Life Science Informatics Publications
Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences
Journal Home page http://www.rjlbpcs.com/
RJLBPCS
ISSN 2454-6348

Original Research Article **DOI: 10.26479/2019.0504.03**

PHOSPHODIESTERASE 3B (PDE3B) A POTENT TARGET IN VASCULAR DISEASE

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ABSTRACT: Cilostazol is a potent phopsphodiesterse 3 inhibitor. A computational study was carried on with the aim of optimizing it's pharmacokinetic properties. A series of congeneric compounds was computationally generated. Series construction was based on computational and experimental data regarding Cilostazol interaction with target molecule and some Cilostazol effect enhancers (enzyme inhibitors). Results show a selective inhibition for PDE3A. Discussion state that potentially by inhibiting PDE3B same therapeutic effect should be obtained. Conclusions illustrate that Cilostazol is a potent PDE3 Ainhibitor.

KEYWORDS: Cilostzol, phosphodiesterase 3 inhibitor, CYPP3A4, CYP2C19.

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1.INTRODUCTION

Pheripherial arterial disease (PAD) [1] is an increasingly spreading condition. Pheripherial vasodilatation together with platelet antiagregation plays an important role in PAD therapy. Vasodilatation is dose dependent on Cilostazol active metabolite witch determines an increase level of cyclic Adenosine Mono Phosphate (cAMP)[2,3]. Cyclic nucleotide phosphodiesterases (PDEs) are important regulators of signal transduction processes mediated by cAMP and cGMP [4,5].Phosphodiesterase 3 (PDE3) exists in two isoforms (PDE3A and PDE3B) and is known to act as cGMP-inhibited cAMP-degrading PDE. Therefore, PDE3 is involved in interaction between two second messenger pathways. NO-sensitive guanylyl cyclase (NO-GC) is the most important

cytosolic generator of cGMP. PDE3A expression is shown in murine aortic smooth muscle, platelets, and heart tissue. Expression and activity of PDE3A in aorta from GCKO and SMC-GCKO mice was reduced by approx. 50% compared to that in control animals[5]. PDE3A down regulation can be linked to the reduction in NO-GC and is not an effect of the increased blood pressure levels resulting from NO-GC deletion. Despite the different PDE3A expression levels, smooth muscle relaxation induced by forskolin to stimulate cAMP signaling was similar in all genotypes. Inhibitors of PDE3, a family of dual-specificity cyclic nucleotide phosphodiesterases, are used clinically to increase cardiac contractility by raising intracellular cAMP content in cardiac myocytes and to reduce vascular resistance by increasing intracellular cGMP content in vascular smooth muscle myocytes. When used in the treatment of patients with heart failure, PDE3 inhibitors are effective in the acute setting but increase sudden cardiac death with long-term administration, possibly reflecting pro-apoptotic and pro-hypertrophic consequences of increased cAMP-mediated signaling in cardiac myocytes. Four variants/isoforms of PDE3 (PDE3A1, PDE3A2, PDE3A3, and PDE3B) are expressed in cardiac myocytes, and new experimental results have demonstrated that these isoforms, which are differentially localized intracellularly through unique protein-protein interactions, control different physiologic responses. While catalytic regions of these isoforms may be too similar to allow catalytic activity of each isoform to be selectively inhibited, targeting their unique protein-protein interactions may allow desired responses to be elicited without adverse consequences that limit the usefulness of existing PDE3 inhibitors[6]. Classical phosphodiesterase 3A (PDE3A) inhibitors provide relaxation of the vasculature system via increasing the cellular level of cyclic adenosine monophosphate (cAMP) and proved to be useful in management of congestive heart failure. Moreover, it considerably improves contractility of cardiac muscles without altering heart beat frequency in experimental subjects [7]. Although inhibition of cyclic nucleotide phosphodiesterase type 3 (PDE3) has been reported to protect rodent heart against ischemia/reperfusion (I/R) injury, neither the specific PDE3 isoform involved nor –underlying mechanisms have been identified. Targeted disruption of PDE3 subfamily B (PDE3B), but not of PDE3 subfamily A (PDE3A), protected mouse heart from I/R injury in vivo and in vitro, with reduced infarct size and improved cardiac function. Proteomics analyses indicated that PDE3B heart mitochondria fractions were enriched in buoyant ischemia-induced caveolin-3-enriched fractions (ICEFs) containing cardioprotective proteins. Accumulation of proteins into ICEFs was PKA dependent and was achieved by ischemic preconditioning or treatment of WT heart with the PDE3 inhibitor cilostamide. Taken together, these findings indicate that PDE3B deletion confers cardioprotective effects because of cAMP/PKA-induced preconditioning, which is associated with accumulation of proteins with cardioprotective function in ICEFs [8].

