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Research Article

SYNTHESIS AND MOLECULAR DOCKING STUDIES OF DIETHYL 2-{[3-(2,4,6- TRIMETHYL BENZYL)-1-PHENYLSULFONYL-1H-INDOL-2- YL]METHYL-IDENE}PROPANEDIOATE AGAINST HYPERTENSIVE PROTEIN AS A POTENTIAL TARGET

SARAVANAN B.* AKHILESH UPGADE ANUSHA BHASKAR MANIVANNAN V.

Centre for Research & Development, PRIST University Thanjavur, Tamilnadu, India. Email: saran197209@gmail.com

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ABSTRACT

High blood pressure and cardiac failure is the most common ailments which directly affect the health economy of the country. An estimated 68 million people are suffering from hypertension which leads to cardiac arrest and kidney malfunction. This pilot study is designed in which Diethyl 2-{[3-(2,4,6-trimethylbenzyl)-1-phenylsulfonyl-1H-indol-2-yl]methylidene}propanedioate an indole based compound was synthesized in our laboratory, and was studied for antihypertensive activity using a renin protein which plays a major role in high blood pressure. Docking studies were performed on structure activity relationship to show the synthesized compound having a renin inhibitory activity which has the promising results.

Keywords: renin, high BP, Diethyl 2-{[3-(2, 4, 6-trimethylbenzyl)-1-phenylsulfonyl-1H-indol-2-yl]methylidene}propanedioate,molecular docking, hypertension, targeted drug delivery

INTRODUCTION

Hypertension is a major risk factor for stroke and myocardial infarction and is interlinked to chronic kidney disorders[1] Hypertension is mainly due to low-renin hypertensive and nonmodulators. Aldosterone, the principal human mineral corticoid. The hormone is the product of a series of biosynthetic reactions are recognized as playing a significant role in cardiovascular morbidity [2,3], High BP remains an essential and critical unsolved problem that affects 10 in 15 adult human being [3].Current evidence suggests that excessive circulating levels of aldosterone cause left ventricular hypertrophy, obviously affecting the cardiac volume. Secondary hyperaldosteronism associated with cardiac failure, suggests a major role in cardiovascular injury due to surplus production of the aldosterone. This concept of aldosterone-mediated cardiac injury has led to studies that have explored the use of selective aldosterone receptor antagonists in heart patients [4] Angiotensin II is a potent constrictor of all blood vessels. It acts on the smooth muscle and, increases the power of resistance exerted by these arteries to the heart which increases heart load and it functions vigorously, ultimately resulting in high BP. An over-active renin-angiotensin system causes vasoconstriction and retention of sodium and water which leads to hypertension. Therefore, renin inhibitors can be used for the treatment of hypertension [5]

MATERIALS AND METHODS

Synthesis of the compounds

To a solution of diethyl-2-((3-(bromo methyl)-1-(phenyl sulfonyl) -1H-indol-2-yl)methylene)malonate (0.3 g, 0.57 mmol) in dry 1,2-dichloroethane (15 ml), anhydrous ZnBr (0.25 g, 1.11 mmol) and mesitylene (0.19 ml, 1.41 mmol) were added. It was then refluxed for 4 h under N_2 atmosphere. The solvent was removed and the residue was quenched with ice-water (50 ml) containing 1 ml of conc. HCl, extracted with chloroform (2 × 10 ml) and dried (Na_2SO_4). Removal of solvent followed by flash column chromatographic purification (n-hexane/ethyl acetate 98:2) led to the isolation of product as colourless crystal.

X ray diffraction analysis

Data was collected on a Bruker Kappa APEX II diffractometer using ω and φ scan mode with the range reflections 2.4 to 24.3° using MoKα radiation. A total of 26660 reflections were collected, resulting in 7349 independent reflections of which 3201 had I > 2σ(I). The intensities were corrected for Lorentz and polarization effects. The structure was solved by direct methods using SHELX 97 program and final R-factor was 0.042[6,7]

Computational molecular docking studies

Crystallographic structures of protein 2IKO were retrieved from the RCSB database with PDB ID 2IKO.Computational analysis was done to compute ligand protein binding affinity of the compound. Docking calculations were carried out using Docking Server [8]. The MMFF94 force field [*9*] was used for energy minimization of ligand using Docking Server. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged and rotatable bonds were defined.

