Chapter 5

Molecular Insights for the Inhibitor Selectivity between PDE4 and PDE7: Docking Study
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5.1 Abstract
5.2 Introduction

Phosphodiesterase4 (PDE4) are ubiquitously distributed in mammalian inflammatory tissues and play a major role in cellular signaling by regulating 3',5'-cyclic adenosine monophosphate (cAMP) concentrations. Although PDE4 is a focal target for inflammatory related diseases the dose limiting side effects of the PDE4 inhibitors hampered its development. As discussed in the earlier chapter for improving the therapeutic ratio and safety of the PDE4 inhibitors several strategies are proposed. They include (a) selectivity towards low affinity versus high affinity rolipram binding form, (b) low BBB permeable drugs, (c) disease activated drugs, (d) subtype selective PDE4 drugs, and (e) selectivity towards broader PDE family.

Among them targeting the broader PDE family is the new hope of direction to overcome the side effects. This is rather a novel approach as it can enhance efficacy and eliminate off-target effects. Of the 11 PDE enzymes that have been unequivocally identified, dual-selective compounds that inhibit PDE4 as well as PDE1, PDE3, PDE5 or PDE7 could offer potential opportunities to enhance clinical efficacy. Dual-selective inhibitors of PDE1C and PDE4 may target proliferating airways smooth muscle cells (that arrest remodeling process) and arrest inflammation (via PDE4 inhibition). PDE3 and PDE4 dual-selective inhibitors may provide more bronchodilator and anti-inflammatory activity. Inhibitors that block both PDE4 and PDE5 may possibly have beneficial effects on hypoxic pulmonary hypertension and vascular remodeling. While dual-selective inhibitors of PDE4 and PDE7 were superior over others because of PDE7’s isolation, characterization and tissue distribution in T-cells and inflammatory cells of asthma and COPD patients. The highly specific nature towards cAMP and its
distribution in proinflammatory and T-cells provoked PDE7 as new potential target for inflammatory and immunological diseases.\textsuperscript{11,12} While a cAMP-specific PDE8 is excluded due to its differential cellular distribution (\textbf{Table 5.1}).

PDE7 is coded by two genes (A and B) and have six isoforms so far.\textsuperscript{13} PDE7A is abundantly expressed in the lung and immune system where as PDE7B is enriched in pancreas, brain, heart, thyroid and skeletal muscle (\textbf{Table 5.1}). The catalytic activity of these subtypes PDE7A and PDE7B can be distinguished by sensitivity to the non selective PDE inhibitor isobutyl methyl xanthine (IBMX) and resistance to the PDE4 selective inhibitor rolipram.\textsuperscript{14} On the other hand PDE4 subtypes (4B and 4D) almost have similar activity towards inhibitors (chapter 4).

\textbf{Table 5.1}. The subtypes, isoforms, cellular distribution, regulatory function, inhibitors and available number of structures in PDB along with theoretical models (PDB ID labeled in italics) are mentioned for the cAMP specific phosphodiesterases PDE4, PDE7 and PDE8.

<table>
<thead>
<tr>
<th>PDE</th>
<th>Subtypes</th>
<th>Isoforms</th>
<th>Tissue expression</th>
<th>Regulatory function</th>
<th>Inhibitors</th>
<th>Number of Structures</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>4 (A, B, C, D)</td>
<td>20</td>
<td>Inflammatory cells</td>
<td>Asthma</td>
<td>Rolipram analogs, Xanthine and Pyrazole analogs etc</td>
<td>39</td>
</tr>
<tr>
<td>7</td>
<td>2 (A, B)</td>
<td>4</td>
<td>Muscle, heart, brain, T-cells</td>
<td>Muscle, cell transduction</td>
<td>Etazolate, pyrimidines, thiazoles, Purine analogs, BRL-50481</td>
<td>1 (1ZKL) (1LHQ)</td>
</tr>
<tr>
<td>8</td>
<td>2 (A, B)</td>
<td>10</td>
<td>Endocrinal, neural</td>
<td>Alzheimers disease</td>
<td>Dipyridimole</td>
<td>3 (3ECM, 3ECN) (1LHW)</td>
</tr>
</tbody>
</table>

There were about 60 X-ray crystal structures compromising nine PDE families (1-5, 7, 8, 9 and 10) deposited in protein data bank (PDB) in last ten years. These PDEs have
PDE4 and PDE7