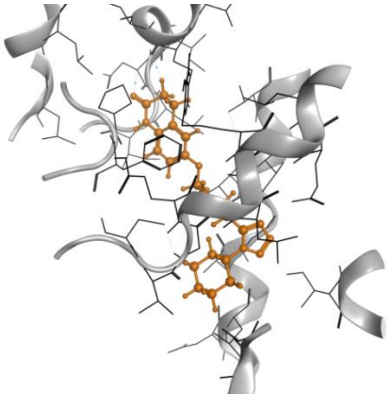
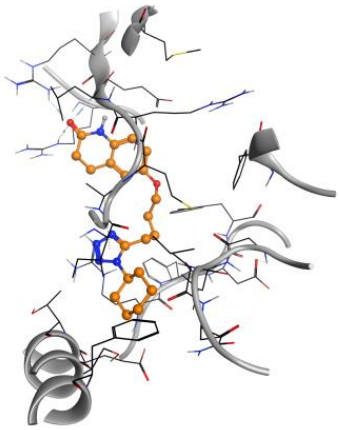
2. MATERIALS AND METHODS

Structural PDB models for phosphodiesterase 3 isoforms A and B was computed using homology modeling having as template UniProtseqences Q14432 [9, 10,11] and Q13370 [12]. Resulted structures were optimized using Schrodinger software package. In order to explore interactions with cytochrome P450PDB models of CYPP3A4 [13,14,15] CYP2C19 [16,17,18] were imported from RCSB data base. Congeneric compounds for Cilostazol were computed in silico. Structures were optimized and stored in sdf format. Binding sites were computed using Schrodinger software. All binding sites were explored. For PDE3A and PDE3B 5 complexes with Cilostazole were retained. All PDE3 cilostazole complexes were energetically minimized using MMFF94 force field and protonated. For each complex descriptors were computed. A comparison between free Cilostazole poses energy and bound complex PDE3 Cilostazole was performed.

3. RESULTS AND DISCUSSION

Binding sites for PDE 3A and 3 B, are shown in supplemental materials. Cilostazole interaction with Cyp and PDE3 is shown in table 1. Cilostazol interacts with Cyp 2C19: Thr 302 to form an hydrogen bound. An has steric interactions with Ile 178, Gly 437, Ala 441, Leu 294, Thr 302, Glu 444, Pro 427, Gln 356, Leu 361, Asp 360. Total energy -332.14 kcal/mol. Cylostazole interaction with Cyp 3A4 : Cilostazole interact with Arg 212, Arg 375, Glu 374 to forms hydrogen bounds and interacts sterically with Arg 372 and Glu 374. Cilostazole interaction with PD3A. Hydrogen bounds with Asp 909 is observed together with a steric interaction with Ile 902. Cilostazole in complex with PDE3B. Hydrogen bounds with Tyr 736, Glu 851 and Asp 894 are formed. Steric interactions with Asn830 and Asp 894 are observed. Best poses for PDE3 A and B are shown in table 2 together with energies.