Docking calculations were carried out on rennin angiotensin II protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools. Affinity (grid) maps of 20×20×20 Å grid points and 0.375 Å spacing were generated using the Autogrid program [10]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms respectively.
Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method [*11*]. Initial position, orientation, and torsions of the ligand molecules were set randomly. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.[16]

RESULTS

X ray Diffraction analysis

CRYSTAL AND EXPERIMENTAL DATA

EMPIRICAL FORMULA	$C_{32}H_{33}NO_6S$
TEMPERATURE	295 K
FORMULA WEIGHT	559.65
WAVELENGTH	0.71073 Å
CRYSTAL SYSTEM	Triclinic

SPACE GROUP	*P1*
UNIT CELL DIMENSIONS (Å)	a= 8.5103 (4) A° b = 8.9540 (4) A° c = 19.6546 (10) A° α= 78.456 (3) β= 87.236 (4) ° γ=86.736(3)
VOLUME Å^3	1463.99 (12)Å^3
Z	2
CALCULATED DENSITY	1.270mg/m^3
ABSORPTION COEFFICIENT	0.16mm^{-1}
REFINEMENT METHOD	Full matrix
F(000)	592
CRYSTAL SIZE	0.22X0.18X0.16 mm
θ – RANGE FOR DATA COLLECTION	2.4 to 24.3°
GOODNESS-OF-FIT ON F^2	1.03
REFLECTIONS COLLECTED/UNIQUE	26660/4328
R-FACTOR	0.042

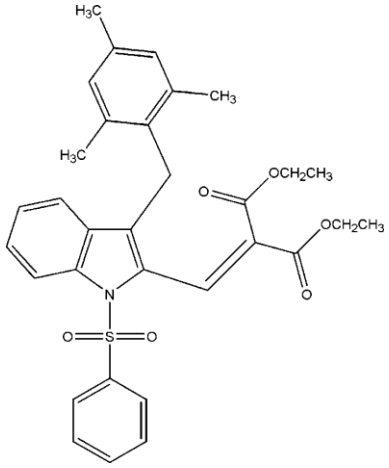

Fig 1: Ligand (Diethyl 2-{[3-(2,4,6-trimethylbenzyl)-1-phenylsulfonyl-1H-indol-2-yl]methyl-idene}propanedioate compound)

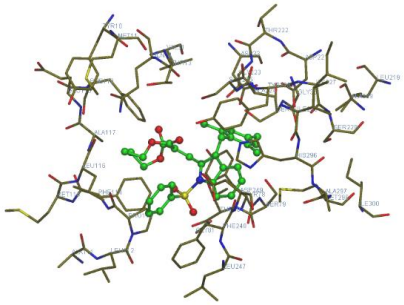

Figure2: Lignad residue interaction Molecular docking Studies

The structures of the ligand Diethyl 2-{[3-(2,4,6-trimethylbenzyl)-1-phenylsulfonyl-1H-indol-2-yl]methyl-idene}propanedioate compound were drawn using tool Chembiodraw 11.0. and converted into PDB format using molecular conversion tool VCC lab online server[12,13]. The crystallographic structures of renin were retrieved from the RCSB database with PDB ID 2IKO was docked with ligand using Auto dock 4.0 with authenticated server.

Lamarckian genetic algorithm is clearly depicted in the docking (Fig.2, 3,4) The interaction shows efficient docked score viz.,-6.51 kcal/mol which is considered as a satisfactory score in ligand-protein interactions (table 1). Hydrogen bonding in docking plays a significant role in interaction studies which is described in Fig. 5 and table 2, The strong interaction between particular residues with a specific bond length, and hydrophobic interaction shown (table 3)