A common core catalytic domain with 300 amino acids and a compact α-helical structure consisting of 16 helices that can be divide into three subdomains. The crystal structures explain the mechanism of cAMP catalysis and substrate specificity (Glutamine switch mechanism), for each class of PDE family. For example PDE7A co-crystal structure explains nonselectivity towards rolipram is likely due to the steric interactions of cyclopentoxy group with Ile in the Q2 pocket and loss of crucial hydrogen bond between Gln and Ser residues. Further site directed mutagenesis studies showed that the single mutations of S373Y, S377T and I412S in PDE7 or Y329S in PDE4 produce a several fold gain or loss of sensitivity to rolipram and mesopram. Although, inhibitor selectivity of PDE7 is explored there are some more questions puzzling such as (a) How does cAMP substrate bind to PDE7? (b) Why is PDE7A sensitive towards other rolipram analogs such as piclimilast cilomilast and filaminast? and (c) What are the crucial elements that control the inhibitor selectivity towards PDE4 and PDE7?

In view of the above questions and progress towards achieving the dual-specific PDE4-7 inhibitors, a molecular docking of the PDE4B, PDE4D and PDE7A X-ray crystal structures with substrate (cAMP), a nonselective inhibitor isobutyl methyl xanthine (IBMX), PDE4 inhibitors such as rolipram, piclimilast, cilomilast and PDE7 inhibitors such as BRL-50481, spiroquinazolinones are studied (Scheme 5.1). Autodock is used for docking and the docked energies obtained are well correlated to specificity of the inhibitors. Based on the docking and active site exploration the modified rolipram and pyrazole analogs (Scheme 5.1) are further docked to understand the inhibitor selectivity for PDE4 and PDE7.
Scheme 5.1. Ligand structures that are docked to PDE4B, 4D and PDE7 proteins.
5.3 Details of Computational Methods

5.3.1 Preparation of Protein and Ligand for Docking: The X-ray crystal structures like catalytic domain 1F0J,\textsuperscript{26a} cAMP co-crystal structures 1ROR,\textsuperscript{26b} 1PTW,\textsuperscript{26c} Rolipram co-crystal structures 1RO6,\textsuperscript{26b} 1OYN\textsuperscript{26d} and IBMX co-crystal structures 1ZKN\textsuperscript{27a} and 1ZKL,\textsuperscript{17} pyrazole analogs 1Y2E, 1Y2J and 1Y2K respectively are downloaded from PDB.\textsuperscript{27b} The water molecules are excluded and ligands are extracted. The atom types of ligand are checked before adding all hydrogens and further assigned with Gasteiger-Huckel charges. These structures are minimized with conjugate gradient method with 0.01 derivative using Tripos force field and others settings are default as in SYBYL7.0.\textsuperscript{28} BRL-50481, spiroquinazolinone ligands are drawn using SYBYL7.0 and minimized similarly to other ligands. The proteins have charged termini with only polar hydrogens been added. The proteins are assigned with Kollman-Uni charges and metals with Gasteiger-Huckel charges and are minimized with Powell method (0.05 gradient) using Tripos force field.

5.3.2 Preparation of GPF and DPF in AUTODOCK: ADT tools of Autodock 3.05 version is used for the ligand and protein input preparation as PDBQ file. A grid of 60x60x60 with grid center as the center of the ligand for the X-ray co-crystal structures is considered. The grid centers in the PDE4B (1F0J) protein structure is -0.414, 4.407, -10.849. The Zn atom is assigned as M parameter type with R\textsubscript{ij} as 0.87 and \epsilon\textsubscript{ij} as 0.35 values. Lamarckian genetic algorithm is used for search of conformations as it is superior over simulated annealing procedure in reproducing various experimental structures.\textsuperscript{29} 150 runs are carried out (ga_run =150) with population size of 100 in each run and with 50,000 energy evaluations. The GA run was for utmost 27000 generations.
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(\text{ga\_num\_generations} \ 27000) \text{ by preserving one as best in each generation} (\text{ga\_elitism} = 1), \text{ mutation rate as 0.02, crossover rate as 0.8 and the GA's selection window to 10 generations are considered. The local search method of Solis and Wets algorithm was performed at 300 iterations.}

5.4 Results and Discussions

5.4.1 Structural Comparison of PDE4 and PDE7

The catalytic domain of PDE7 has \(~32\%\) sequence identity with PDE4 and \(~72\%\) sequence identity with respect to the active site.\(^{15}\) \text{ The secondary structural comparison of PDE4D and PDE7A show missing of only two 3\text{\_}10\text{-helices.}^{17}\text{ The superimposition of Ca atoms of PDE4D and PDE7 X-ray structures have a 1.79 }\AA\text{ root mean square deviation. The most significant difference is the N-terminal portion of helix H11 has a positional displacement of 2.8 }\AA.\text{ While amongst the three loop regions (10/11, 12/13 and M-loop region) of PDE4, the ERK docking site (a }\beta\text{-turn, 12/13 region) is absent in PDE7. This suggests that there may be lack of protein–protein interactions in PDE7 (Figure 5.1).}

