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RESEARCH ARTICLE

STRUCTURE BASED RATIONAL DRUG DESIGN OF SELECTIVE PHOSPHODIESTERASE-4 LIGANDS AS ANTI-INFLAMMATORY MOLECULES

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Phosphodiesterase-4 enzyme (PDE4) has been gaining increasing attention for the last two decades as a pharmacotherapeutic target, as it is involved in the etiology of a variety of pathologies that comprise a majority of inflammation problems concerning respiratory pathway in major aspect. Intense efforts have been directed towards the development of effective and selective PDE4b inhibitors, but not much success has been reported till yet. This is because of the structural similarity between the two isoforms of PDE4, PDE4b (therapeutic effect) and PDE4d (side effect of emesis). Analogues of 1,2-dihydroxy-xanthen-9*H*-one were designed as selective ligands for PDE4b using the structure based drug design. The selectivity was determined by docking of xanthone analogues in PDE4b and PDE4d active sites respectively using GLIDE docking programme from Schrodinger Inc. ADME properties of the designed ligands were also predicted using QikProp from Schrodinger Inc. Interpretation of protein-ligand interactions and binding modes of xanthone analogues showed that these ligands are more selective for PDE4b than for PDE4d.

Key words: PDE-4 (phosphodiesterase-4), Drug design, Molecular docking, ADME prediction.

INTRODUCTION

Type 4 c-AMP-specific phosphodiesterase (PDE4) is an enzyme responsible for the hydrolysis of the second messenger c-AMP to AMP in many cell types. Inhibition of this enzyme can significantly increase the intracellular c-AMP concentration, leading to major alterations in cell biochemistry and function. In particular, some inflammatory processes can be attenuated with PDE4 inhibitors. For example, LPS (lipopolysaccharides)-stimulated TNF-R (tumour necrosis factor-R) release in human blood mononuclear cells can be blocked with PDE4 inhibitors (Jin and Conti, 2002). Antigen-induced bronchospasm is another pharmacological event that can be attenuated using PDE4 inhibitors (Macdonald *et al* 2000).

The two isoforms of PDE4 *i.e.* PDE4b and PDE4d closely resemble each other (80%). Among the active site residues of PDE4b Glu413, His278, Asp275, Glu304, His274, Asp392, His238, Gln443, Gly280, His234, Phe414, Met411 and Gln417 are absolutely conserved residues in the two isoforms which play active role in getting hydrophobic and H-bond interaction with the ligand. Inhibition of PDE4b is responsible for the therapeutic effect while that of PDE4d for the side effects (Burnouf *et al* 1998). So to overcome those side effects it is needed to come up with specific inhibitors of PDE4b.

The proposed work consists of PDE4b, as the potential target for anti inflammatory molecules. The aim is to utilize the *in silico* techniques to design selective ligands for PDE4b, targeting

specific cavity. Objectives of the present study were to design inhibitors for PDE4b specific pocket and establish their selectivity for PDE4b by molecular docking and *in silico* ADME/T prediction of designed molecules. Structural similarity aspects of both the isoforms is clear from the literature search but besides this, the finding of a recent research stated that both these isoforms differ at C-terminal (Kranz *et al* 2009). So the C-terminal residues which lie in the catalytic cavity can also be targeted to explore the fact that if the specificity can be generated in the ligands with targeting of the dissimilar C-terminal residues, specificity can be there with predefined catalytic site.

X-ray crystal structures of PDE4b and PDE4d were obtained from the Protein data bank (PDB). The complexes were selected on the basis of resolution factor and number of C-terminal residues present. Among the available PDBs of PDE4b, 1XM6 with a resolution of 1.92 A° was found to have more number of C-terminal residues so selected for the further molecular docking studies. For PDE4d, 1Y2K PDB was considered for further analysis having lowest resolution 1.36 A°, in all of the reported PDBs.

