukin-1β molecule.

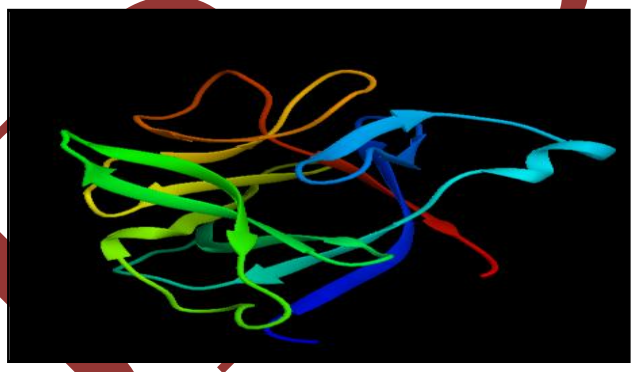
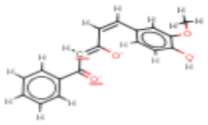
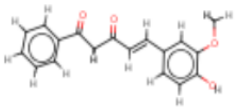
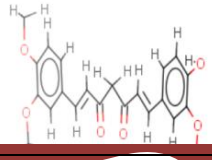
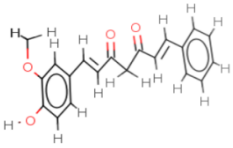
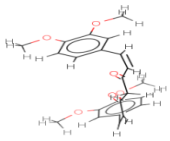
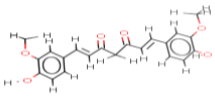


Figure 2. IL-1β (9ILB) crystal structure in Chimera tool.

2. Designing Drug Library: Curcumin compound was selected from PubChem as natural inhibitor for IL-1β. The chemical compound was subjected to ZINC database to generate analogues for designing drug library. Total five analogues were generated from ZINC database. The molecular descriptors were calculated and visualized by MarvinView package which shown in Table 1.

Table 1. Chemical structural representation by MarvinView

Chemical_ID	Chemical Structure
zinc_33799538	
zinc_33799539	
zinc_38424733	
zinc_40828260	
zinc_44608728	
zinc_curcumin	

3. Virtual Screening by Lipinski rule of Five: Drug likeliness properties of curcumin and its analogues were calculated in Table 2. Lipinski's Rules rule for the molecule should have less than 5 H-bond donar , no more than 10 H-bond acceptor ,LogP value not greater than 5. As per Table No.2 the all five analogues were predicted as Drug likeness properties on the basis of Lipinski's Rule. Further these compounds were passed for the ADMET screening.

Table 2. Drugliklіness Properties using Lipinski 5 Screening

Compound ID	Mol. wt	Log P	Rotatable bonds	H-bond acceptor	H-bond donar	TPSA
zinc_33799538	295.314	3.34	5	4	1	70
zinc_33799539	295.314	3.34	5	4	1	70
zinc_38424733	295.314	3.34	5	4	1	70
zinc_40828260	321.352	3.71	6	4	1	70
zinc_44608728	395.431	3.66	9	6	0	77
zinc_curcumin	367.377	3.05	7	6	2	99

4. Virtual Screening by ADME Toxicity: The ADME and Toxicity (Absorption, Distribution, Metabolism, and Excretion) properties of the chemical compounds were predicted using admetSAR server. The ADMET properties were Blood-Brain Barrier (BBB) penetration, HIA (Human Intestinal Absorption), and AMES toxicity were calculated. The predicted ADMET data were summarized in Table 2.

Table 2. ADME and Toxicity Properties

Molecule Name	Blood-Brain Barrier	CYP450 1A2 Inhibitor	AMES Toxicity	Carcinogens	Acute Oral Toxicity
zinc_33799538	0.7143	0.8595	Non Toxic	Non-carciogenic	0.4692
zinc_33799539	0.8413	0.8533	Non Toxic	Non-carciogenic	0.5741
zinc_38424733	0.8313	0.7633	Non Toxic	Non-carciogenic	0.5429
zinc_40828260	0.8413	0.8533	Non Toxic	Non-carciogenic	0.5741
zinc_44608728	0.9090	0.9124	Non Toxic	Non-carciogenic	0.6133
zinc_curcumin	0.8182	0.9105	Non Toxic	Non-carciogenic	0.6349