The active site of PDE4 can be divided into three pockets\(^{26}\) (a) a metal-binding pocket (M-pocket), (b) a solvent-filled side pocket (S-pocket), and (c) a pocket containing the purine-selective glutamine (Q-pocket). The M-pocket contains the two metal ions (Zn, Mg) and has highly conserved hydrophobic and polar residues which coordinate to the metal ions. The S-pocket consists mainly of hydrophilic amino acids and is filled with a network of water molecules. The Q-pocket consist of an glutamine that have hydrogen bond interactions with the ligand and is flanked by two asymmetrical hydrophobic sub pockets as Q1 and Q2 (Figure 5.1).
Out of the 31 active site residues that are lining the three pockets of PDE4 only eleven residues are different in PDE7 (Table 5.2). Most of these varying residues are seen in Q2 pocket and belong to helix 14 (Figure 5.1). All the metal interacting histidines and aspartic acids are conserved. Thus mechanism for the catalytic function may remain similar in PDE4 and PDE7. There is only one residue different (Asn\textsuperscript{260}/Ser\textsuperscript{208}) in solvent binding pocket and its significance is reported recently.

Table 5.2. The active site residues of PDE4B, PDE4D and PDE7A are tabulated.

<table>
<thead>
<tr>
<th>Pocket Type</th>
<th>PDE4B</th>
<th>PDE4D</th>
<th>PDE7A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal binding</td>
<td>His\textsuperscript{234}, His\textsuperscript{238}, His\textsuperscript{274}, Asp\textsuperscript{275}, His\textsuperscript{278}, Asn\textsuperscript{283}, Leu\textsuperscript{303}, Glu\textsuperscript{304}, Asp\textsuperscript{346}, Met\textsuperscript{347}, Asp\textsuperscript{392}</td>
<td>His\textsuperscript{160}, His\textsuperscript{164}, His\textsuperscript{200}, Asp\textsuperscript{201}, His\textsuperscript{204}, Asn\textsuperscript{209}, Leu\textsuperscript{229}, Glu\textsuperscript{230}, Asp\textsuperscript{272}, Met\textsuperscript{273}, Asp\textsuperscript{318}</td>
<td>His\textsuperscript{212}, His\textsuperscript{216}, His\textsuperscript{222}, Asp\textsuperscript{253}, His\textsuperscript{256}, Gln\textsuperscript{261}, Leu\textsuperscript{333}, Asp\textsuperscript{362}</td>
</tr>
<tr>
<td>Solvent binding</td>
<td>Gly\textsuperscript{280}, Ser\textsuperscript{282}, Glu\textsuperscript{413}, Gln\textsuperscript{417}, Ser\textsuperscript{429}, Cys\textsuperscript{432}</td>
<td>Gly\textsuperscript{206}, Ser\textsuperscript{208}, Glu\textsuperscript{319}, Gln\textsuperscript{343}, Ser\textsuperscript{355}, Cys\textsuperscript{358}</td>
<td>Gly\textsuperscript{258}, Asn\textsuperscript{260}, Glu\textsuperscript{383}, Gln\textsuperscript{388}, Ser\textsuperscript{399}, Cys\textsuperscript{402}</td>
</tr>
<tr>
<td>Gln residue (as Q), Q1 pocket</td>
<td>Tyr\textsuperscript{233}, Leu\textsuperscript{395}, Asn\textsuperscript{395}, Pro\textsuperscript{396}, Tyr\textsuperscript{403}, Trp\textsuperscript{406}, Thr\textsuperscript{407}, Ile\textsuperscript{410}, Met\textsuperscript{411}, Phe\textsuperscript{414}, Met\textsuperscript{431}, Val\textsuperscript{439}, Ser\textsuperscript{442}, Gln\textsuperscript{443}, Phe\textsuperscript{446}, Ser\textsuperscript{468}, Gln\textsuperscript{469}, Phe\textsuperscript{472}</td>
<td>Tyr\textsuperscript{293}, Leu\textsuperscript{319}, Asn\textsuperscript{321}, Pro\textsuperscript{322}, Tyr\textsuperscript{329}, Trp\textsuperscript{332}, Thr\textsuperscript{333}, Ile\textsuperscript{336}, Met\textsuperscript{337}, Phe\textsuperscript{340}, Met\textsuperscript{357}, Val\textsuperscript{365}, Ser\textsuperscript{366}, Gln\textsuperscript{369}, Met\textsuperscript{372}, Phe\textsuperscript{372}</td>
<td>Tyr\textsuperscript{211}, Ile\textsuperscript{363}, Asn\textsuperscript{365}, Ser\textsuperscript{377}, Val\textsuperscript{380}, Thr\textsuperscript{381}, Pro\textsuperscript{366}, Ser\textsuperscript{373}, Trp\textsuperscript{376}</td>
</tr>
<tr>
<td>Gln residue (as Q), Q2 pocket</td>
<td>Pro\textsuperscript{396}, Tyr\textsuperscript{403}, Trp\textsuperscript{406}, Thr\textsuperscript{407}, Ile\textsuperscript{410}, Met\textsuperscript{411}, Phe\textsuperscript{414}, Met\textsuperscript{431}, Val\textsuperscript{439}, Ser\textsuperscript{442}, Gln\textsuperscript{443}, Phe\textsuperscript{446}, Ser\textsuperscript{468}, Gln\textsuperscript{469}, Phe\textsuperscript{472}</td>
<td>Pro\textsuperscript{322}, Tyr\textsuperscript{329}, Trp\textsuperscript{332}, Thr\textsuperscript{333}, Ile\textsuperscript{336}, Met\textsuperscript{337}, Phe\textsuperscript{340}, Met\textsuperscript{357}, Val\textsuperscript{365}, Ser\textsuperscript{366}, Gln\textsuperscript{369}, Met\textsuperscript{372}, Phe\textsuperscript{372}</td>
<td>Pro\textsuperscript{366}, Ser\textsuperscript{373}, Trp\textsuperscript{376}, Ser\textsuperscript{377}, Val\textsuperscript{380}, Thr\textsuperscript{381}, Pro\textsuperscript{366}, Ser\textsuperscript{373}, Trp\textsuperscript{376}, Ser\textsuperscript{377}, Val\textsuperscript{380}, Thr\textsuperscript{381}</td>
</tr>
</tbody>
</table>

