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Research Article

SYNTHESIS AND MOLECULAR DOCKING STUDIES OF ETHYL 1-BENZENESULFONYL -2-[(E)-2-(2 METHYLPHENYL) ETHENYL] INDOLE -3-CARBOXYLATE WITH HUMAN RENIN COMPLEXED WITH INHIBITOR

C. RAMATHILAGAM[1*], AKHILESH UPGADE[2], ANUSHA BHASKAR[2], P.R.UMARANI[3], V.MANIVANNAN[4]

[1]Department of Physics, AMET University, Kanathur, Chennai - [2]Center for Research and Development, CACB, PRIST University,Thanjavur-[3]Kunthavai Naacchiyar Govt Arts College for Women, Thanjavur-[4]Center for Research and Development, PRIST University, Thanjavur-
Email: cramathilagam@gmail.com

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ABSTRACT

Various proteins play important roles in hypertension and a number of drugs have been tested for their efficacy in modulating hypertension. Renin is an aspartyl protease involved in the production of angiotensin II, a potent vasoconstrictor. Renin inhibitors can prevent blood vessel constriction and therefore could be useful for the treatment of hypertension. With this rationale, some new indole compound are synthesized and evaluated for their antihypertensive activity. Docking studies using Molegro Virtual Docker (MVD) on the human Renin complexed with inhibitor (PDB ID : 2IKO) show their role in the antihypertensive activity of the molecule and explain the higher potency of compound based on ReRanking score and binding poses of the molecule.

Keywords: Indole, Human Renin complexed with inhibitor, Molegro Virtual Docker, Antihypertensive, Single XRD.

INTRODUCTION

Hypertension is a major contributor to avoidable death and disease in India. Over 140 million people are believed to be suffering from high blood pressure in the country and the number is expected to cross the 214 million mark in 2030 (Zoccali et al., 2002[1]).Increase in blood pressure (BP) increases the risk of developing heart disease, obesity (Chow et al., 2000[2], kidney disease (Johnson et al., 2007[3]), eye damage, and stroke (Stokes et al., 1987[4]). A WHO estimate in 2008 suggested 33 per cent men and 32 per cent women older than 25 years had hypertension in India [5].Currently the hypertension attracts the kidney diseases, in which excess fluid in kidney damages the walls and exerts the pressures resulted in (ESRD) End stage renal disease. Hence root cause is taken for surveillance and targeted. Hypertension cycle in renal part can be ceased by inhibiting the aggravating enzymes like Angiotensin. In current study synthesis and computational molecular docking studies of (Ethyl 1-benzenesulfonyl-2-[(E)-2-(2methylphenyl)ethenyl] indole-3- carboxylate(code: CR1) with Human Renin complexed with inhibitor (PDB ID: 2IKO) using Molegro Virtual Docker software done to understand the basic mechanism of inhibition.

Importance of indole

The indole subunit is a near-ubiquitous component of biologically active natural products, and its study has been a major focus of research for generations. Compounds having indole groups are biologically important compounds. Indole along with their several derivatives finds a prominent place in synthetic organic chemistry, as they found to be potent pharmacophores. Indole derivatives have displayed versatile pharmacological properties such as antimicrobial, antiviral, antifungal, antiHIV, anti-tubercular, antihypertensive, anti-inflammatory and analgesic, anticancer, antidiabetic and anticonvulsant agents[6-22]. These effects have been well-proven in randomized controlled studies. Substituted indoles have frequently been referred to as privileged structures since they are capable of binding to multiple receptors with high affinity, and thus have applications across a wide range of therapeutic areas. Due to this activity, it is not surprising that the indole ring system has become an important building block or intermediate in the synthesis of many pharmaceutical agents.

Because of the wide variety of the biological applications of the indoles, the synthesis of several substituted indoles and the study of their crystal, molecular structure and molecular docking studies, continue to be an interesting field of research. With this idea, crystals of indole derivative are synthesized. The synthesized compound are subjected to single crystal X-ray studies in order to investigate their molecular structure. Using Molegro Virtual Docker (MVD), docking studies of the title derivative have been carried out to understand the possibility of these compound to act as effective target for hypertensive. Docking is frequently used to predict the binding orientation of small drug candidate to their protein targets in order to predict the affinity and activity of the small molecule [17].

