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Research Article

Computational Insights on Novel Rock-1 Inhibitors from Marine Compounds Towards Anti-Glaucoma Therapeutics

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ABSTRACT

Glaucoma is a severe condition that causes damage to the eyes and is characterized by an excavated cupping appearance of the optic nerve with progressive thinning of retinal nerve fibre layer tissue and loss of vision. The disease affects millions of individual and is a leading cause of irreversible blindness. Intraocular pressure (IOP) has been recognized as a key risk factor for glaucoma. Among the Rho kinase activator, ROCK-1 (Rho-associated protein kinase-1) is reported as a key target in glaucoma therapeutics. ROCK's are a class of serine/threonine kinases and is involved in the regulation of smooth muscle contraction and specific ROCK-1 inhibitors can decrease IOP. The aim of this computational study is to screen and identify novel ROCK-1 inhibitors from marine compounds. The molecular docking protocol is followed with the three-dimensional structure of human ROCK-1 and the selected ligand dataset of marine compounds. The docking results was evaluated by least binding energy and hydrogen bonds interactions that confirmed the effective inhibition of caulerpenyne. Molecular dynamics studies also confirmed the stability of ROCK-1 caulerpenyne complex. Our study will decipher the structural inhibition mechanism with best marine compound caulerpenyne from Caulerpa sp. and aid in the development of novel ROCK-1inhibitor that can act as anti-glaucoma agents.

Keywords: ROCK-1, Glaucoma, Molecular docking and Binding energy.

INTRODUCTION

Glaucoma is an ocular neuropathic disease, in which at least one eye has accelerated ganglion cell death characterized by excavated cupping appearance of the optic nerve with progressive thinning of retinal nerve fiber layer tissue and corresponding subsequent loss of visual field. As the leading cause of irreversible blindness worldwide, this disease has affected more than 60.5 million individuals in 2010, an estimate extrapolated from multiple population-based surveys, and is projected to reach 79.6 million by the year 2020[1,2]. Historically, intraocular pressure (IOP) has been recognized as the primary modifiable risk factor and is now accepted as a continuous, fluctuating variable. The goal of concurrent therapy is to reduce IOP to a range that preserves structural and functional testing results[2-4]. IOP can be lowered by affecting inflow and outflow via pharmacologic agents (five current classes include prostaglandin analogs, beta-blockers, carbonic anhydrase inhibitors, sympathomimetics, and miotics), laser trabeculoplasty, and incisional surgery, which can be divided into standard filtration surgery or various newer minimally invasive procedures[5,6]. The Rho family consists of three small guanosine triphosphate (GTP)-binding proteins (RhoA, RhoB, RhoC), which Rho kinase inhibitors and glaucoma regulate aspects of cell shape, motility, proliferation, and apoptosis throughout the body[7]. On activation by binding to GTP, Rho activates its effector molecules (Rho kinase ROCK1 and 2), which downstream signal molecules to polymerize actin fibers in the cardiovascular, pulmonary, and renal systems[8,9]. Rho GTPases were first hypothesized to function in aqueous humor outflow in 2001 because of their expression in TM, with the ability to induce calcium sensitization in smooth muscle contraction in rabbit eyes[10,11]. Since then, significantly elevated levels of RhoA have been detected by immunostaining in the optic nerve head of glaucomatous eyes compared with age-matched controls, reinforcing the association of Rho proteins and glaucoma pathophysiology[12]. ROCKs are serine/threonine kinases that regulate smooth muscle contraction in which selective ROCK inhibitors could increase aqueous humor drainage through the TM, leading to a decrease in IOP[13]. In mammals, ROCKs exist as two isoforms: ROCK1, which is located on chromosome 18 and contains 1,354 amino acids and ROCK2, which is located on chromosome 12 and encodes a 1,388-amino acid product[14,15]. Overall, ROCK1 and ROCK2 share 65% homology in amino acid sequence with 92% homology in their kinase domains. In humans, ROCK1 and ROCK2 tend to be expressed in the majority of tissues, including human TM and ciliary muscle cells[14]. Structurally, ROCKs are composed of three major domains: An N-terminal kinase domain that phosphorylates protein targets, a C-terminal autoinhibitory domain that limits kinase activity via intramolecular interactions, and a coiled-coil Rho-binding domain that appears to facilitate the switch from the inactive to active