In our study we had optimised substituted xanthone (1, 2-dihydroxy-xanthen-9*H*-one) as the core molecule. Rational behind choosing the xanthone was the fact that many naturally occurring xanthones as well as their synthetic derivatives have shown a broad spectrum of biological activities including anti-inflammatory activity (Librowski *et al* 2005; Chung *et al* 2002; Lin *et al* 1996; Marona, 1998; Shankaranarayan *et al* 1979).

MATERIALS AND METHODS

All computational experiments were carried out using LigPrep2.3, GLIDE 5.5 and QikProp3.2 from (Maestro 9.0.111 (2009, update I), Schrodinger, LLC, New York, NY) on window based system. Besides these the other software used were ClustalW for sequence based profile search, DaliLite V.3 server for structure based similarity search, MODELLER 8v2 for designing the missing residues of the PDB 1XM6. All the softwares were used at window based system.

Molecular docking studies

Docking studies were done in GLIDE (Grid based Ligand Docking with Energetics) (Maestro 9.0.111(2009, update I), Schrodinger, LLC, New York, NY). GLIDE uses a series of hierarchical filters to search for possible locations of the ligand in the active-site region of the receptor (Friesner *et al* 2004). Coordinates for each structure were taken from the RCSB Protein Data Bank (PDB) and prepared using Protein Preparation Wizard, which is part of the Maestro software package (Maestro 9.0.111 (2009, update I), Schrodinger, LLC, New York, NY). Bond order, formal charges for heterogroups and hydrogens to all atoms in the system were added. To optimise the hydrogen bond network, His tautomer and ionisation states were predicted. 180° rotation of the terminal χ angle of Asn, Gln and their residue were assigned and hydroxyl, thiol hydrogens were sampled. Water molecules were removed. A brief relaxation was performed using an all-atom constrained minimisation carried out with the impact refinement module (Impref) using the OPLS-2001 force field to alleviate steric clashes that may exist in the original PDB structures. The minimisation when the energy converged or the rmsd reached a maximum cut off of 0.3 A°. GLIDE energy grids were generated for prepared complexes of PDBs of both PDE4b and PDE4d. The binding site was defined by a rectangular box surrounding the X-ray ligand. Ligand preparation was done in LigPrep2.3 (Maestro 9.0.111(2009, update I), Schrodinger, LLC, New York, NY) using force field OPLS-2005. Ligands were refined using the "refine" option in GLIDE and the option to output GLIDE 5.5 XP descriptor information was chosen. Default setting was used for the refinement and scoring.

ADME studies

The designed inhibitors as well as already reported PDE4 inhibitors were sketched and prepared using LigPrep 2.3. Using QikProp3.2 (Maestro 9.0.111(2009, update I), Schrodinger, LLC, New York, NY) the ADME properties were calculated using fast mode. The desirable properties were screened from the overall output and considered for further analysis.

RESULTS

Struture based rational drug design

The known inhibitors of PDE4b till date *e.g.* Rolipram, Mesopram (first generation), Roflumilast, Cilomilast, Filamilast, Piclamilast (second generation) showed promising therapeutic effect as bronchodilators but besides this they also carry a side effect of nausea because all of them interacted with both PDBs in the conserved pose both in terms of hydrophobic and H-bond interactions (**Figure 1a, b**).

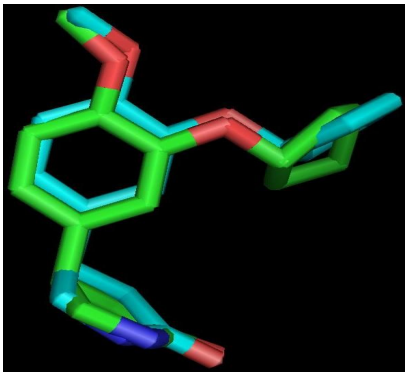

Fig. 1a. Allignment of rolipram in PDE4b (green) and PDE4d (Cyan)

