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Identification of EPAC (Exchange Protein Activated by cAMP) bioinformatically as a potential signalling biomarker in Cardiovascular Disease (CVD) and its molecular docking by a lead molecule

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Abstract:
The present work delineates the combinatorial approach of firstly, creation of a centralized data-set comprising signalling proteins identified on the basis of altered expression, such as over-expression or repression of a set of signalling protein(s) leading to the cause of the disease, which is based on published reports screened through Pubmed and secondly, in the in silico creation of novel lead (drug) molecules and docking of identified signalling biomarkers using such drugs to investigate possibility of their future application in the model systems eventually. EPAC (Exchange Protein Activated by cAMP) emerges as a signalling biomarker in cases studied presently. Brefeldin, the known inhibitor of EPAC, though the mechanism yet unexplored, has been the molecule used as the pharmacophore for creation of lead drug molecule. Various modifications have been incorporated into the pharmacophore to increase the hydrophobic interactions for increasing the binding efficiency of the generated lead molecule. Side-chain modifications of the pharmacophore and refinement of data through firedock upon docking of EPAC with the modified pharmacophore yielded best results on the bases of atomic contact energy, van der Waal and partial electrostatic interactions as well as additional estimations of the binding free energy. Modifications of CH3 at C15 with COOH and H at C2 with OH in brefeldin showed the best docking results on the basis of protein-drug interaction parameters. The present work provides a clue in rational design of EPAC inhibitors which could be developed as drug lead in combating CVD.

Keywords: CVD, EPAC, Brefeldin, Pharmacophore, Inhibitors

Background:
Cardiovascular disease (CVD) is known as the leading cause of human deaths the world over. Strategies encompassing drug docking using genome and proteome interfaces and wet lab-based drug applications on animal models and cells in vitro are underway in most of the laboratories as strategies to control the disease. However, mortality rate of the affected human population due to CVD is still high. Consequently, a better management of the disease is required. Given the importance of cell signalling in the basic understanding of a pathway and its involvement in the exploration of the disease, its applicability in the identification of CVD hot-spots could precisely be pinpointed by identification of novel protein targets and creating suitable drug leads to modulate their expression. The disease management can be achieved better if there is availability of a central repository of data comprising molecules which are critical to the cause of such diseases. The created data-set will provide ease in the planning and execution of experimental strategy for systematic testing and prioritization of candidate molecules for drug discovery and allow in silico creation of better lead molecules for targeting the disease comprehensively. EPAC is a guanine nucleotide exchange factor catalysing exchange of GDP for GTP and has been pivotal in controlling a number of cellular processes through sensing the cAMP levels in cells involved in focal adhesion formation, migration, apoptosis and axon growth and guidance **[1, 2, 3]**. The factors contributing to abnormal cAMP levels in the cells range from hyperactive receptors (metabotropic receptors) **[4]**, aberrant expression of polycystin, which is crucial in the cause of autosomal dominant polycystic kidney disease (ADPKD) **[5]** and various cyclic nucleotide phosphodiesterases (PDEs), of which 11 families are known and which catalyze the hydrolysis of cAMP **[6]**.

In various Pubmed based data analysis criteria through a bioinformatic approach carried out presently, EPAC emerged out to be a biomarker in some CVD cases of myrocardial hypertrophy **[7, 8]** and hence had been the target for lead molecule generation. Brefeldin, initially isolated as an anti-viral antibiotic and a known drug for activating a GTPase of a GEF called Arf1p, which is involved in the formation of transport vesicles by recruiting coat proteins to intracellular membranes, was employed for generation of the pharmacophore.

Materials & Methodology:
There is a vast body of literature available in PubMed on the given subject. Refinement in the searches were made by specific keywords, which make the information more relevant to our requirement **(Figure 1)**. Data-sets comprising Molecular Interaction, Receptor(s) (membrane-bound) involved, Critical Interaction leading to over-expression or repression and the PubMed reference link (PMID) were created on the excel sheet. The biomarker was identified using the criteria of catalysed molecule and its case specific occurrence manner. Pharmacophore was generated from the reported drugs for the identified biomarker and side chain modifications were made based on logistics worked out on the basis of the existing interactions of the natural ligand for the biomarker (protein). For docking and refinement, online free wares such as Patchdock **[9]** and Firedock **[10, 11, 12]** were used, respectively. For highlighting interactions, Ligplot **[13]** was used as a tool.

