Current Trends in Biotechnology and Pharmacy
Vol. 17 (4A) 15 - 21, October 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.4s.85

Identification of New Dual PDE4/5 Inhibitors Using Pharmacophore Based Virtual Screening

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Abstract

Chronic Obstructive Pulmonary Disease (COPD) is a progressive airway obstruction disease caused due to inflammation of the airway mucosal layer. Current therapies have proved to be inadequate and thus there is high unmet medical need. Research has provided evidence that dual inhibition of Phosphodiesterase 4/5 (PDE4/5) is an effective strategy in suppressing inflammatory mediator release from cell types which express PDE4 and PDE5. In this study ligand-based pharmacophore models of PDE4 and PDE5 were developed and validated followed by their application in virtual screening of Universal Natural Products Database (UNPD) to identify UNPD192494 as a new dual inhibitor of PDE4/5 from natural origin.

Keywords: dual inhibitors; pharmacophore-based; virtual screening; COPD; inflammation of mucosal layer.

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality with increasing prevalence. Short-Acting Beta Agonists (SABA) are the first-line treatment while combination of Long-Acting Muscarinic-Antagonists (LAMA) and Long-Acting Beta Agonists (LABA) is the maintenance therapy. A combination of LABA and Inhaled Corticosteroids (ICS) is commonly used in treating COPD patients to reduce the mucosal layer inflammation (1–8). The current therapeutic options to treat COPD only provide modest benefits and do not slow down the disease progression (9,10). Chronic use of steroids increases the risk of relapse in COPD patient and makes them more susceptible to infections such as pneumonia.

Among the newly approved medications for treating COPD, orally administered PDE 4 inhibitors have shown promise, however their use is also associated with drawbacks such as gastrointestinal adverse effects. Thus, there is a high unmet medical for new and effective therapies for COPD (11). There is growing evidence to suggest that dual inhibition of Phosphodiesterase 4/5 (PDE4/5) can be additive or even synergistic at suppressing inflammatory mediator release from cell types that express PDE4 and PDE5, while limiting adverse effects have not been reported. This diverse spectrum of biological effects has thus implicated dual PDE4/5 inhibitors as potential therapeutic agents for a range of disease indications involving inflammation and altered mucus production such as COPD, asthma, and cystic fibrosis.

In this paper we report the identification of new dual PDE4/5 inhibitors via the application of Pharmacophore Based Virtual Screening.

Material and Methods

Pharmacophore Modelling

A literature search was carried out to find out the inhibitors of PDE4 and PDE5, the identified compounds were distributed into a training set and test set.

Ligand-based pharmacophore modelling was carried out as it has proved to be an efficient approach for virtual screening (12). Ligand Scout 4.4 was used to generate the pharmacophore models (13).

Pharmacophore Validation and Optimization

The validation of the pharmacophore model required a setup of a database as a decoy set and a test set. Anefficient pharmacophore model should be able to distinguish the test set compounds from the decoy set compounds. The ability of developed pharmacophore model to achieve this was tested.

Virtual Screening

After the validation of pharmacophore models, the models were used to perform virtual screening. Both the models were used for virtual screening of compounds present in Universal Natural Products Database (UNPD) to enrich the available compounds that has inhibitory effects on both PDE 4 and PDE 5. Since the identified compounds are expected to be usedorally, Lipinski's rule of five was applied to narrow down the selection of compounds and obtain compounds which are suitable for oral mode of administration. Generally, the compounds that fulfilled RO5 have significant permeability and solubility allowing them to be used orally (14–16).

Molecular Docking

The affinity of the identified compounds, with PDE4 and PDE5 was determined using docking studies. The interaction of identified compounds with PDE4 and PDE5 were observed at atomic level and the binding affinities were obtained (17,18).

The structures of PDE4 and PDE5 were obtained from Protein Data Bank (PDB) in (.pdb) format. The location of co-crystallized ligand was used to identify the binding site to be used for docking. Protein preparation was carried out in Discovery Studio to optimize the protein structure for molecular docking. PyRx 0.9 was used for molecular docking, whereby grid of size 40x40x40 was used to cover the binding site and exhaustiveness was set at 6.

