Phosphodiesterase 3B (PDE3B) a potent target in vascular disease: a comparative study with Cyp system and phosphodiesterase 3A(PDE3A)

Claudiu N. Lungu, Mihai Creteanu

1 Department of Chemistry, Faculty of Chemistry and Chemical Engineering, Babes-Bolyai University, 400028 Cluj, Romania
2 Department of Radiology. County Emergency Clinical Hospital "Sfantul Ioan cel Nou", 720224 Suceava, Romania

Abstract: Cilostazol is a potent phosphodiesterase 3 inhibitor. A computational study was carried out with the aim of optimizing its pharmacokinetic properties. A series of congeneric compounds was computationally generated. Series construction was based on computational and experimental data regarding Cilostazol interaction with the target molecule and some Cilostazol effect enhancementors (enzyme inhibitors). Results show a selective inhibition of PDE3A by Cilostazol and suggest that, by inhibiting PDE3B, a same therapeutic effect should be obtained.

Key words: Cilostazol, phosphodiesterase 3 inhibitor, CYPP3A4, CYP2C19.

1. Introduction

Peripheric arterial disease (PAD) [1] is an increasingly spreading problem. Peripheric vasodilatation together with platelet anti-aggregation plays an important role in PAD therapy. Vasodilatation is a dose dependent on Cilostazol active metabolite which determines an increase level of cyclic adenosine monophosphate (cAMP)[2-4].

2. Methods

Structural PDB models for phosphodiesterase 3 isoforms A and B were computed using homology modeling having as template UniProtseqences Q14432 [5] and Q13370 [6]. The resulted structures were optimized using Schrodinger software package. In order to explore interactions with cytochrome P450, PDB models of CYPP3A4 and CYP2C19 where 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 Cilostazol were retained. All PDE3-cilostazol complexes were energetically minimized using MMFF94 force field and protonated. For each complex, several descriptors were computed. A comparison between free Cilostazol poses energy and bound complex PDE3-Cilostazol was performed.

3. Results

Binding sites for PDE 3A and 3 B, are shown in supplemental materials. Cilostazol interaction with Cyp and PDE3 is shown in Table 1. Cilostazol interacts with Cyp 2C19:Thr 302 to form hydrogen bonds. It 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. Cilostazol interaction with Cyp 3a4 : Cilostazol interact with Arg 212, Arg 375, Glu 374 to form hydrogen bonds and interacts sterically with Arg 372 and Glu 374. Cilostazol interaction with PD3A: hydrogen bonds with Asp 909 is observed together with a steric interaction with Ile 902. Cilostazol in complex with PDE3B: hydrogen bonds with Tyr 736, Glu 851 and Asp 894 are formed. Steric interactions with Asn 830 and Asp 894 are observed. Best poses for PDE3 A and B are shown in Table 2 together with the corresponding energies.