Methodology

Compund Synthesis

To a suspension of hexane (5 ml) washed NaH (0.29 g, 6.10 mmol) in dry THF (10 ml) at -10° C under N_2 atmosphere was slowly added the solution of diethyl (3-(ethoxycarbonyl)-1-phenylsulfonyl-1*H*-indol-2-yl)methylphosphonate (0.97 g, 2.03mmol) in dry THF (5 ml) via syringe and stirred for 15 min. Then a solution of 2-methylbenzaldehyde (0.28 g, 2.32 mmol) in dry THF (5 ml) was added and allowed to stir for additional 2 h. After completion of the product formation (monitored by TLC), it was then poured over crushed ice (100 g) containing conc. HCl (3 ml). The solid formed was filtered and recrystalized with MeOH to afford ethyl 2-(2-methylstyryl)-1-phenylsulfonyl-1*H*-indole-3-carboxylate as bright yellow crystals [0.70 g, 78%].

X-ray crystallographic analyses

A single crystal of the title complex suitable for X–ray structural analysis is selected from the crystals obtained above. All measurements were made on a Bruker Kappa APEX-II diffractometer with graphite monochromated Mo- Kα radiation (0.71073 Å). The structure was solved by direct methods and refined by full–matrix least squares on F2. All non-hydrogen atoms were refined anisotropically. The H atoms were introduced in calculated positions and refined with fixed geometry with respect to their carrier atoms [23].

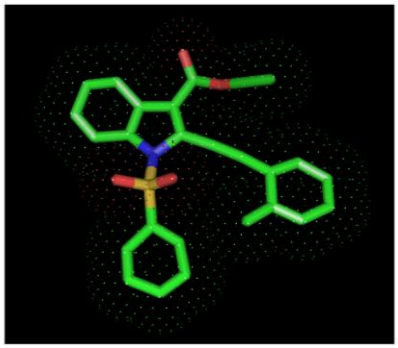


Fig.1: Schematic diagram of Ethyl 1-benzenesulfonyl-2-[(E)-2-(2- methylphenyl)ethenyl]indole-3-carboxylate

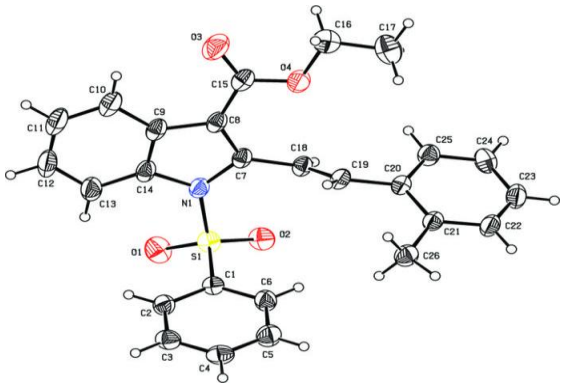


Fig. 2: ORTEP diagram of Ethyl 1-benzenesulfonyl-2-[(E)-2-(2-methylphenyl)ethenyl]indole-3-carboxylate

Preparation of Ligand Structure

The ligand structure was constructed using Chem3D ultra 8.0 software (Molecular Modeling and Analysis; Cambridge Soft Corporation, USA, 2004) and saved as MDL molFile (.mol).

Preparation of Protein structure

The 3-Dimensional crystal structure of Human Renin complexed with inhibitor - A Chain (PDB code: 2IKO) was selected from the Protein Data Bank (PDB) [24] as the receptor/target model in virtual screening process.

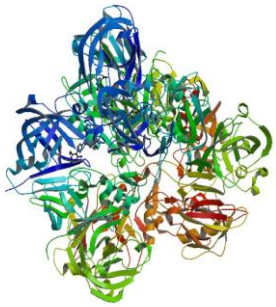


Fig.3: crystal structure of human Renin complexed with inhibitor

Active site identification

The structure of the drug target was obtained from Protein Data Bank. The PDB file was loaded into Pocket-Finder [25] to identify the active site (pocket) detection on Renin complexed with inhibitor protein (PDB ID: 2IKO).

Site 1: Min Coords : (-6,-5,-57)

Max Coords: (27, 35, -24)

Site volume: 2481 Å^3

Protein volume: 62711 Å^3

Computational molecular docking studies

Molegro Virtual Docker (Version- 5.5)

In this work Molegro Virtual Docker (MVD) [26] has been used for the prediction of protein- ligand interactions study [27].

In the present investigation, an attempt was made to understand the ligand-receptor interactions of indole derivative(code:CR1) against human Renin complexed with inhibitor(PDB ID: 2IKO) as a target enzyme, by performing docking studies using Molegro Virtual Docker (MVD) version 5.5, probably the most accurate predictive tool of binding geometry today.