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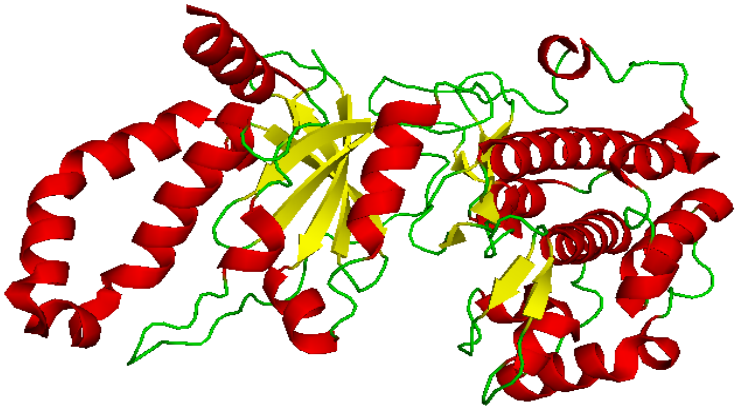
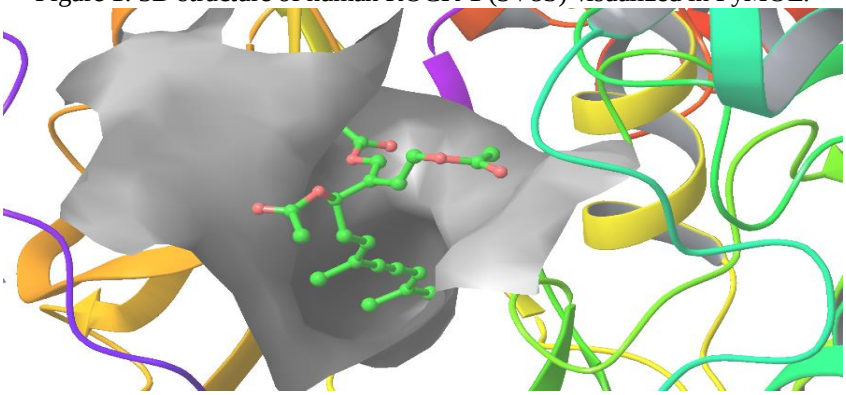


Figure 1: 3D structure of human ROCK-1 (3V8S) visualized in PyMOL.



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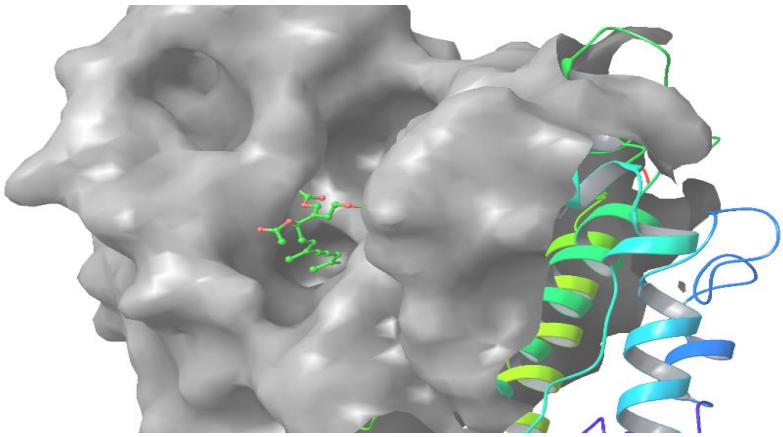

2b

Figure 2a & 2b: Docked complex of ROCK lead compound.

Table 1: Virtual screening result of ligand dataset from AutoDock Vina.

Compound	Binding affinity (kcal/mol)	Conformation
Caulerpenyne	-9.5	4
Coibamide A	-8.2	1
Scytonemin	-6.6	2
Apratoxin A	-6.5	9
Stypoldione	-5.9	10
Cyptophycin 1	-5.0	2

conformation[16,17]. On binding to Rho, the catalytic activity of ROCKs is moderately enhanced, although activation of the kinase can also occur in response to lipids such as arachidonic acid[18].

MATERIALS AND METHODS

Dataset

The experimental 3D structure of human Rho-Associated Protein Kinase 1 (ROCK-1) with PDB code: 38VS was used for the computational analysis. The ROCK-1 contains following amino acids Gly85, Gly88, Glu89, Val90, Ala103, Glu154, Tyr155, Met156, Leu205 and Asp216 as active site residues. The co-ordinates of the ROCK-1 binding pocket were X- 0.73, Y- 2.4 and Z- 6.2 Å.

