Computational Biology and Chemistry 67 (2017) 84–91

Contents lists available at ScienceDirect

Computational Biology and Chemistry

journal homepage: www.elsevier.com/locate/compbiolchem

Research Article

The *in silico* identification of small molecules for protein-protein interaction inhibition in AKAP-Lbc–RhoA signaling complex

Asifullah Khan[a,b,*], Mehwish Munir[a], Sara Aiman[a], Abdul Wadood[a], Arif-ullah Khan[c]

[a] Department of Biochemistry, Abdul Wali Khan University Mardan, Mardan, 23200, Pakistan
[b] Chinese Academy of Sciences (CAS) Key Laboratory of Computational Biology, CAS-MPG Partner Institute for Computational Biology (PICB), Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China
[c] Ripha Institute of Pharmaceutical Sciences, Ripha International University Islamabad, Pakistan

ARTICLE INFO

Article history:
Received 4 July 2016
Received in revised form 22 December 2016
Accepted 30 December 2016
Available online 31 December 2016

Keywords:
AKAP-Lbc
Protein-protein interaction inhibitors
Molecular docking
Drug designing

ABSTRACT

The rational design of small molecules that mimic key residues at the interface of interacting proteins can be a successful approach to target certain biological signaling cascades causing pathophysiological outcome. The A-Kinase Anchoring Protein, i.e. AKAP-Lbc, catalyses nucleotide exchange on RhoA and is involved in cardiac repolarization. The oncogenic AKAP-Lbc induces the RhoA GTPase hyperactivity and aberrantly amplifies the signaling pathway leading to hypertrophic cardiomyocytes. We took advantage of the AKAP-Lbc–RhoA complex crystal structure to design *in silico* small molecules predicted to inhibit the associated pathological signaling cascade. We adopted the strategies of pharmacophore building, virtual screening and molecular docking to identify the small molecules capable to target AKAP-Lbc and RhoA interactions. The pharmacophore model based virtual screening unveils two lead compounds from the TIMBAL database of small molecules modulating the targeted protein-protein interactions. The molecular docking analysis revealed the lead compounds' potentialities to establish the essential chemical interactions with the key interactive residues of the complex. These features provided a road map for designing additional potent chemical derivatives and fragments of the original lead compounds to perturb the AKAP-Lbc and RhoA interactions. Experimental validations may elucidate the therapeutic potential of these lead chemical scaffolds to deal with aberrant AKAP-Lbc signaling based cardiac hypertrophy.

1. Introduction

Modulation of biological interactions by small molecules holds potential in pharmaceutical research to identify and investigate novel therapies for various human diseases (Cardinale et al., 2010). The protein-protein interaction (PPI) control processes is involved in both normal and pathological pathways, including signal transduction, cell adhesion, cellular proliferation, differentiation, programmed cell death, and cytoskeleton structure (Huart et al., 2012). Among these, the process of cellular transduction contains many proteins as privileged drug targets (e.g., G protein-coupled receptors and kinases) whose activation/inhibition by drug-like compounds is likely to correct or reverse the pathological states (Groner et al., 2012).

AKAP-Lbc (also known as AKAP13) is a member of the A-kinase-anchoring proteins that have a common feature of binding the PKA (protein kinase A) regulatory subunit and control its localization (Diviani et al., 2001). Several AKAPs are found to be expressed in cardiac tissue and contribute to cardiac repolarization and remodeling during stress responses. Cardiac diseases such as rhythm disorder, long-QT syndrome, cardiac hypertrophy and heart failure are associated with mutations and polymorphisms in proteins of this family (Soni et al., 2014; Chen et al., 2007). AKAP-Lbc contains a conserved RhoGEF (Rho GTPase guanine-nucleotide-exchange factor) domain, which activate the Rho GTPases by catalyzing nucleotide exchange and promote Rho–GTP formation for downstream cellular signaling pathways (Olson et al., 1997). The AKAP-Lbc protein is also known as a mediator of cardiac hypertrophy through Rho GTPase activity and studies reported

* Corresponding author at: Department of Biochemistry, Abdul Wali Khan University Mardan, Mardan, 23200, Pakistan.
E-mail addresses: asif@awkum.edu.pk, asifullah111@gmail.com (A. Khan).

http://dx.doi.org/10.1016/j.compbiolchem.2016.12.014
1476-9271/

two-fold increase level of AKAP-Lbc in patients with hypertrophic cardiomyopathy. In addition, a truncated form of AKAP-Lbc has been identified as an oncoprotein that causes overactivation of RhoGEF activity (Rubino et al., 1998; Zheng et al., 1995; Schwartz et al., 1996).