The replacement of Tyr\textsuperscript{329} in PDE4D to Ser\textsuperscript{373} residue has increased the size of the Q1 pocket (lined by Phe\textsuperscript{416}, Ser\textsuperscript{417}, Pro\textsuperscript{366} and Ile\textsuperscript{363}) in PDE7 (Figure 5.1) and has changed the hydrogen bonding interactions of glutamine residue in Q-pocket. In PDE7A the CO group of Gln residue is forming hydrogen bond with Ser\textsuperscript{377} residue\textsuperscript{17} and in PDE4D it is forming with Tyr\textsuperscript{329}. The replacement of Ile\textsuperscript{336}, Met\textsuperscript{377} and Ser\textsuperscript{368} residues in PDE4D to Val\textsuperscript{380}, Thr\textsuperscript{381} and Ile\textsuperscript{412} respectively in the PDE7 has decreased the size of the
Q2 pocket. It may also reduce hydrophobic interactions with the inhibitors or create a hydrophilic environment due to Thr$^{381}$ residue in PDE7 (Figure 5.1). Further Met$^{357}$ and Val$^{365}$ residues that are present on the surface of Q2 pocket (in the M-loop region) are also varied to Leu$^{401}$ and Ile$^{409}$ residues in PDE7 and can also alter the shape of the Q2 pocket (Table 5.2).

In summary, the structural comparison of PDE4 and PDE7 shows a major change in the Q1 and Q2 pocket architectures but have a conserved metal and solvent binding pocket (Figure 5.1). To further characterize about the active site, docking studies of the substrate (cAMP) and inhibitors (IBMX, Rolipram and BRL-50481) are performed.

**Figure 5.1.** The protein structure of PDE4D is displayed in red color and PDE7A is in green color. The yellow and purple colors represent fast Connolly channel surface for PDE4D and PDE7A structures respectively. The IBMX ligand in the active site is shown in ball and stick.
5.4.2 Docking

The docking studies are promising tools in rational drug design as it helps in enlightening the type of interactions existing between the enzyme and ligands. The docking results with substrate specific cAMP, nonselective inhibitor IBMX, PDE4 specific inhibitor rolipram and PDE7 specific inhibitors such as BRL-50481 and spiroquinazolinones are discussed. Later modified rolipram and pyrazole analogs that have shown comparable binding affinity towards PDE4 and PDE7 are presented.