Design of Novel PDE4/5 Dual Inhibitors

The compound with the best binding affinity from the molecular docking studies were selected for further modification to increase the binding affinity of the ligand to target site. The structural modifications of the compounds were performed based on the structure activity relationships. The modifications were performed by replacing relevant functional groups in the compounds to increase the binding affinity of the compound to both target enzymes.

Results and Discussion

Ligand-based Pharmacophore Modelling

After applying inclusion criteria, 20 PDE4 inhibitors were selected, 13 were included in the training set while 7 were included in the test set. Likewise, 20 PDE5 inhibitors were selected, and 13 were included in the training set and 7 were included in the Test set. 10 sets of pharmacophore model were generated for each PDE 4 and 5 inhibitors. Pharmacophore validation resulted in the identification of validated PDE 4 inhibitor model consisting of two hydrogen bond acceptors, one hydrophobic feature and one aromatic ring. Meanwhile, the validated PDE 5 inhibitor model consisted of two hydrogen bond acceptors and two aromatic rings (Table 1).

Virtual Screening

Before initiating virtual screening, Lipinski's rule of five was applied to UNPD using LigandScout 4.4. The resulting set of compounds was subjected to virtual screening using the validated pharmacophore models for PDE4 and PDE5. A set of 10 compounds were identified in this manner and all the compounds

were further investigated using molecular docking.

Molecular Docking

The binding affinities of each of the compounds, identified in previous steps, to the target proteins i.e., PDE4 and PDE5 were calculated in PyRx 0.9 (12). The protein structures were obtained from RCSB Protein Data Bank, PDE 4 (PDB ID: 1XMU) and PDE 5 (PDB ID: 1UDT).

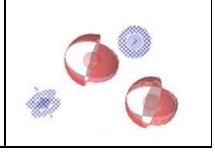
The compounds with the overall highest binding affinity for both protein structures were selected for further analysis (19,20). UNPD192494 demonstrated the highest overall binding affinity for both PDE4 and PDE5 i.e., of -9.7 kcal/mol and -10.1 kcal/mol respectively (Table 2). The lower value of binding free energy indicateshigher affinity between the protein and the ligand (21).

Visualization of the Protein-Ligand Interactions PDE 4 inhibitors Visualization

Biovia Discovery Studio visualiser was used to visualise the interaction between the UNPD192494 and the target proteins (Figure 1 and Figure 2).

A hydrogen bond between UNDP192494 and Asp392 was observed, while Tyr233 and Phe446 were involved in the critical pi-pi interactions with UNPD192494, both being part of the catalytic domain of PDE4. UNPD192494 also showed pi-pi interaction with

Table 1: Pharmacophore models validated and their features. (HBA: red, AR: blue, HPB: yellow)

Pharmacophore	HBA	HBD	AR	HPB	Pharmacophore Model
PDE4 inhibitors	2	0	1	1	
PDE5 inhibitors	2	0	2	-	

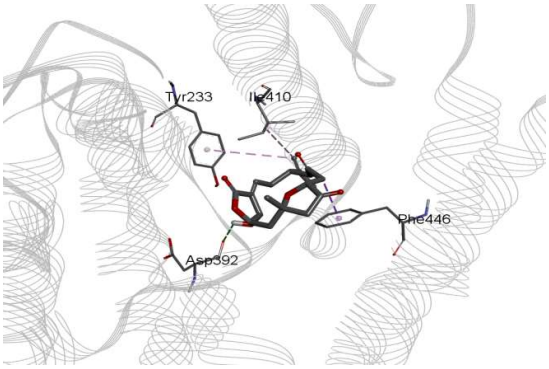


Figure 1: 3D presentation of the interaction between UNPD192494 (dark grey) and PDE 4 protein structure (PDB: IXMU) using Discovery Studio software.