Virtual screening

The software AutoDock Vina in PyMOL plugin was used for the identification of hits. The input of both protein and ligands were saved in PDB format and used for the docking procedures. To have large search space for the docking, the

Table 2: Re-docking result of top hits from AutoDock.

Compound	Binding energy, kcal/mol	Hydrogen bonds	Residues involved
Caulerpenyne	-8.22	5	Gly88, Glu89, Val90, Leu205, Asp216
Coibamide A	-6.58	2	Asp200, Glu100

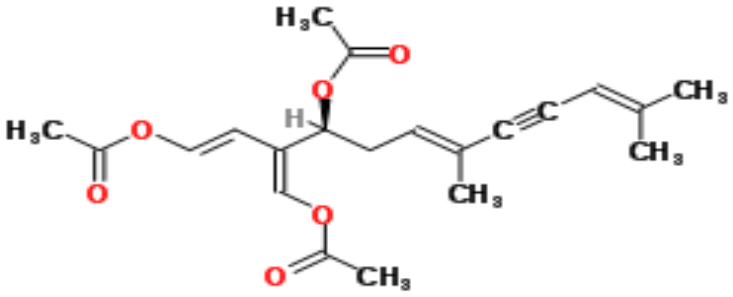

Figure 3: Chemical structures of top lead candidate caulerpenyne.

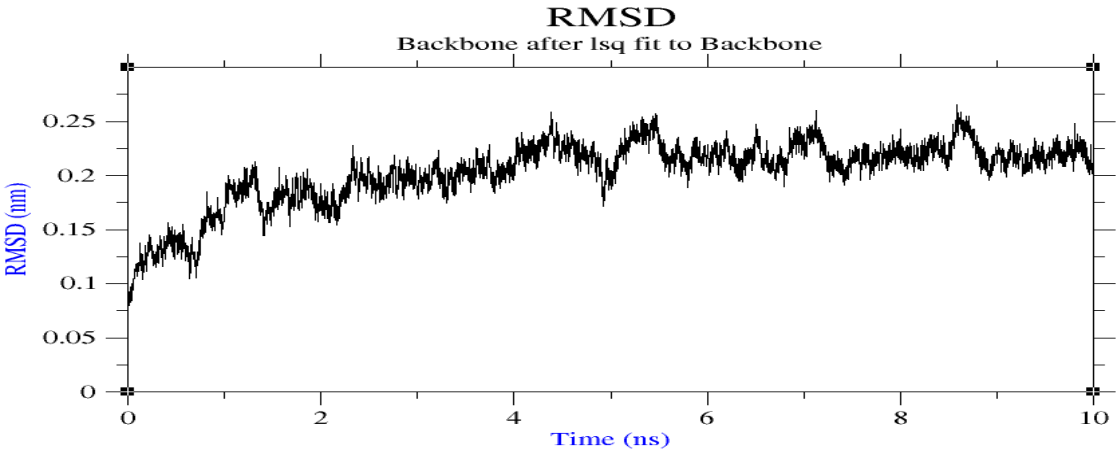

Figure 4: RMSD of protein backbone atoms.

volume of the grid box was fixed to 27000 Å. The docking grid box was constructed with spacing 0.375 Å which targets the active binding pocket of ROCK-1. The molecular docking run was set to 10. The scoring function of AutoDock Vina was categorized by binding affinity expressed in kcal/mol and ligands with least binding affinity were identified as top hits[19].

Molecular docking

The re-docking on hits was performed in software AutoDock 4.2 in PyMOL plugin for the identification of lead candidates. All the nonpolar hydrogens were merged and water molecules were removed. For the molecular docking, same grid parameters were used as in Vina. Using the genetic algorithm, 10 possible binding conformations were generated in AutoDock. A default protocol was applied in a genetic algorithm, with a population size of 150 randomly placed individuals, the maximum number of 2.5 x 10^5 energy evaluations, the maximum number of 2.7 x 10^4 generations, gene mutation rate of 0.02 and crossover rate of 0.8 were used. The docking results were evaluated by based on the number of H-bond interactions between protein-ligand in docked complex. H-bond interactions were analyzed in PyMOL[20].