5.4.2.1 Substrate (cAMP)

The adenine moiety of the cAMP adopts an anti conformation and orients to the hydrophobic pocket made up of residues Tyr$^{159}$, Leu$^{319}$, Asn$^{321}$, Thr$^{333}$, Ile$^{336}$, Gln$^{369}$ and Phe$^{372}$. In all X-ray crystal structures, adenine moiety forms two hydrogen bonds with each side chains of Gln$^{369}$ and Asn$^{321}$ and one with Tyr$^{159}$ residue.$^{15,21}$ The bicyclic (adenine) ring also has stacking interactions with Ile$^{336}$ and Phe$^{372}$ residues. While ribose sugar stacks with Tyr$^{159}$ residue and has van der Waal’s contacts with residues His$^{160}$, Met$^{273}$, Ile$^{336}$ and Phe$^{340}$. The phosphate group of cAMP coordinates with both metal ions and forms hydrogen bonds with His$^{160}$ and Asp$^{201}$ residues. Similar types of interactions are found when the cAMP is docked to PDE4B, 4D and 7A (1F0J, 1ROR, 1PTW and 1ZKN) structures (Figure 5.2). The docked conformation in 4B (1ROR) and 4D (1PTW) are similar to its X-ray crystal conformation with a RMS deviation of 1.38 and 0.98 Å and shows high clustering among various sampled (Table 5.3). Inspite of variations such as Ser$^{362}$ to Ile$^{412}$ and Tyr$^{329}$ to Ser$^{373}$ residues in the Q1 and Q2 pocket of PDE7 the Gln$^{413}$ conformation is maintained because CO group of Gln forms hydrogen bond with OH group of Ser$^{377}$ and Thr$^{381}$. So the variation of Tyr$^{329}$/Ser$^{373}$ residue does not
influence the binding mode of cAMP. The similar interactions of phosphate group in metal-pocket as PDE4 indicate possibility of similar mechanism for cAMP catalysis (Figure 5.2).

![Figure 5.2](image)

**Figure 5.2**. The active site residues of 1ROR, 1PTW, 1F0J and 1ZKL with cAMP are shown. The crystal and docked conformations of the ligand are in violet and green color capped sticks. The hydrogen bonding interactions with protein residues are shown in orange color.

5.4.2.2 Nonselective Inhibitor IBMX (Isobutyl Methyl Xanthine)

IBMX is a purine analog (Scheme 5.1) and is a weak nonselective inhibitor of the PDE4 and the PDE7A. IBMX is nonselective because it binds at common core of active site pocket and interacts with highly conserved residues of all PDEs. The six membered ring of this ligand has hydrogen bond donors and acceptors similar that of cGMP. The purine analog shows hydrogen-bonding interactions with Gln^{369}, Asn^{321} and Tyr^{159}.
residues and stacking interactions with Phe\textsuperscript{372} residue. The isopropyl group of IBMX has hydrophobic interactions with Tyr\textsuperscript{159}, His\textsuperscript{160} and Ile\textsuperscript{336} residues in the PD4D crystal structure\textsuperscript{17,22}. The docking of IBMX to PDE4B, 4D and 7A (1F0J, 1ZKN and 1ZKL) have shown slight difference in similar hydrogen bond, stacking interactions and different orientation of isopropyl group (Figure 5.3). In the docked conformation bicyclic ring is displaced to deep into pocket and isopropyl group orients towards Ile\textsuperscript{336}/Val\textsuperscript{380} in the PDE7 and towards the Met\textsuperscript{347} in PDE4B for hydrophobic interactions. The change in positions of hydrogen bonding acceptors and donors of the IBMX with respect to cAMP results in lesser number of interactions and lower docked energies. Thus indicate its weak binding similar to cGMP for these proteins.

![Image]

**Figure 5.3.** The active site residues of 1F0J, 1ZKN and 1ZKL are shown with IBMX ligand. The crystal and docked conformations of the ligand are in violet and green color capped sticks. The hydrogen bonding interactions with protein residues are shown in orange color.

### 5.4.2.2 PDE4 Inhibitors

Rolipram is the first PDE4 specific inhibitor. The keen interactions in binding mode of rolipram are hydrogen-bonding interactions of the alkoxy oxygen atoms with Gln\textsuperscript{369} residue, stacking interactions of benzene with Phe\textsuperscript{372} residue and pyrolidine with Tyr\textsuperscript{159} residue\textsuperscript{17,21} and the hydrophobic interactions of cyclopentoxy group with Ile\textsuperscript{336},
Met$_{337}$, Phe$_{340}$ and Met$_{357}$ residues in the Q2 pocket$^{17}$ and methoxy group with Tyr$_{403}$, Pro$_{396}$ residues in the Q1 pocket. Rolipram does not interact with the divalent metals.

Table 5.3. The docked and intermolecular energies are mentioned in kcal/mole. The estimated inhibitors binding constant ($K_i$) and number of conformations in the cluster are tabulated for the PDE4B, PDE4D and PDE7A crystal structures that are docked with ligands cAMP, IBMX, rolipram, filaminast, piclimilast, BRL-50481, spiroquinazolinones, modified rolipram and pyrazole analogs respectively.