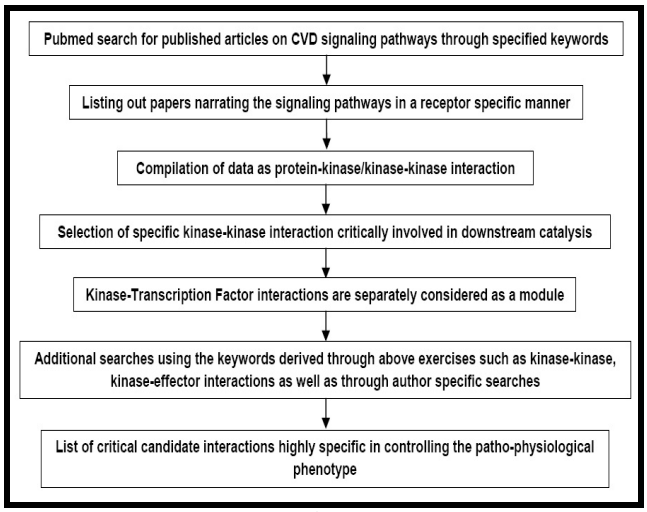

Figure 1: Data curation protocol for cataloguing the signaling pathway molecules

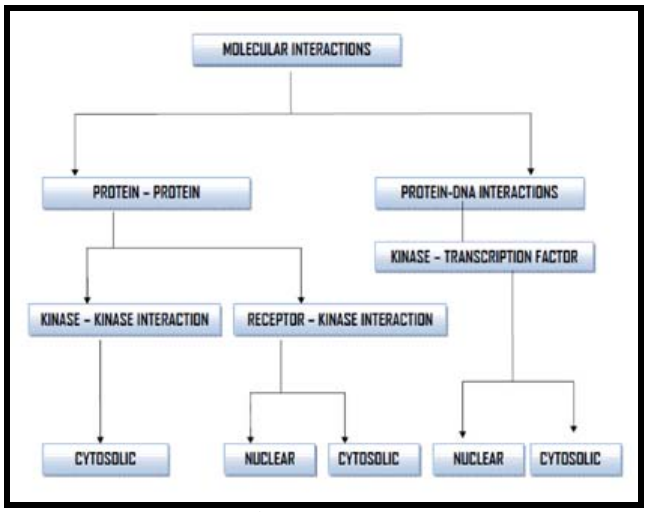

Figure 2: The criteria adopted for curating the signalling molecules.

Results and Discussion:

Data Annotation criteria adopted for cataloguing the biomarker molecules:

A search in the Pubmed through the keyword "Signal transduction and Cardiovascular disease" typically fetches over 13000 references, of which around 4500 belongs to the review category, while the rest belongs to the research articles. A careful reading of majority of such articles yielded data on the above mentioned aspects. **Figure 1 & 2** highlight the criteria adopted for the purpose. All the molecules participating in the signalling network were annotated as Molecular Interaction and the molecules associated directly or indirectly with an effector for upregulation or downregulation of the effector are considered as critical interaction. Of the critical interaction, protein-protein interactions at the cellular and nuclear levels were annotated separately considering the fact that they were crucial in the up-regulation or down-regulation of genes.

Data-set of proteins involved and their downstream effects:

There are over 500 proteins annotated to be involved in molecular interactions in CVD pathways. Of these, 320 protein categories are of G-protein coupled receptor (GPCR) mediated responses, while rest belongs to the category of receptor tyrosine kinase (RTK) mediated and other or indirect receptor-mediated effects. The GPCR-mediated response has been able to culminate its effect primarily on GPCR-response Kinase (GRK) in isoform-specific manner, NFAT and EPAC in the cell line based studies in CVD cases. The RTK mediated response effects in activation of NFkB primarily for CVD cases considered presently. The critical module of protein-protein interaction in the present case envisage that kinase-kinase and kinase-transcription factor (Elk/PAK-NFkB) are important interactions delineating the pathway of signalling process, which leads to activation of the response genes.

Protein-Protein Interaction data-set:

A total of 250 interactions of proteins out of 500 proteins annotated to be involved in CVD pathways. The protein-protein interactions were divided into two modules, such as kinase-kinase/adaptor/GEF interactions and kinase-transcription factor interactions **(Figure 2)**. Of these, there were more than 200 interactions, which belonged to kinase-kinase/adaptor/GEF category and 30 interactions were of kinase-transcription factor category as regards CVD pathways. Of these interactions, the modules considered critical in the sense that they controlled the pathway execution in a specific manner and the alteration in their expression was mainly responsible for cause of CVD.