Molecular dynamics (MD) simulations

MD simulations were performed in GROMACS 4.5.6 for refinement of binding affinity, analysis the stability of the complex and evaluate the conformational changes in ROCK-1 after ligand binding. The force field GROMOS96 43a1 was used for all simulations and the energy minimization of the protein-caulerpenyne complex was performed with the steepest algorithm. Initially, the topology of caulerpenyne from the docked complex was generated using PRODRG server and partial charges were added for the ligand preparation. After topology generation, the solvation of complex was performed in a dynamic system with cubic box size 1.0 nm and distance between periodic images with a minimum of 2.0 nm. The particular water model spc216 was used for the aqueous environment in the dynamic system. The neutralization of the system was done by adding six sodium ions and periodic boundary conditions were applied in all directions. The cutoff range was set to 14 Å and 12 Å for van der Waals interactions and electrostatic interactions respectively. All bond lengths of the protein were constrained by LINCS algorithm and geometry of water molecules were constrained by SETTLE algorithm. Initial simulations well equilibrated the complex in two phases namely NVT and NPT. In the case of NVT, the complex was simulated at 300 K and with a coupling constant of 0.1 ps for duration 100 ps. After NVT, the complex was equilibrated with a constant pressure of 1 bar was employed with a coupling constant of 5 ps for duration 100 ps. Berendsen coupling scheme was applied for both NVT and NPT equilibration ensembles. Finally, the MD

production run was performed for duration 10 ns and all MD trajectories were analyzed[21].

Analysis

g_rms to analyze the structural deviation through RMSD plot. g_hbond to evaluate inter-hydrogen bond interactions between two groups by NH plot. All plots from MD trajectories were plotted using Xmgrace.

RESULTS AND DISCUSSION

The three dimensional structure of human ROCK-1contains well-defined helices in red color, sheets in yellow color and loops in the green color (Fig. 1). Totally six ligands from marine source were subjected to phase-1 virtual screening in AutoDock Vina. The results showed the top two hits with a least binding affinity that confirmed the strong binding in the respective active site of ROCK-1. The range of binding affinity -9.5 to -5 kcal/mol and the corresponding best conformation of each hits were mentioned in Table1. For the two top hits, the ligands were subjected to re-docking in AutoDock. The re-docking results showed the top two hits with least binding energy that confirmed the strong binding and its binding mode in the respective active site of ROCK-1. The range of binding energy of both hits was in -8.22 and -6.58 respectively. Top hit caulerpenyne showed five hydrogen bonds and the corresponding critical residues and identified as best lead compound in Table 2 (Fig 2A & 2B). Other hit didn't interact with active site residues of ROCK-1. The 2D chemical structure of lead compound caulerpenyne was shown in Fig.3. MD simulations were performed for the ROCK-1-caulerpenyne complex with the binding pose from the docked complex. After running 10 ns simulations, the trajectories were analyzed based on RMSD and inter-hydrogen bonds. Root mean square deviation (RMSD) was evaluated for the convergence of the protein structure towards an equilibrium state after lead binding. From protein RMSD plot based on backbone atoms, Caulerpenyne complex showed till 2ns the structure was equilibrated well and started to converge with RMSD range near to 0.25 nm. The RMSD value of caulerpenyne complex was a smaller amount and clearly explained the less structural deviation after ligand binding (Fig. 4). Inter-hydrogen bond interactions between protein and ligands were evaluated for the ROCK-1-caulerpenyne complexes. NH plot results showed a range of two to six hydrogen bond interactions were observed throughout 10 ns simulation and a maximum of six hydrogen bonds. NH analysis confirmed strong inhibition of ROCK-1 by caulerpenyne in dynamic system same as docking results inferred with five hydrogen bonds. The inter-hydrogen bond interactions pattern suggested the plausible mode of strong binding of caulerpenyne with ROCK-1 favored the inhibition mechanism.

CONCLUSION

Marine compounds are acts as current active agents in the treatment of many dreadful diseases. With the use of rational inhibitor design, novel ROCK-1 inhibitors are identified from the marine compounds. Two steps of docking were applied and screened the top compound from the dataset based on least binding energy and hydrogen bond interactions. The active site residues were blocked with caulerpenyne that effectively inhibit the ROCK-1. The stability of the ROCK-1-caulerpenyne complex evaluated the strong inhibition in a dynamic system. In conclusion, our computational findings suggested caulerpenyne as a novel ROCK-1 inhibitor for glaucoma treatment.

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