<table>
<thead>
<tr>
<th>PDB ID</th>
<th>PDE type</th>
<th>Ligand</th>
<th>Docked energy</th>
<th>Intermolecular Energy</th>
<th>$K_i$</th>
<th>Cluster Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1F0J</td>
<td>4B</td>
<td>cAMP</td>
<td>-14.45</td>
<td>-14.55</td>
<td>1.76x10$^{-10}$</td>
<td>29</td>
</tr>
<tr>
<td>1ROR</td>
<td>4B</td>
<td>IBMX</td>
<td>-12.31</td>
<td>-10.81</td>
<td>5.79x10$^{-8}$</td>
<td>75</td>
</tr>
<tr>
<td>1PTW</td>
<td>4D</td>
<td>Rolipram</td>
<td>-12.34</td>
<td>-12.20</td>
<td>9.36x10$^{-9}$</td>
<td>102</td>
</tr>
<tr>
<td>1ZKL</td>
<td>7A</td>
<td>Filaminast</td>
<td>-12.83</td>
<td>-12.62</td>
<td>4.59x10$^{-9}$</td>
<td>81</td>
</tr>
<tr>
<td>1F0J</td>
<td>4B</td>
<td>BRL-50481</td>
<td>-8.42</td>
<td>-9.48</td>
<td>9.17x10$^{-7}$</td>
<td>70</td>
</tr>
<tr>
<td>1RO6</td>
<td>4B</td>
<td>Piclimilast</td>
<td>-9.31</td>
<td>-10.08</td>
<td>3.37x10$^{-7}$</td>
<td>127</td>
</tr>
<tr>
<td>1OYN</td>
<td>4D</td>
<td>Spiroquinazolinones</td>
<td>-10.32</td>
<td>-10.18</td>
<td>8.13x10$^{-7}$</td>
<td>34</td>
</tr>
<tr>
<td>1ZKL</td>
<td>7A</td>
<td>Modified rolipram</td>
<td>-9.02</td>
<td>-10.10</td>
<td>3.16x10$^{-7}$</td>
<td>88</td>
</tr>
<tr>
<td>1XZKL</td>
<td>7A</td>
<td>Para-aminoPhPCEE</td>
<td>-9.69</td>
<td>-10.09</td>
<td>1.06x10$^{-6}$</td>
<td>11</td>
</tr>
<tr>
<td>1ZKL</td>
<td>7A</td>
<td>Meta-nitro PhPCEE</td>
<td>-9.45</td>
<td>-9.66</td>
<td>7.56x10$^{-6}$</td>
<td>66</td>
</tr>
</tbody>
</table>

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The docking of this ligand to the PDE4B, 4D and 7A (1F0J, 1RO6 and 1OYN) has similar binding mode as X-ray structures with RMS deviation of 1.03 and 0.75 Å (Figure 5.4). In the case of PDE4B catalytic domain (1F0J) the docking pose has close resemblance to binding mode of 4B (1RO6). While the lowest docked conformation of PDE7 has pushed cyclopentoxy group away from the Q2 pocket. This is likely due to (a) unfavorable steric interaction of Ile$^{412}$ and (b) less hydrophobic nature of Val$^{380}$ and Thr$^{381}$ residues in the Q2 pocket.$^{17}$ This also results in loss of hydrogen bond between dialkoxy atoms of rolipram to Gln$^{413}$ residue. These variations of Q2 pocket explain its reduced interactions, low docked energy (-4.26 kcal/mole) and insensitivity towards rolipram (Table 5.3). As the variation of residues alter the size, shape and nature of Q2 pocket may be less hydrophobic group such as CH$_3$, or hydrophilic groups such as CF$_3$, CH$_2$CH$_2$OH could increase binding affinity towards the PDE7 protein.

On the other hand the second generation rolipram analogs like filaminast, piclimilast and cilomilast also have 3,4-dialkoxyphenyl (catechol) pharmacophore but with variation of substituents (Scheme 5.1). All these ligands have similar interactions and cyclopentoxy group is present in Q2 pocket. The improved affinity of them over rolipram is because they reach deep into M-pocket and have electrostatic interactions with metal ions and mediated hydrogen bonds through water molecules to the residues.$^{19}$ Docking of filaminast and piclimilast have shown similar binding mode but slight variation in placing substituents of catechol in PDE4. In PDE7 these ligands conformation indicate again a shift of cyclopentoxy group from Q2 pocket and loss of crucial hydrogen bond with Gln$^{413}$ residue but there is gain in electrostatic interactions with metal ions. Unlike rolipram, these ligands still have comparable docked energies.
(-11.37,-10.89 kcal/mol) in PDE4 and PDE7 (-10.09, -10.32 kcal/mol) because peripheral residues and external conditions influence ligand binding ability such as phosphorylated form of enzyme in PDE4D has much affinity towards cilomilast and piclimilast. The influence of selectivity of piclimilast, cilomilast may also dependent on phosphorylated form of PDE7.