Protein-transcription factor data-set:

Of the total protein-protein interactions mentioned above, 30 interactions belonged to protein-transcription factor category for CVD. In the CVD related signalling pathway, 12 interactions were mediated upon GPCR activation, while 18 interactions were the outcome of RTK activation.

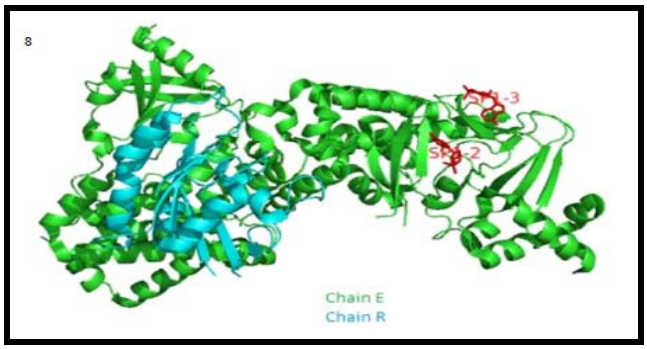

Figure 3: PDB ID: 3CF6, Epac protein (Shown in Green is Epac2) is activated by binding of the second messenger cAMP (Shown in Red,It is an analogue of cAMP) and then act as guanine nucleotide exchange factors for Rap proteins (Shown in Blue,It is Rap1B) **[21]**.

Drug Docking of the Marker Molecule:

In totality 3 molecules emerged as markers of CVD as per the criteria envisaged in the present analysis, which are EPAC, GRK-2 & GRK-5 as well as NFkB. Drug Docking using Patchdock **[9]** to identify the lead drug molecules were performed with EPAC (3CF6) presently, which is a known guanine exchange factor activated by cAMP. Brefeldin A, the currently known natural inhibitor of EPAC **[14]** was used as the template for generation of lead molecule. There is binding of cAMP in the natural EPAC molecule **(Figure 3)**. The topology of 3 CF6 using PDBsum revealed the residues interacting with SP12 E (a natural ligand, cAMP for 3CF6, Fig not shown) and the interactions were refined using Ligplot **[13]**. The interacting atoms or groups available in the binding sites of cAMP, the natural ligand for EPAC, were chosen as reference for incorporating similar modifications in brefeldin (the chosen ligand for the present work) to increase the hydrophobic interactions and increase the binding efficiency of the generated brefeldin lead molecule (BR2). Of the various interactions occurring in the docked interface and scored through Ligplot **[13]**, hydrophobic interactions were prevalent, revealing the stability of the interaction. In all, 12 different modifications were made and BR2 molecule generated by replacement of -CH3 at C15 and -H at C2 with –COOH and –OH, respectively yielded H- bond interactions between BR2 and Ala 407 (E), which in turn induced additional H-bond formation between –OH

at C1 and C7 and Ala 416 and Cys 395, respectively. These interactions induce further stability in the complex by improved hydrophobic interactions **(Figure 4A-D)**.