Table 1 Cilostazol interaction with Cyp and PDE 3

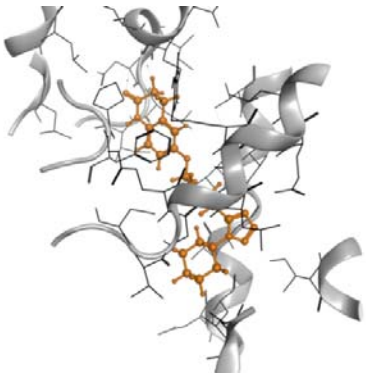
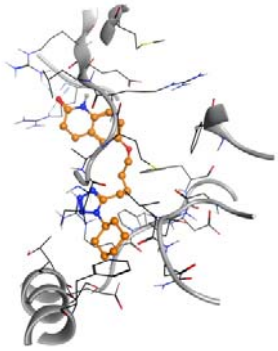
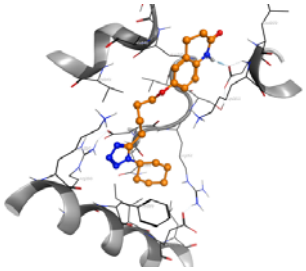
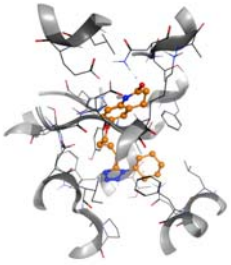
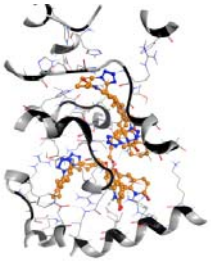
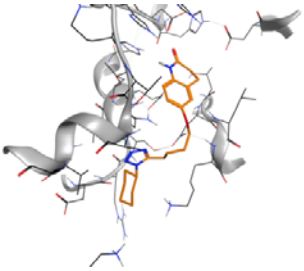
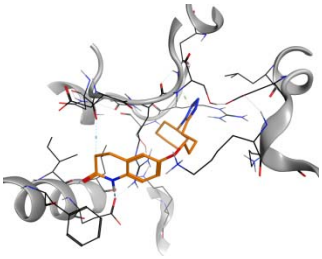
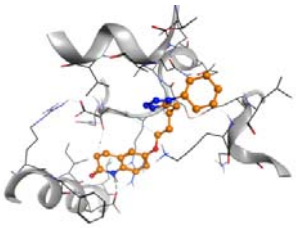
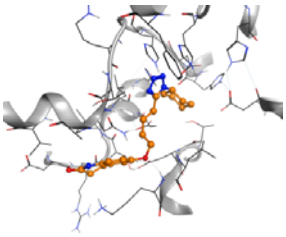
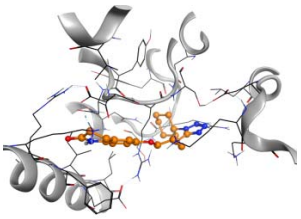
	
Cyp 2C19; Total energy -331.530kcal/mol	Cyp 3A4; Total energy -130.672 kcal/mol
	
PDE3 A	PDE3 B

Table 2 Cilostazol in complex with PDE3 A

		
All poses	1	2

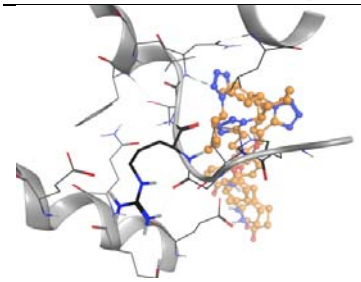
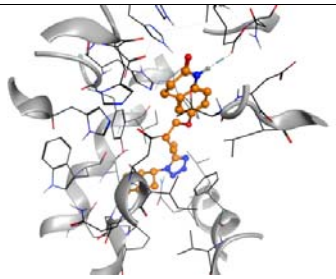
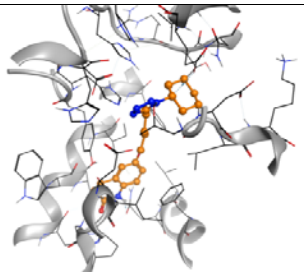
3 4 5

Binding energies for PDE3 A are shown in Table 3

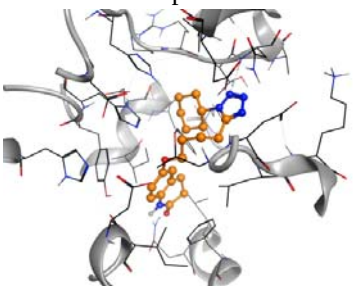
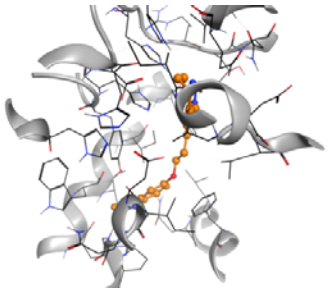
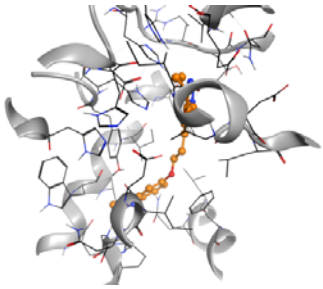
Table 3 Binding energies for the complex PDE3 A : Cilostazol (kcal/mol)

Pose	Total energy	Steric energy	Hydrogen bond	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 Cilostazol in complex with PDE3 B

All poses **1** **2**

3 **4** **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 isoforms A and B are shown in Table 6. For both isoforms, the 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 Waals energy, CP-critical packing parameter, Enb-nonbonded 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

4. Discussion

As shown in the presented results, according to total complex energies, (computationally) PDE3A seems to form with Cilostazol more energetically favorable compounds than PDE3B (Figure 1).