Figure 5.4. The active site residues of 1RO6, 1OYN, 1F0J and 1ZKL with rolipram are shown. The crystal and docked conformations of the ligand are in violet and green color capped sticks. The hydrogen bonding interactions with protein residues are shown in orange color.
5.4.2.4 PDE7 inhibitors

There are various classes of the PDE7 specific inhibitors developed in the recent times. They are BRL-50481, benzothiadiazines, purine analogs, thiadiazoles, spiroquinazolinones etc. BRL-50481 (Benzene sulphanamide, Scheme 5.1) has a secondary amine with methyl substitutions, which can have hydrophobic interactions. The oxygen atoms of the SO\textsubscript{2} and NO\textsubscript{2} groups of the ligand can act as hydrogen bond acceptors and benzene ring can have stacking interactions with the residues of protein. Docking of this inhibitor revealed absolutely different docking conformations for PDE4B and PDE7 where it is placed in Q pocket for PDE4B and in M-pocket for PDE7. In PDE4B, there are two hydrogen bonding interactions (oxygen atom of SO\textsubscript{2} group has hydrogen bond with NH group of Gln\textsuperscript{443} residue, NO\textsubscript{2} group of the ligand is placed in the solvent pocket), a hydrophobic interactions of secondary amine alkyl groups with residues of the Q1 pocket and stacking interactions of benzene ring with Phe\textsuperscript{372} residue. In contrast PDE7 has the NO\textsubscript{2} group facing towards Asn\textsuperscript{365}, and SO\textsubscript{2} group and its secondary amine are in metal binding pocket surrounded by His\textsuperscript{212}, His\textsuperscript{216} residues and Zn metal (Figure 5.5). The difference in docked energies of PDE4B and PDE7A is due to strong electrostatic interactions of ligand in PDE7 which explain its selectivity to PDE7 (Table 5.3).

Spiroquinazolinones also have bicyclic ring similar to IBMX (Scheme 5.1). Docking of this ligand to the PDE4B (1F0J) and PDE7A crystal structures indicated similar binding mode and interactions. The docked conformation has a hydrogen bonding interaction with Cl atom and NH group of ligand with Asn\textsuperscript{321} and Asp\textsuperscript{318} residues in the active site of the PDE4 and PDE7 protein. The replacement of Cl atom with CH\textsubscript{2}COOH


has preserved its placement in the Q2 pocket. This supports that the shape of Q2 pocket is governing factor for the selectivity of PDE4 and PDE7 inhibitors. There is no significant change in docked energies between PDE4 (-11.10) and PDE7 (-10.87) (Table 5.3). Further to gain insight of inhibitor selectivity to PDE7 may be water molecules inside the protein should be considered.

**Figure 5.5.** The active sites of 1F0J and 1ZKL with BRl-50481 ligand are shown. The docked conformation of the ligand is in green color capped sticks. The hydrogen bonding interactions with protein residues are shown in orange color.

### 5.4.2.5 Dual-Selective PDE4 and PDE7 Inhibitors

There are several dual-specific PDE4 and PDE7 inhibitors reported in the recent years. But the main difference that we observed in the PDE4 and the PDE7 active sites from structural comparison (MOLCAD surfaces) and docking studies is the shapes of the Q1 and Q2 pocket. PDE4 has smaller Q1 and bigger Q2 pockets and PDE7 due to variations of residues (Tyr<sup>329</sup>/Ser<sup>373</sup>, Ser<sup>368</sup>/Ile<sup>412</sup>, Ile<sup>336</sup>/Val<sup>380</sup> and Met<sup>377</sup>/Thr<sup>381</sup>) has slightly bigger Q1 and smaller Q2 pockets. These variations also affected the size, shape and nature of pockets. In order to understand the influences of Q1 and Q2 pockets less
hydrophobic substituents of ligands are been docked such as modified rolipram and pyrazole analogs.