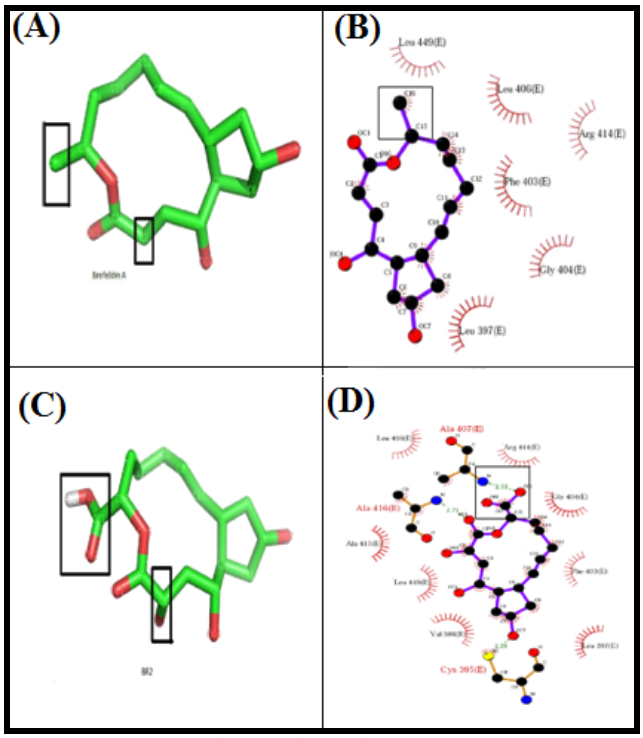

Figure 4(A): Structure of unmodified brefeldin; **4(B):** Interactions between unmodified Brefeldin and residues of Chain E of EPAC. Fewer groups are found to be involved in hydrophobic interaction and no H-bond is observed; **4(C):** Structure of modified brefeldin (BR2); **4(D):** The interactions between BR2 (modified brefeldin) and residues of Chain E of EPAC are shown. Of the 12 different modifications involving C2, C10 and C15 of brefeldin pharmacophore, the replacement of -CH3 at C15 and -H at C2 with – COOH and –OH, respectively yielded H- bond interactions between BR2 and Ala 407 (E) [highlighted as boxed area], which in turn induced additional H-bond formation between –OH at C1 and C7 and Ala 416 and Cys 395, respectively. These interactions induce further stability in the complex by improved hydrophobic interactions.

Molecular modelling approaches in designing and devising new drug leads have been a recent approach in addressing the disease problems. Diseases such as cancer **[15]**, gout **[16]**, cardiac damage **[17]** and various others **[18, 19]** have been the subject of studies on the lines of the present work. An emerging trend is thus approaching towards dealing with the disease bioinformatically on the first hand basis because of the ease and sufficiency of the technology involved in data annotation process. However, the biomarkers identified and the drug leads created need to be validated through targeted approaches by the support of systems medicine approach **[20]**.

Conclusion:

EPAC is the signalling biomarker identified in cardiovascular (CVD) cases through a bioinformatic approach undertaken presently and drug leads were generated using existing online softwares, which could be useful in synthesis of chemical drugs for further analysis in controlling the disease. It is assumed that the present work would contribute towards augmenting the repertoire of knowledge available on formulations of drug for combating CVD.

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References:

[1] Lyle KS *et al. Cell Signal*. 2008 **20**:1104 [PMID: 18346875]
[2] Branham MT *et al. J Biol Chem*. 2009 **284**:24825 [PMID:19546222]
[3] Murray AJ & Shewan DA. *Mol Cell Neurosci*. 2008 **38**:578 [PMID:18583150]
[4] Reid SNM *et al. J Neurosc.* 1996 **16:**7619 [PMID: 8922418]
[5] Grande JP. *Kidney International* 2000 **57:**1770 [PMID:10760114]
[6] Murray F *et al. Am J Physiol. Lung Cell Mol. Physiol.* 2007 **292**: L294 [PMID:16980375]
[7] Métrich M *et al. Cell Signal*. 2010 **22**:1459 [PMID:20576488]
[8] Szabo-Fresnais N *et al. Cell Signal*. 2010 **22**:1143 [PMID:20227492]
[9] Schneidman-Duhovny D *et al. Nucleic Acids Res.* 2005 **33**:363 [PMID:15980490]
[10] Zhang C *et al. J Mol Biol*. 1997 **267**:707 [PMID:9126848]
[11] Kingsford CL *et al. Bioinformatics* 2005 **21**:1028 [PMID:15546935]
[12] Andrusier N *et al. Proteins*. 2007 **69**:139 [PMID: 17598144]
[13] Wallace AC *et al. Protein Eng.* 1995 **8**:127 [PMID:7630882]
[14] Ster J *et al. J Physiol.* 2009 **587**:101 [PMID:19001039]
[15] Geromichalos GD *et al. Anticancer Res.* 2011 **31**:831 [PMID:21498703]
[16] Sathisha KR *et al. Bioorg Med Chem.* 2011 **19**:211 [PMID: 21163661]
[17] Jiang B *et al. BMC Pharmacol.* 2010 **10**:10 [PMID:20735854]
[18] Shanmugam A & Natarajan J. *J Mol Model*. 2011 [PMID: 21491188]
[19] Tasso B *et al. Eur J Med Chem.* 2011 **46**: 2170 [PMID:21459491]
[20] Harsha HC *et al. PLoS Med.* 2009 **6**: e1000046 [PMID: 19360088]
[21] Rehmann H *et al. Nature*. 2008 **455**: 124 [PMID:18660803]

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