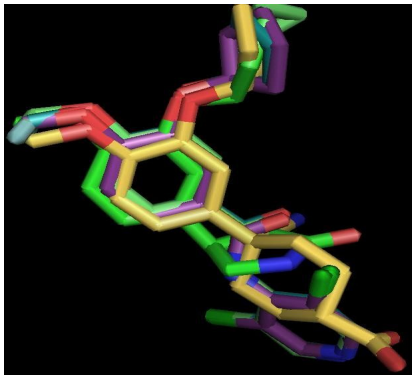

Fig. 1b. Allignment of cilomilast (Violet), piclamilast (yellow), roflumilast (Blue) and rolipram (green)

The rationally designed substituted xanthone *i.e.* 1,2-dihydroxy xanthone had exploited the catalytic cavity of PDE4b, C-1-OH showed the H-bond interaction with O-atom of Asn395 and C-2-OH picked up the H-bond with Asp392 and Tyr233. Carbonyl group got the interaction with Gln443 (**Figure 2a**), but failed to exploit the catalytic cavity of PDE4d *i.e.* the core did not get any interaction in PDE4d (**Figure 2b**).

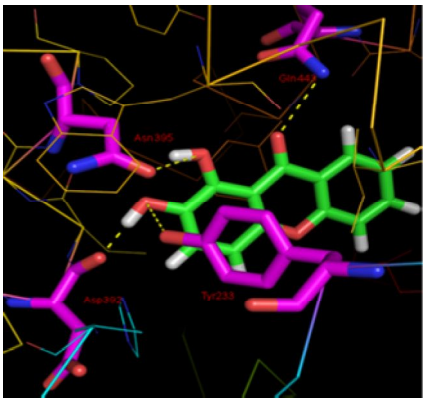

Fig. 2a. Interaction of designed core (1,2-dihydroxy-xanthen-9*H*-one) in PDE4b

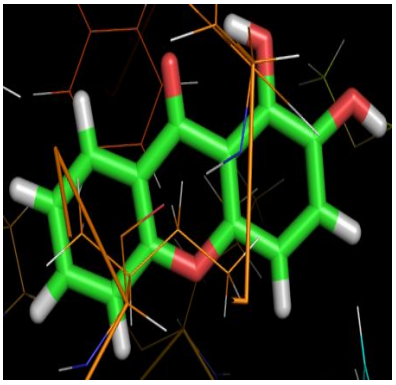

Fig. 2b. Interaction of designed core (1,2-dihydroxy-xanthen-9*H*-one) in PDE4d

The binding pose of the designed ligand was conserved neither with that of the binding pose of the docked ligand in PDE4d nor with that of the co-crystallised ligand Mesopram (**Figure 3a, 3b**). So the designed core was analysed to be optimum scaffold to design the selective ligands for PDE4b (**Table 1**).

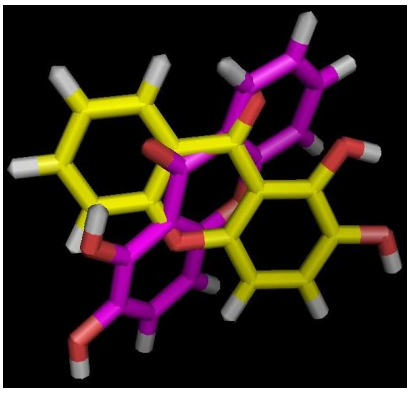

Fig. 3a. Alligned docked Core in PDE4b (yellow) and PDE4d (purple)

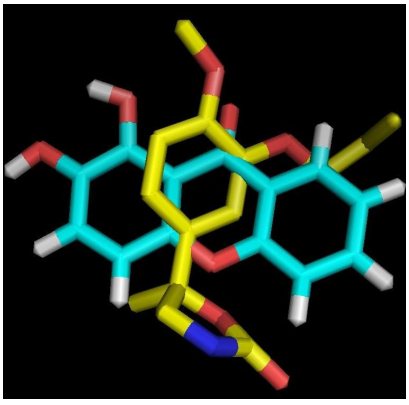

Fig. 3b. Alligned docked core in the PDE4b (cyan) and cocrysatallized mesopram (yellow) of PDB 1XM6