Drug discovery projects with a known target structure can be initiated by structure-based pharmacophore modeling and high-throughput screening of databases of drug-like compounds. These efforts predict novel and potent drug-like molecules capable to inhibit the targets (Lyne, 2002). Here we pursued pharmacophore modeling and virtual screening approaches to identify small drug-like molecules that putatively inhibit RhoGEF activity of AKAP-Lbc by modulating the molecular interactions between AKAP-Lbc and RhoA proteins. Though inhibition of AKAP-mediated PKA localization has attracted much attention as reviewed by Calejo and Tasken (2015). Nonetheless, the dysfunction of AKAP-Lbc based Rho GTPase activation may also be implicated in serious genetic disorders and diseases. The small molecules and scaffolds addressed in this study will be worthwhile in drug discovery for cardiac hypertrophy, pulmonary arterial hypertension and certain cancers associated with AKAP-Lbc mediated Rho GTPase hyperactivity.

2. Material and methods

2.1. AKAP-Lbc–Rho complex X-ray structure retrieval

The AKAP-Lbc–Rho complex X-ray crystal structure coordinates with resolution of 2.1 Å was retrieved from Protein Data Bank with PDB ID, i.e. 4D0N.

2.2. Energy minimization and structure protonation

The complex X-ray structure energy-minimization and 3D protonation was performed with the Molecular operating environment (MOE) package to keep the complex structure ready for downstream analyses. This add in the right geometric orientation of the hydrogen atoms present in the enzyme as well as in the environment. The calculation was performed using the MMFF94x force field to parameterize a system comprise of large proteins and small organic molecules. Once a proper orientation of the hydrogen atoms in the enzyme and environment are obtained, the complex structure then kept under the PDB extension to maintain proper connectivity and guidance.

2.3. Structure based pharmacophore model generation

LigandScout 3.1 (Wolber and Langer, 2005) was used to generate a pharmacophore model. LigandScout is a recommended tool for structure based pharmacophore model generation from a biological complex data, and allows fine-tuning to build selective pharmacophoric screening filters for a specific target. It offers a wide range of chemical features including hydrogen bonding vectors, chargeable groups, aromatic plane and aromatic-positive ionizable interactions. An advance alignment algorithm of LignadScout allows overlying pharmacophore and molecule so that the common binding modes may be detected and shared chemical features can interpolate. During pharmacophore model generation, the critical residues of AKAP-Lbc been reported to play a major role in building interactions with RhoA protein i.e., Val2159 and Leu2160 were selected. The pharmacophore model was checked and satisfied for all essential chemical features required for virtual screening.

2.4. TIMBAL library generation and screening

TIMBAL-v2 is a dedicated database containing 6896 distinct small molecules of molecular weight <1200 Da that potentially modulate PPI (Higueruelo et al., 2013). The compounds library was prepared for pharmacophore-based virtual screening using the internal library preparation features of the LigandScout.

2.5. Molecular docking

The docking algorithm of the Molecular Operating Environment (MOE) used to dock the selected lead compounds within the AKAP-Lbc and RhoA binding pockets. The site finder implemented in MOE used for prediction a nearby pocket or active site able to anchor a small molecule. The MOE provides information to obtain minimum energy structure. Ten different conformations of protein ligand interactions were generated and the top ranked pose was selected

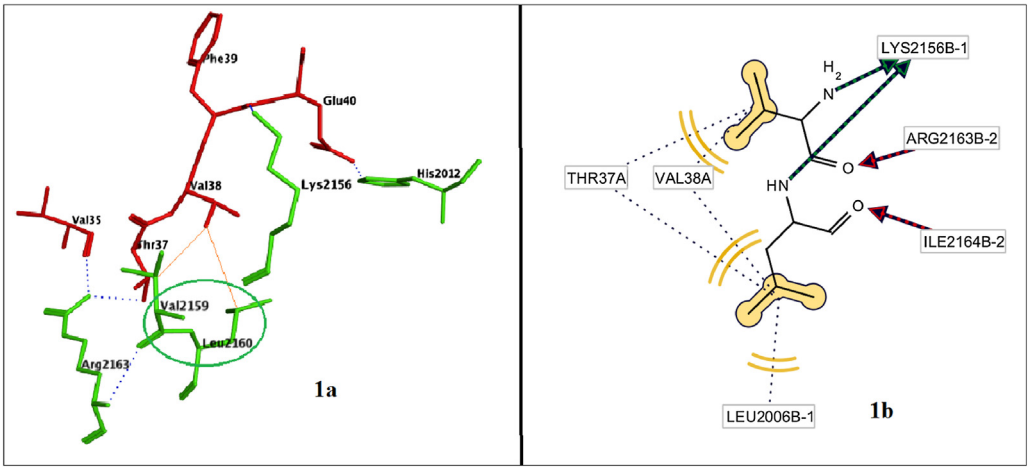

Fig. 1. 1a. The 3D diagram of the prominent binding residues of RhoA and AKAP-Lbc. The AKAP-Lbc residues are shown in green while RhoA residues are shown in red color. The blue dotted line indicates the hydrogen bonds while the straight line represents hydrophobic interactions. The encircled Leu2160 and Val2159 residues are selected for pharmacophore model generation. 1b. The two dimensional view of pharmacophore model. The red dotted arrows indicate the hydrogen bond acceptor and the green dotted arrows indicate the hydrogen bond donor features. The yellow spheres representing the hydrophobic features in the pharmacophore model. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)