Table 1 Cilostazole interaction with Cyp and PDE3

	
Cyp 2C19 Total energy -331.530kcal/mol	**Cyp 3A4 Total energy -130.672 kcal/mol**

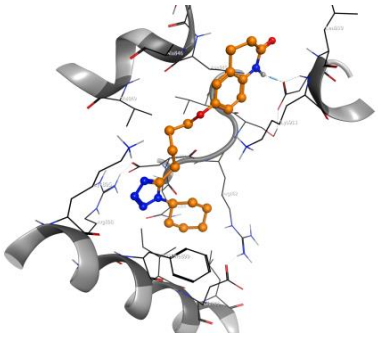
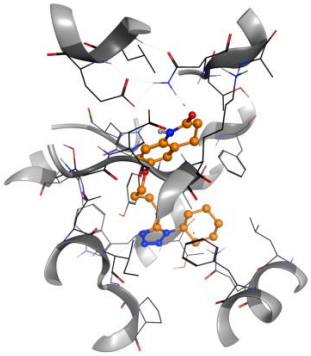
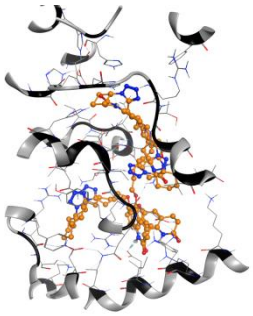
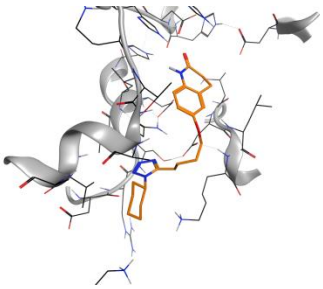
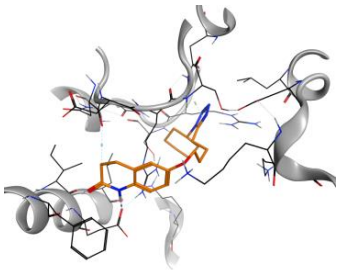
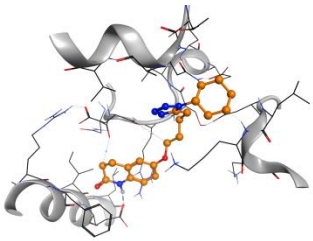
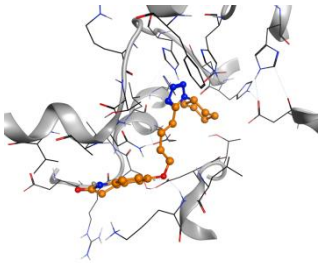
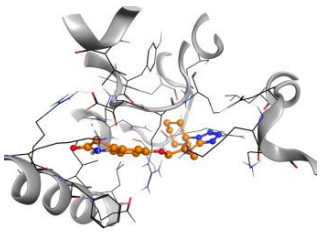
	
PDE3A	**PDE3B**

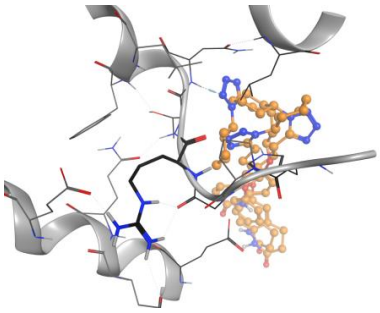
Table 2:Cilostazole in complex with PDE3A

		
All poses	1	2
		
3	4	5

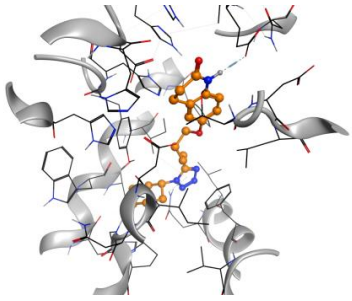
Binding energies for PDE3A are shown in table 3

Table 3 binding energies for PDE3A Cilostazole (kcal/mol)				
Pose	Total energy	Steric energy	Hydrogen bound	Steric energy
1	-267.731	-273.090	-9.960	7.423
2	-276.392	-266.911	-26.594	10.947
3	-274.415	-282.814	-8.811	11.217
4	-265.916	-278.104	-10.385	13.564
5	-265.450	-273.674	-13.728	12.631