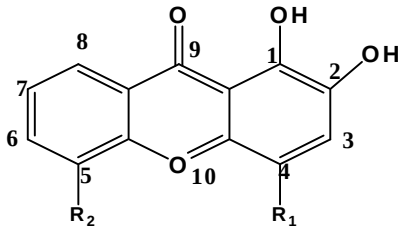

Table 1. Designed analogues of the core

Ligands	R_1	R_2
P4_1	*p*-Nitrohexane	-H
P4_2	*p*-Cyclohexanoic acid	-H
P4_3	5-Methyl-oxazolidin-2-one	-H
P4_4	-H	1-(Hexa-2,4-diene-2-sulfonyl)-piperazine
P4_5	-H	1-(3-Ethoxy-benzenesulfonyl)-4-ethyl-piperazine
P4_6	*p*-Cyclohexyl-phosphonamidic-acid	-H
P4_7	5-Methyl-1*H*-pyrazole-3-ol	-H
P4_8	-H	(4-Methyl-piperazin-1-yl)-phenyl-methanone
P4_9	-H	(4-Methyl-piperazin-1-yl)-phenyl-phosphinic acid
P4_10	1,2,4-Triazole	-H
P4_11	5-Hydroxy-tetrahydro-pyran-2-one	-H
P4_12	-H	Pyrrolidin-2-one
P4_13	5-Hydroxy-dihydro-pyran-3-one	-H
P4_14	N-(3,5-Dichloro-pyridin-4-yl)acetamide	-H
P4_15	*m*-Cyclohexanoic acid	-H

Molecular docking

Sixteen ligands/PDE4b and PDE4d complexes were docked using GLIDE. The well docked complexes in the top-ranked pose (lowest docked energy) were enumerated. The best was identified and tabulated the number of well docked pose. Comparative study of the docking score, glide energy and H-bond interactions in PDE4b and PDE4d is discussed in **Table 2**. The superposition of all the designed inhibitors bound to PDE4b, had revealed with astounding clarity that there is a highly conserved binding mode among all the inhibitors of drastically different chemo types (**Figure 4a, 4b**).

Table 2. Comparative study of the docking score, glide energy, H-bond interactions in PDE4b and PDE4d

Ligand	Glide score in PDE4b	Glide energy	H-bonding residues in PDE4b	Glide score in PDE4d	Glide energy	H-bonding residues in PDE4d
Core	10.2	-23.67	Gln443, Asn395, Asp392, Tyr233	7.3	-30.5	No interaction
P4_1	11.3	-46.0	Gln443, Asn395, Asp392	7.4	-38.3	No interaction
P4_2	11.6	-53.1	Gln443, Asn395, Asp392 Asp275,Tyr233, Thr345	7.2	-41.1	No interaction
P4_3	11.1	-42.2	Gln443, Asn395, Asp392, Thr345	6.4	-43.2	No interaction
P4_4	10.5	-37.3	Gln443, Thr345, Asn283	6.3	-41.5	No interaction
P4_5	10.9	-32.6	Gln443, Thr345, Asn283	6.8	-48.2	No interaction
P4_6	12.4	-55.5	Gln443, Asn395, Asp392, Glu304	7.3	-43.2	Ser368
P4_7	11.2	-44.5	Gln443, Asn395, Asp392	7.3	-41.5	Gln369, His160
P4_8	11.1	-36.9	Gln443, Thr345, Asn283	6.2	-42.2	No interaction
P4_9	12.3	-52.7	Gln443, Thr345, Asn283, Glu304, His234	5.6	-35.6	No interaction