**Modified Rolipram structure:** It has replacement of cyclopentoxy group with the CH₂CH₂OH (Scheme 5.1). Unlike rolipram, docking of this ligand to the PDE4B, 4D and 7A (1F0J, 1OYN and 1ZKN) protein structures indicated smaller differences in docking energies (Table 5.3). The docked conformations of PDE4B and 4D have similar catechol scaffold interactions, but CH₂CH₂OH is placed in the Q2 pocket and Q1 pocket for PDE4D and PDE4B respectively. The CH₂CH₂OH group of ligand in 4D gains only hydrophobic interactions and PDE4B has gained two more hydrogen bonds (CO group of Gln⁴⁴³ residue and with OH group of Thr⁴⁰⁷ residue). The docked conformation of PDE7 has CH₂CH₂OH group in the Q1 pocket and gains hydrogen bond with Gln⁴¹³ residue and improved the binding affinity. This is expected due to alteration in size of the pockets (Figure 5.6).

![Figure 5.6](image)

**Figure 5.6.** The active site residues of 1F0J, 1OYN and 1ZKL are shown with modified rolipram ligand. The docked conformation of the rolipram ligand is in green color capped sticks. The hydrogen bonding interactions with protein residues are shown in orange color.

**Pyrazole analogs:** The pyrazole analogs PhPCEE (3,5-dimethyl-1-phenyl-1H-pyrazole-4-carboxylic acid ethyl ester) are the new class of PDE4 inhibitors that are designed
based on scaffold based drug design (Scheme 5.1). The carboxy group of the ligand form hydrogen bonds with Gln\(^{369}\) residue and pyrazole and the phenyl ring have stacking interactions with Phe\(^{372}\) and Tyr\(^{159}\) residues similar to rolipram. The difference in the substitution of phenyl group at para and meta position of pyrazole analog has influenced the binding affinity\(^{36}\) to PDE4B and PDE4D. This is because the nitro group in M-pocket gains electrostatic interactions and para-amino group is instead in solvent pocket.\(^{17}\)

![Figure 5.7](image)

**Figure 5.7.** The active site residue of 1Y2E is with para amino PHCEE ligand, 1Y2J and 1Y2K are with meta-nitro PHCEE ligand and 1ZKL with both ligands are shown. The crystal and docked conformations of the ligand are in violet and green color capped sticks rendering. The hydrogen bonding interactions with protein residues are shown in orange color.

In contrast to rolipram, hydrophobic interactions are only due to ethoxy group with residues in the Q1 pocket and CH\(_3\) group with residues of Q2 pocket. CH\(_3\) group has reducing hydrophobic interactions in the Q2 pocket as compared to rolipram analogs.
Thus, these are docked to test its affinity towards the PDE7A. The PDE4B and 4D (1Y2J, 1Y2E and 1Y2K) crystallized with these ligands and the PDE7A are docked with these two PhPCEE analogs. The docked conformation has similar binding mode as in crystal structures of the PDE4B and 4D (1Y2J, 1Y2E and 1Y2K) with RMSD of 1.96, 1.39 and 1.43Å respectively. The docked energies of the PDE7 are comparable to the PDE4 structures. The docked conformation also shows that ethoxy group is placed in the Q1 pocket and CH₃ in the Q2 pocket (Figure 5.7).

5.5 Conclusions

A molecular docking of the PDE4 and PDE7 structures with the cAMP, IBMX, rolipram and BRL-50481 ligands were studied using AUTODOCK. The crystal structures deposited with cAMP, rolipram, piclamilast and filaminast were reproducible. The docked energy values are also well correlated to its specificity. The factors influencing the cAMP binding in PDE7 was due to the maintenance of Gln⁴⁴³/⁴¹³ conformation similar to that in PDE4 and conservation of the metal binding residues. The small difference in docking energies of rolipram analogs and spiroquinazolinones cannot explain the selectivity as external conditions, water and peripheral residues can also influence binding. While the inhibitor selectivity of rolipram was due to the change in size and nature of the Q1 and Q2 pocket due to Tyr³²⁹/Ser³⁷³ and Ser³⁶⁸/Ile⁴¹² and Ile³³⁶/Val³⁸⁰, Met³³⁷/Thr³⁸¹ residues. In view of these changes in pocket a modified rolipram structure where cyclopentoxo group is replaced by CH₂CH₂OH and pyrazole ligand has been docked and it indicate increase in the binding affinity of the PDE7 and reduction in the affinity for PDE4. The docked energies of these ligands have very small difference between PDE4 and PDE7. This supports the hypothesis that an optimized hydrophobic
group in the Q2 pocket is parameter to obtain a PDE4, PDE7 or dual specific PDE4-7 inhibitors.

5.6 References


25. **AUTODOCK**, 10550, North Torrey Pines Road, La Jolla, CA 92037-1000, USA.


28. **SYBYL 7.0**, Tripos Inc., 1699 South Hanley Rd., St. Louis, Missouri, 63144, U. S. A.