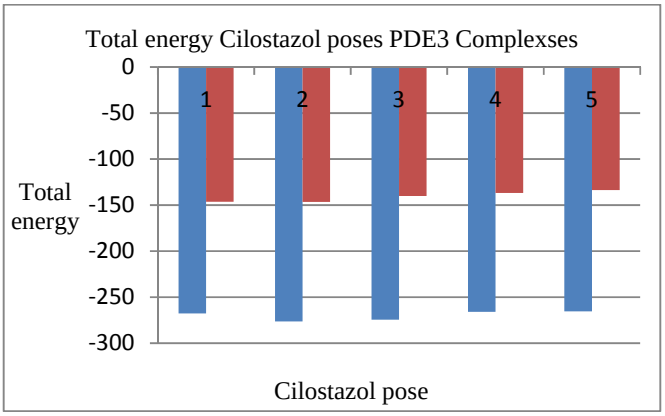


Figure 1 Cilostazol PDE3 complexes total energy (PDE3A in blue, PDE3B in red).

These findings conclude with the experimental findings, cilostazol being cited as a PDE3A inhibitor by FDA.

However, when computing PDE3A and PDE3B complexes with cilostazol, same values for descriptors were obtained. Judging by cilostazol mechanism of action [7] only the external surface of the PDE3A cilostazol complex is important in the interaction with cAMP. These findings suggest that isoenzyme PDE3B is capable of producing the same effects as PDE3A when a proper specific inhibitor interacts with it [8-9].

Computational results are explained by the fact that the docking procedure compensate for the energetic need in the complex forming and by that allows the same protein surface changes like in PDE3A to occur.

Cilostazol computationally has a high affinity for Cyp 2C19 rather that Cyp 3A4. However, these two enzymes metabolize cilostazol efficiently. In some studies, by inhibiting Cyp, cilostazol concentration increases by around 50%.[10].

5. Conclusion

Cilostazol is a potent PDE3A inhibitor. Cyp 2c19 plays a major role in cilostazol metabolism. PDE3B is a valuable target with potentially identical effect like PDE3A.

References

1. Violi, F, Basili, S, Berger, JS, Hiatt, WR). *Antiplatelet therapy in peripheral artery disease. Handbook of Experimental Pharmacology*. **210**. pp. 547–63, **2012**

2. Lee DH, Chun EJ, Oh TJ, Kim KM, Moon JH, Choi SH, Park KS, Jang HC, Lim S, Effect of Cilostazol, a Phosphodiesterase-3 Inhibitor, on Coronary Artery Stenosis and Plaque Characteristics in Patients with Type 2 Diabetes (ESCAPE Study)., , **2019**.

3. Liu Y[1], Shakur Y, Yoshitake M, Kambayashi Ji J. Cilostazol (pletal): a dual inhibitor of cyclic nucleotide phosphodiesterase type 3 and adenosine uptake., Cardiovasc Drug Rev.19(4):369-86, **2001..**

4. Ikeda Y., Antiplatelet therapy using cilostazol, a specific PDE3 inhibitor., Thromb Haemost.82(2):435-8. **1999.**

5 https://www.uniprot.org/uniprot/Q14432 March **2019.**

6 https://www.uniprot.org/uniprot/Q13370 March **2019.**

7. Goto S., Cilostazol: potential mechanism of action for antithrombotic effects accompanied by a low rate of bleeding., Atheroscler Suppl. 15;6(4):3-11, **2005**.

8. Matthew Movsesian, Faiyaz Ahmad, and Emilio Hirsch . Functions of PDE3 Isoforms in Cardiac Muscle, J Cardiovasc Dev Dis. 5(1): 10, **2018.**

9. Movsesian M., Novel approaches to targeting PDE3 in cardiovascular disease. Pharmacol Ther. ;163:74-81, **2016.**

10. FDA. https://www.accessdata.fda.gov/drugsatfda_docs/nda/99/20863.cfm March **2019.**