P4_10	11.1	-41.5	Gln443, Asn395, Asp392, Tyr233	6.8	-34.7	No interaction
P4_11	11.6	-46.7	Gln443, Asn395, Asp392	7.3	-32.3	Tyr159
P4_12	10.8	-25.1	Gln443, Thr345	4.7	-35.7	No interaction
P4_13	11.9	-45.9	Gln443,Asn395, Asp392 His234	7.8	-36.9	No interaction
P4_14	11.3	-52.5	Gln443, Asn395, Asp392, His234	7.3	-42.2	No interaction
P4_15	11.9	-49.4	Gln443, Asn395, Asp392	7	-37.9	Tyr159, His160

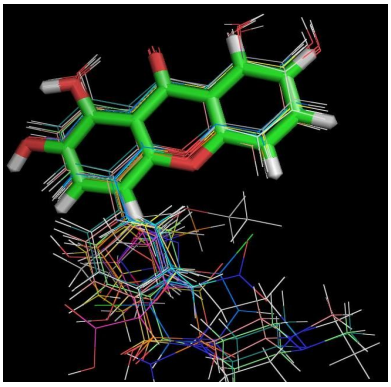

Fig. 4a. Allignment of docked pose of all designed inhibitors in PDE4b

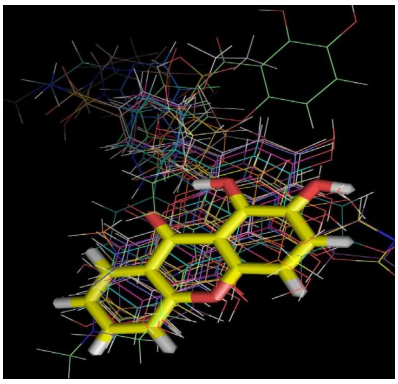

Fig. 4b. Allignment of docked pose of all the designed inhibitors in PDE4d

All these inhibitors share a core binding site distal to the bimetal ions that can be characterized by a planar ring sandwiched by the hydrophobic clamp and the formation of an H-bond with an invariant glutamine. The substructure of the inhibitors that bind to this core represents a framework upon which more potent and selective inhibitors can be developed. One of the contributors to inhibitor binding involves hydrophobic interactions with residues lining the active site pocket. The complete hydrophobic clamp formed by Phe446-4b, Ile410-4b, Tyr233-4b, Asn395-4b, Phe414-4b, Met411-4b, Met431-4b (Card *et al* 2004), was interacted by all the ligands successfully in both of the PDBs. The residues involved in nucleotide recognition made up a secondary yet important component in inhibitor binding and also enabled specificity towards the two PDE4 subfamilies. The binding interactions of the core (**Figure 5**), both in PDE4b and PDE4d have been discussed.

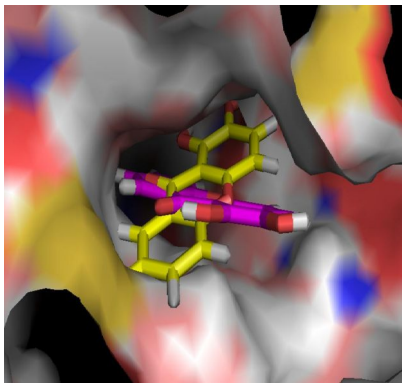

Fig. 5. Surface view of alligned docked core in PDE4b (yellow) and PDE4d (purple)

The nucleotides Gln443, Asn395, Asp392, His234, Glu304, Tyr233, Asp275, Thr345, and Asn283 were analysed to be involved in getting interaction with PDE4b. While in case of PDE4d nucleotide Gln369, Ser368, His160, Tyr159 were analysed to be involved in getting interaction.
The invariant glutamine residue (Gln443-4b, Gln369-4d), is always H bonded to the inhibitors is either a single or a bidentate H bond. The orientation of the gamma-amide group of this invariant glutamine is anchored through an intricate network of H-bonds with nearby residues. This invariant glutamine residue also played a role in the control of specificity toward different inhibitors. In the present case the core made the single H-bond between the carbonyl group with N-atom of the Gln443 shown by all substituted analogues except some of which showed bidentate H-bond with Gln443. P4_4, P4_ 5, P4_ 8, P4_ 9, P4_ 12 had shown the H-bond between C-1-OH group and N-atom of Gln443 along with that of carbonyl group. None of ligands, designed and core had made any H-bond with Gln369 in PDE4d in described way. Only P4_7 had depicted the H-bond with Gln369 but that was with N-atom of pyrazole ring.