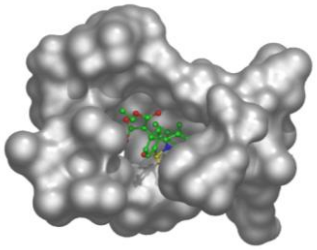

Figure3: Pocket binding lignad

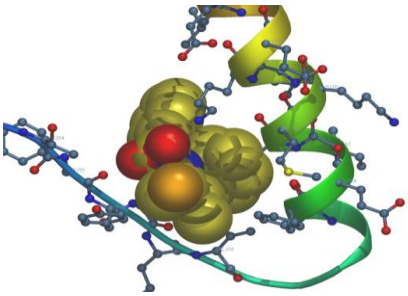

Figure4: Docked image showing protein and ligand interaction

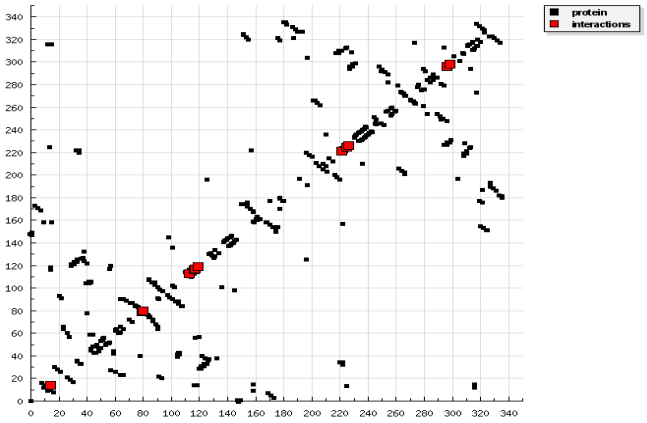

Figure5: Hydrogen Binding Plot

Table1: **Energy values of docked Diethyl 2-{[3-(2,4,6-trimethylbenzyl)-1phenylsulfonyl-1H-indol- 2yl]methyl-idene}propanedioate compound ligand with renin**

Rank	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	vdW + Hbond + desolv Energy	Electrostatic Energy	Total Intermolec. Energy	Frequency	Interact. Surface
1.	-6.70 kcal/mol	12.24 uM	-9.88 kcal/mol	-0.04 kcal/mol	-9.93 kcal/mol	50%	1254.348

Table2: Binding sites of the pocket in proteins target interacting residues

14:00	GLN
79:00:00	SER
80:00:00	THR
113:00:00	PRO
116:00:00	LEU
117:00:00	ALA
119:00:00	PHE
221:00:00	ASP
225:00:00	SER
226:00:00	TYR
296:00:00	HIS
298:00:00	MET

Table 3: possible interaction of the polar and non polar amino acid

polar	hydrophobic	other
TYR226 (-1.3471)	HIS296 (-1.1371)	THR80 (-1.5765)
GLN14 (-0.7291)	LEU116 (-0.8166)	SER225 (-0.8069)
	PRO113 (-0.7217)	SER79 (-0.3915)
	ALA117 (-0.1862)	PHE119 (-0.3196)
	MET298 (-0.1003)	ASP221 (-0.1766)

DISCUSSION

Renin-angiotensin system (RAS) is one of the targets in case of the hypertension related diseases because recent studies state that there is the increase in the obesity which leads to the hypertension. [(14)] The renin enzyme circulates in the blood stream and breaks down (hydrolyzes) angiotensinogen a peptide secreted from the liver into the peptide angiotensin I. Angiotensin I is further cleaved in the lungs by endothelial-bound angiotensin-converting enzyme(ACE) into angiotensin II, this angiotensin II plays a role of hormone and more vasoconstion occurs in smooth muscles. Ultimately the heart tries to overcome this excess flow and vigorously works. This mechanism leads to high blood pressure. Hence the ACE inhibitors are the more point of attraction as a target in therapeutics. Various RAS inhibitors available in market directly block the angiotensin II receptor which develops the hypotension. [15]

This study targets the angiotensin producer system called rennin so as to avoid the production of angiotensin II by the conversion using ACE, hence we are trying to stop the activity prior to the synthesis of angiotensin II using indole compound. Indole exhibits the hypertensive properties but no previous work has been reported. Hence our newly synthesized compound was subjected to *Insilico* docking studies with respective target.

The compound like indoles are the potent inhibitors used according to the literature cited. In this study a compound synthesized using standards and structure was resolved by universal method of X- ray crystallography to understand the intermolecular bonding. No doubt the crystallographic data plays a key role in predicting the compound.

According to the docking score, and other features revealed by the compound in this study such as binding energy, interaction score and hydrogen bonding with residues, the compound must have a drug like behavior and binding capacity, and further ADMET studies are strongly recommended which leads to clinical trials.

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