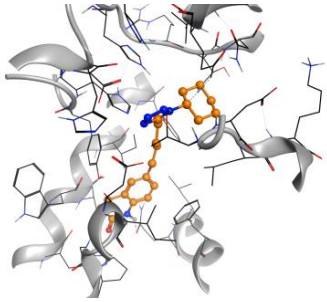
Table 4:Cilostazole in complex with PDE3B



All poses



1



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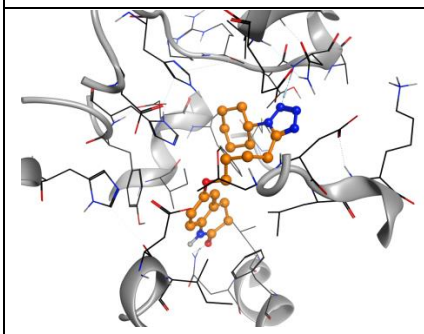

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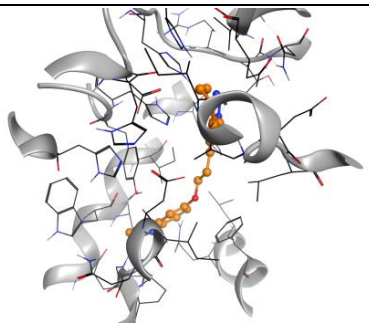

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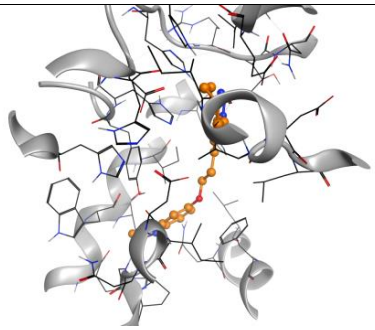

5

Binding energies for PDE3B are shown in table 5 (kcal/mol)

Table 5: binding energies for PDE3B Cilostazole				
Pose	Total energy	Steric energy	Hydrogen bound	Steric energy
1	-146.301	-159.207	-5.673	9.077
2	-146.455	-150416	-7.926	10.687
3	-140.216	-141.297	-6.114	0.508
4	-136.830	-147.893	-7.918	14.200
5	-133.830	-154.650	-4.212	9.242

Descriptors for Cilostazol PDE3 complexes with izoforms A and B are presented in table 6. For both izoforms descriptors present the same values.

Table 6 Cilostazol PDE3 complexes computed descriptors. Density-density, bpol-difference of bounded atoms polarizabilities, E-potential energy, Etor-torsion energy, ASA-water accessible surface area, Glob – globularity, nmol-number of molecules, vsurfR –surface rugosity, Eele-electrostatic energy, Eopp –out of plane energy, Evdw-van der Wallss energy, CP-critical paking parameter, Enb-non bonded energy, Flex-flexibility,Mr-molecular polarizability

Pose	E ele	E oop	Evdw	CP	Enb
1	1709838.5000	0.7145	9.6879	0.0072	9.6897
2	1657740.6250	1.2200	9.6720	0.0070	9.6737
3	1656791.8750	0.9995	9.6787	0.0069	9.6803
4	1758579.3750	1.1742	9.6913	0.0064	9.6930
5	1616807.6250	1.3415	9.6673	0.0071	9.6689

Pose	Density	bpol	E	Etor	ASA	Glob	nmol	Flex	mr	vsurfR
1	0.5934	38118.7734	9.6897	69.9649	79315.9531	0.2446	18550	2.7075	8757.9158	2.8263
2	0.5387	38133.5547	9.6737	5.9509	79396.9219	0.2446	18638	3.0166	8775.8748	2.8227
3	0.5395	38091.2578	9.6803	35.0931	79426.4844	0.2447	18548	2.4194	8757.0967	2.8583
4	0.5398	38048.9883	9.6930	74.0218	79395.4609	0.2446	18495	2.2940	8747.1094	2.8381
5	0.5380	38168.8672	9.6689	11.8905	79378.4531	0.2447	18655	2.8656	8779.1191	2.8195