The other nucleotides of PDE4b e.g. Asp392, His234, Tyr233, Glu304, Thr345 involved in the interaction with their O-atom. Ligands caught interacting with these nucleotides either by the C-1-OH and C-2-OH of the core moiety or with the different functional groups of the substituent of the ligands. Asn283 caught interacting with its N-atom while Asn395 got interactions with both its N and O-atoms in different ligands with their C-1-OH and C-2-OH of the core moiety or H-atom of the substituent ring of the ligands. On the contrary the interaction of these ligands in PDE4d, 12 ligands including core didn't play any part in making H-bond with any of the residue. Regarding the rest four ligands, if a consideration is given to the interaction with residues other than Gln369, only three residues *i.e.* Tyr159, Ser368, His160 were involved in the interaction. The interaction of ligand with Gln443-4b and Gln369-4d, played a vital role in deciding the affinity and selectivity of the ligands to the PDE4b. Absence of this interaction in PDE4d made the view strong that the designed ligands are selective for PDE4b. All the designed ligands were substituted at position C-4 and C-5 only. Because in case of the substitutions at position other than C-4 and C-5, it was found that either there was no interaction with the Gln443 in PDE4b or their desired pose of the core was failed to conserve, i.e. flipping of the ligand was reported in case of the substitution at other position except C-4 and C- 5 (**Figure 6a, 6b**). So, it was concluded that substitution of the core at the position C-4 and C-5 only resulted into ligands having selective affinity for PDE4b.

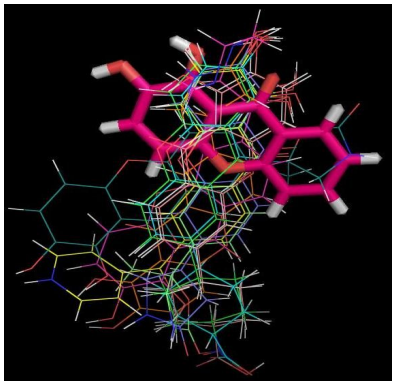

Fig. 6a. Allignment of the docked pose of some of the ligands, substituted at positions other than C-4 and C-5 in PDE4b

ADME studies

The ADME profile of all the designed ligands (**Table 3, 4**) was analysed to be optimum to show a drugeable behaviour. All the ligands had molecular weight within the described range. QPPCaco parameter of the ligands had shown the optimum results.

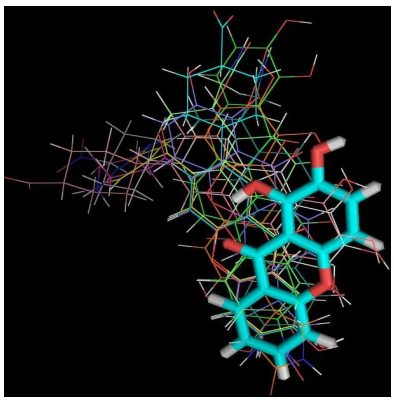

Fig. 6b. Allignment of the docked pose of some of the ligands, substituted at positions other than C-4 and C-5 in both PDE4d

CNS activity parameter is '-2' for all the ligands except core, depicting the negligible CNS effect of the ligands. Hence it can be theoretically analysed that the designed ligands can avoid emesis side effect (Robichaud *et al* 2001). All the rest parameters have the optimum results for the designed ligands except QPlogPo/w parameter for some of the ligands, but since already available inhibitors had also this range of results for this parameter, it can be theoretically inferred that the results for this parameter of the designed ligands were also more towards the drugeable behaviour.