Docking calculations were carried out using MVD on new ligand protein model. The protein structure of 2IKO was imported in MVD, and missing bond orders, hybridization states, and angles were then assigned. To obtain better potential binding sites in the protein, a maximum of five cavities were detected using parameters such as molecular surface (expanded van der Waals), maximum number of cavities (n = 5), minimum cavity volume (10), probe size (1.20), maximum number of ray checks (n = 16), minimum number of ray hits (n = 12), and grid resolution (0.80). The chosen cavity was further refined using side-chain minimization by selecting the add-visible option at a maximum of steps per residue (10000) and a maximum of global steps (10000). The setup for side-chain flexibility by selection of the add-visible option, the setting for the selected flexible side chain during the docking option, and other parameters, all were kept in default. All docking calculations were carried out using the grid-based MolDock score (GRID) function with a grid resolution of 0.30Å. The binding site on the receptor was defined as extending in X , Y, and Z directions around the dock molecule with a radius of approximately 10 to 17 Å . The MolDock optimization search algorithm with a maximum of ten runs was used through the calculations, with all other parameters kept as defaults. One pose per run was retained based on root mean square division clustering using a heavy atom threshold set at 2.0 A° and an energy penalty of 100. All the poses were examined manually and the best poses were retained.

Optimization of the parameter for suitable docking

To obtain better potential binding sites in the protein (2IKO), a maximum of three cavities was detected using default parameters. Out of the detected cavities, cavity number 1 (cavity volume= 531.456 Å^3; Surface=1427.2 Å^2) was selected for further studies. The chosen

cavity was further refined using side-chain minimization by selection of an add-visible option set at a maximum of 10000 steps per residue and at a maximum of 10000 global steps. The side-chain flexibility was set by selecting the add-visible option .The same was selected during docking, and the remaining parameters were kept as fixed variables. Furthermore, the docking

Simulation was run and the best pose for the set derivative was selected on the basis of the MolDock score and ReRank score.

RESULTS AND DISCUSSION

X-ray structure analysis

The crystallographic data and refinement parameters are listed in Table 1. The selected bond distances are given in Table.2

Table 1: Crystal data

Parameters	Data
Molecular Formula	$C_{26}H_{23}NO_4S$
Molecular Weight	445.51
Crystal system	Monoclinic
Z	4
Space group	*P*21/*n*

Volume	2196.63 (15) Å^3
Density	1.347 Mg m^{-3}
Temp	295 K
Unit cell dimensions	a = 10.4248(4) A°
	b = 8.3629(3) A°
	c = 25.2284(11)A°
Final R indices [I>2σ (I)]R1	0.042
Goodness-of-fit on F	1.03
Melting point	371–373 K

Table 2: Selected bond distances (Å)

Bond	Distance(Å)	Bond	Distance(Å)
C1-C2	1.381(2)	C11-C12	1.379(4)
C2-C3	1.379(3)	C12-C13	1.383(3)
C3-C4	1.368(3)	C13-C14	1.388(3)
C4-C5	1.371(3)	C16-C17	1.485(3)
C5-C6	1.379(3)	C18-C19	1.313(2)
C7-C8	1.3662(2)	C19-C20	1.472(2)
C8-C9	1.440(2)	C20-C21	1.408(2)
C9-C10	1.404(3)	C21-C22	1.382(3)
C10-C11	1.368(3)	C22-C23	1.370(3)
N1-S1	1.6789(17)	C23-C24	1.368(3)
O1-S1	1.4217(14)	C24-C25	1.380(3)
O2-S1	1.4194(14)	C1-S1	1.7523(17)

Ligand - Protein Interaction

Table 3: Amino acid residues and atoms of ligand involved in hydrogen bonding and their bond lengths.

Ligand	MolDock Score	Rerank Score	No of Hydrogen bonds	Ligand atom	Residues	Distance(Å)
Code(CR1)	-177.529	-134.508	3	N	Thr80	3.01
				O2	Ser225	3.38
				O4	Ser79	3.18

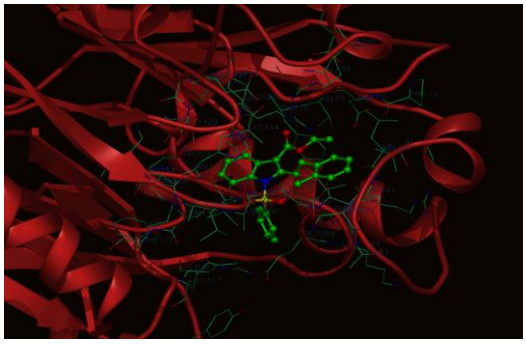


Fig. 4

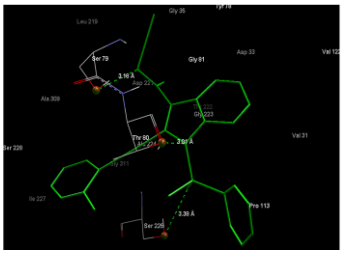


Fig. 4(a)