5. Molecular Docking: Molecular docking studies are computational techniques for exploration of possible binding mode of a ligand (Curcumin) to a given receptor (IL-1β). Docking results of the curcumin and its analogues using Hex molecular docking suite which reveals that interaction between the ligand and receptor. Five analogues and curcumin were docked with IL-1β receptor and binding energy were calculated in Table 3. As per the table binding energy score of (1E, 6E)-1-(3, 4-dimethoxyphenyl)-7-(4-hydroxy-3-methoxy-phenyl) hepta-1, 6-diene-3, 5-Dione [Zinc_38424733 (-297.07)] is better as compared to other derivatives and curcumin molecule (-

256.56). Zinc_38424733 was more compatible for the potential inhibitor with receptor than other ligand molecules. Further the docked complex between the Zinc_38424733 (-297.07) and IL-1β molecule were visualized and shown in Figure 3. The below figure reveals blocking of interacting residues LEU34, PHL133, PRO31, and GLU25 of IL-1β with Zinc_38424733

Table 3. Binding energy score between the Drug molecules with IL-1β

Receptor	**Ligand**	**Docking Score**
IL-1β	Curcumin	-256.56
	zinc_33799538	-216.1
	zinc_33799539	-251.0
	zinc_38424733	-297.07
	zinc_40828260	-261.78
	zinc_44608728	-272.78

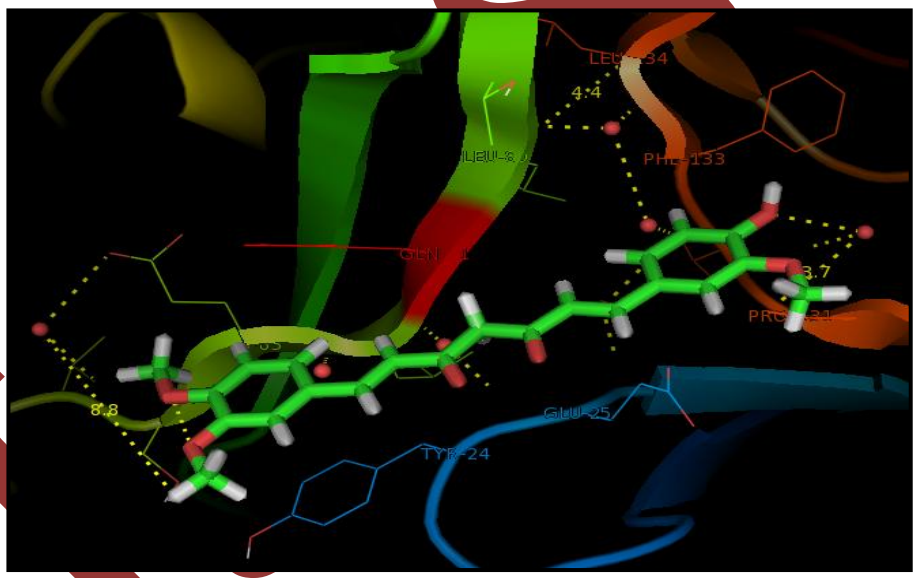


Figure 3. Molecular Docking between IL-1β and Zinc_38424733

CONCLUSION

Coronary Artery Disease is leading cause of death all over world. The present preliminary investigation mainly leads to understand the target protein IL-1β which directly involved in causing coronary artery disease inflammation. The natural inhibitors Curcumin with its analogues were designed by PubChem and ZINC database by *in silico* approach. The curcumin absorption, biodistribution, metabolism and elimination level was overcome to

enhance its bioavailability and many chronic inflammatory diseases will be at the forefront as promising targets for curcumin therapy. Drug descriptors of curcumin analogues were calculated by MarvinView package. Drug likeness properties like Lipinski rule of five and ADME Toxicity were predicted by virtual screening techniques. Further, the molecular docking results of the current study were clearly demonstrated that out of screened compounds the (1E, 6E)-1-(3, 4-dimethoxyphenyl)-7-(4-hydroxy-3-methoxy-phenyl) hepta-1, 6-diene-3, 5-dione of is better inhibitors for IL-1β in Coronory artery disease inflammation. However, this lead should undergo various preclinical analysis and optimization process before going into clinical trials.

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