DISCUSSION

PDE4b as a therapeutic target is becoming of great importance, with a strong need to develop novel chemo types lacking the current profile or the side-effects of the various PDE4 inhibitors that have failed to the clinical trials. The structural analysis of these proteins suggests that Gln443 interaction with the ligand is crucial for it to act as effective PDE4b inhibitor. But besides this Phe446, Asn395, Asp392, Thr345, and His234 are also some of the specific residues of the catalytic cavity region for PDE4b which are conserved in PDE4d also. Selective and specific inhibitors of PDE4b were designed by structure based rational drug design. The analyses of binding site characteristics of newly designed molecules were predicted using molecular docking. The designed molecules showed the key H-bonding interactions with Gln443, Asn395, Asp392, Thr345, His234,

Tyr233, Asn283, Glu304 and maintained a conserved binding pose of the core in PDE4b. While in case of PDE4d only four of the designed ligands had shown H-bond interactions with the protein and only one of them had H-bond with Gln369 but pose of the core failed to conserve.

Table 3. ADME properties of some of the available PDE4 inhibitors

Molecules	MW	QPPCaco	CNS	QPlogKhsa	#metab	%Absorption	QPlogPo/w
Filaminast	292.33	497.536	-2	-0.007	3	88.43	-1.704
Piclamilast	381.25	2333.462	0	0.396	3	100	-4.24
Rolipram	275.34	560.798	0	-0.016	3	90.116	-2.387
Mesopram	263.29	1154.338	0	-0.538	2	91.734	-1.704

Table 4. ADME properties of designed PDE4 inhibitors

Molecule	MW	QPPCaco	CNS	QPlogKhsa	#metab	%Absorption	QPlogPo/w
CORE	228.204	565.816	0	-0.132	2	86.807	-1.8
P4_1	355.346	139.52	-2	0.308	3	79.83	-2.4
P4_2	354.359	32.315	-2	0.147	4	70.83	-2.8
P4_3	327.293	111.283	-2	-0.14	2	70.63	-1.7
P4_4	466.508	25.113	-2	0.053	3	62.03	-1.8
P4_5	524.587	25.349	-2	0.279	4	54.995	-2.7
P4_6	389.344	27.164	-2	-0.353	3	50.052	-1.9
P4_7	324.292	91.627	-2	0.236	3	74.806	-2.1
P4_8	430.459	49.631	-2	0.378	3	72.97	-2.6
P4_9	466.429	26.414	-2	-0.373	3	50.968	-1.72
P4_10	295.254	101.386	-2	-0.237	2	68.473	-1.961
P4_11	342.304	59.973	-2	-0.265	5	63.25	-1.766
P4_12	311.293	32.084	-2	-0.329	4	58.303	-1.751
P4_13	342.304	108.549	-2	-0.378	6	67.331	-1.675
P4_14	417.204	142.119	-2	0.082	5	80.50	-2.567
P4_15	354.359	26.807	-2	0.194	4	65.424	-2.8

The designed ligands showed selectivity and affinity for PDE4b over PDE4d even without taking the C-terminal residues in consideration. Further, only two substituent's positions on the core were found to be selective and effective *i.e.* C-4, C-5. Substitutions at other positions resulted in the flipping of the ligands resulting in an unconserved pose of the core in the designed ligands.

CONCLUSION

Thus from the current research work, it can be concluded that the designed core and its analogues will prove to be effective and selective PDE4b inhibitors. All the designed ligands had shown optimum results for the ADME parameter. The result of CNS activity parameter has also supported our findings that the designed inhibitors will avoid emesis side effect. Moreover, to the best of our knowledge, it is for the first time that the designed core (1,2-dihydroxy-xanthen-9*H*-one) and its analogues are reported as PDE4 inhibitor.

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