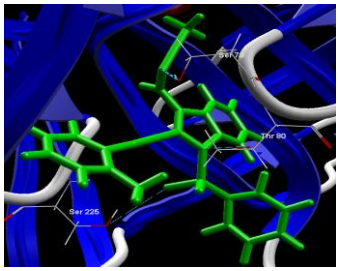
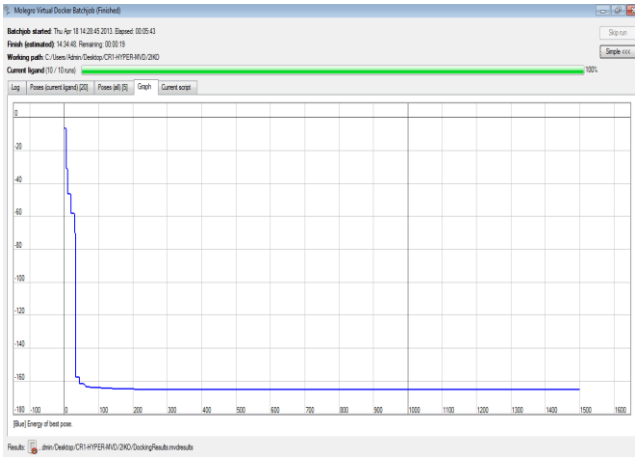


Fig. 4(b)

Atoms of protein (2IKO) and ligand involved in three hydrogen-bond interactions shown as dotted lines (Table.3) showing one hydrogen bond between **Nitrogen** and **Thr80** of distance **3.01Å** and one hydrogen bond between **Oxygen2** to **Ser225**of distance**3.38 Å** and one hydrogen bond between **Oxygen4** to **Ser79** of distance **3.18 Å** . This picture was created with Molegro virtual docker program. The MolDock score and ReRank score of molecule are presented in Table 3. The parameter used for identifying the best ligand binding position was the root-mean square distance (RMSD) value [28, 29].

Schematic view of MolDock score



Binding modes

Active site of 2IKO offers different binding modes for these compounds as they are strongly dependent on the attached substituent. 2IKO bound ligand was docked deeply within the binding pocket region forming interactions with Thr80, Ser225, Ser79 respectively.

Discussion

Most of the world's population is living with hypertension, ultimately leading to death by heart attack. Knowing the key factor is one of the greatest facts and targeting it further difficult. Hence a research has been made to understand the basic mechanism in and around the target. Renin is an aspartyl protease that is specific for only one substrate, angiotensinogen. The renin stored and produced in juxtaglomerular apparatus of the kidney. Angiotensin given by the liver cells undergoes proteolytic cleavage of a 43 amino acid N-terminal and allows conversion to active renin is >99.9% observed i.e. nothing but the angiotensin [30].

Main target is the renin secretion which is necessary to inhibit so that it will neither activate nor develop the pressure in kidney.

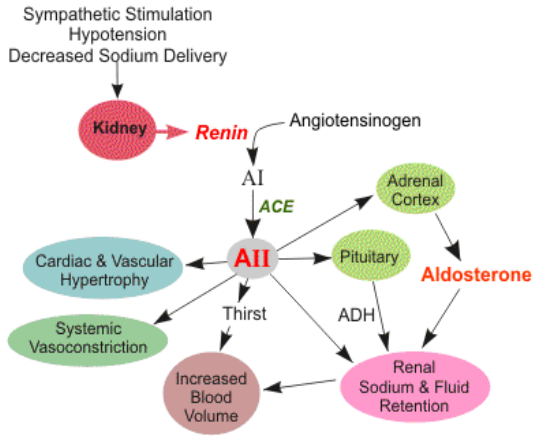


Fig. (5)

Fig.(5) reveals that the AII (AngiotensinII) enzyme clearly depicts that sympathetic stimulation of hypotension release renin which activated as AII and how it performs the different systems, which directly involved in the vasoconstriction and renal fluid retention. This leads to cardiac arrest due to kidney malfunction. Rationale of this issue is still in research to understand and investigate the target. Currently Atenolol and Lisinopril are the only two drugs available in the market. They acts as beta blockers or simply ACE blocker so that renin cannot active and whole RAS system can fail. [31]

The aim of the current study is to design a novel renin inhibitor. Hence the renin protein was designated as a target. Insilico study is done where the advanced computational studies were performed in drug development. Screening methods are routinely and extensively used to reduce cost and time of drug discovery. A docking study was carried out using molegro virtual docker software where the ligand (code: CR1) was docked with human Renin complex with inhibitor. The docking score revealed that the ligand showed highest score and hence a strong binding affinity towards the protein effectively. The compound showed moderate antihypertensive activity, which is suggested for clinical trials.

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