As shown in presented results judging by total complex energies. PDE3A seem to form computationally more energetically favorable compounds that PDE3B with Cilostazole (figure 1)

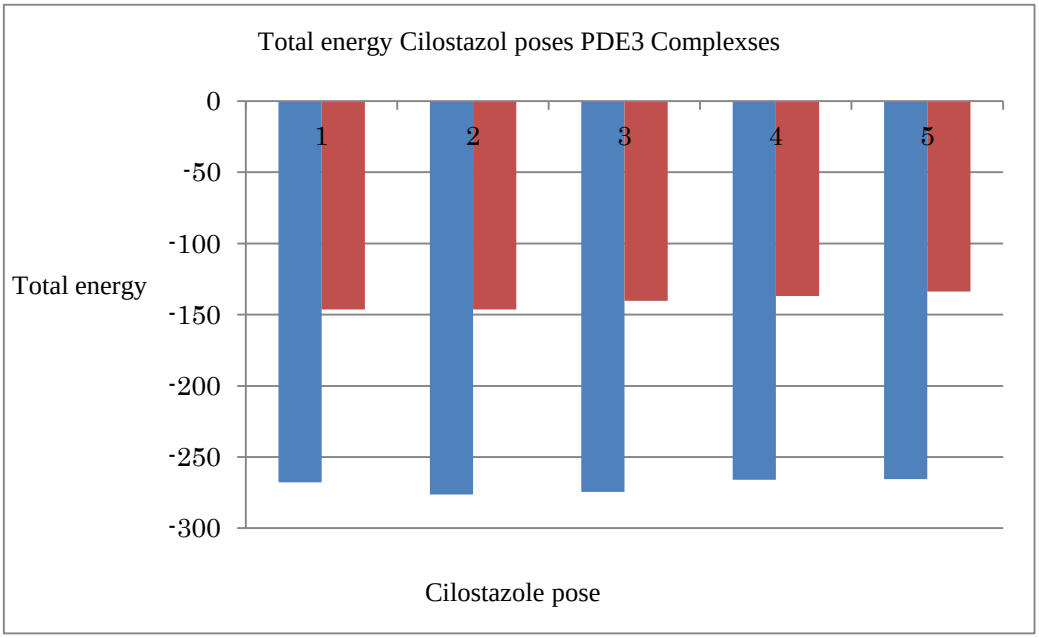

Figure 1: Cliostazol PDE3 complexes total energy. PDE3A is represented in blue, PDE3B in red.

Those findings conclude with experimental findings Cilostazol being cited as a PDE3A inhibitor by FDA. However when computing PDE3A and PDE3B complexes descriptors with Cilostazol same

values were obtained. Judging by Cilostazol mechanism of action [19,20,21,22] only eternal surface of the PDE3A cilostazol complex is important in interacting with cAMP . Those findings suggest that izoenzime PDE3B is capable of producing the same effects as PDE3A when a proper specific inhibitor interacts with it [23,24,25,26]. Computationally results are explained by the fact that the docking procedure compensate for the energetic need for complex forming and by that allows the same protein surface changes like in PDE3A to take place. Cilostazol computationally has a high affinity for Cyp2C19 rather that Cyp3A4. However, those two enzymes metabolise Cilostazole efficentelly. In some studies by inhibiting Cyp, Cilostazol concentration increases by around 50%.[27,28,29]

4. CONCLUSION

Cilostazol is a potent PDE3A inhibitor. Cyp2C19 plays a major role in Cilostazole methabolism. PDE3B is a valuable target with potentially identical effect like PDE3A.

ACKNOWLEDGEMENT

Claudiu N. Lungu and Mihai Creteanu established the conceptual framework, produced the results, discussion and conclusions, and assembled the paper.

CONFLICT OF INTEREST

The authors report no conflict of interests

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