Table 2: Molecular docking results of ligand-based obtained hits

Number	PDE4		PDE5	
	Ligand	Affinity (kcal/mol)	Ligand	Affinity (kcal/mol)
1	UNPD192494	-9.7	UNPD192494	-10.1
2	UNPD189758	-9.4	UNPD189758	-9.6
3	UNPD163500	-8.5	UNPD163500	-9.5
4	UNPD187771	-8.5	UNPD187771	-9
5	UNPD139031	-8.3	UNPD13662	-8.9
6	UNPD177821	-8.1	UNPD158364	-8.7
7	UNPD13662	-7.9	UNPD139031	-8.6
8	UNPD171105	-7.9	UNPD171105	-8.3
9	UNPD158364	-7.7	UNPD135212	-8.2
10	UNPD135212	-7.7	UNPD177821	-7.9

Table 3: Key interactions between UNPD192494 (dark grey) and PDE 4 protein structure (PDB: IXMU)

Compound	No. of Interaction	Residues
UNPD192494	4	Pi-interactions: Phe446, Ile410, Tyr233 Hydrogen bond: Asp392

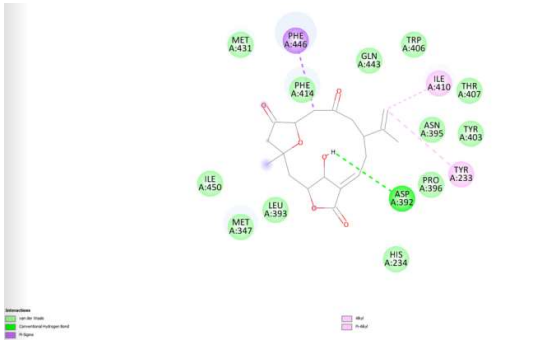


Figure 2: 2D presentation of interaction between UNPD192494 (dark grey) and PDE 4 protein structure (PDB: IXMU) using Discovery Studio software.

Ile410, which is crucial for higher binding affinity with PDE4. Further, there is a potential to increase the activity with proper structural modifications of UNPD192494 (Table 3) (22).

PDE5 inhibitors Visualization

Visualisation of the interactions of UNPD192494 with PDE5 showed that non-polar interactions were formed with Leu804 and Phe820, while a non-conventional(C-H) hydrogen bonds were formed with His613. However, UNPD192494 did not interact with Gln817, this contrasts with other PDE5 inhibitors (Figure 3 and Figure 4) (Table 4) (23).

Design of novel PDE4/5 inhibitos

UNPD192494 has molecular formula of $C_{19}H_{24}O_6$ with molecular weight of 348.4

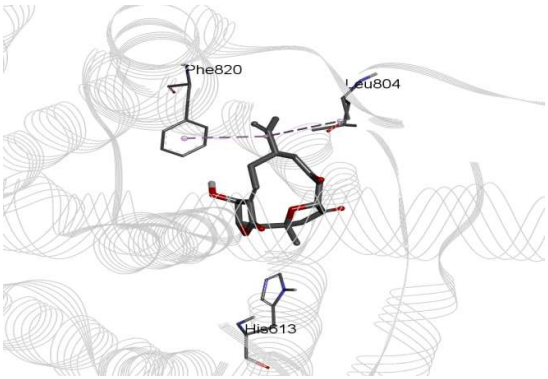


Figure 3: 3D presentation of the interaction between UNPD192494 (dark grey) and PDE 5 protein structure (PDB: 1UDT).

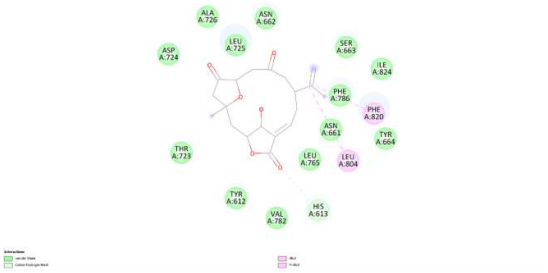


Figure 4: 2D presentation of interaction between UNPD192494 (dark grey) and PDE 5 protein structure (PDB: 1UDT) using Discovery Studio software.

(Figure 5) and its structure comprises of 1 hydrogen bond donor and 6 hydrogen bond acceptors. According to the interaction of UNPD192494 and target site of PDE4 and PDE5 enzyme, some structural modifications were possible to improve the binding affinity. By using UNPD192494 as a lead compound, 14 variations were introduced using different functional groups.

Structural modifications were carried out to improve the binding affinity while retaining the core chemical features of the compound. Therefore, some chemical features were exchanged based on the visualization of the protein-ligand interaction to achieve better binding affinity. The modified models were then redocked to assess the

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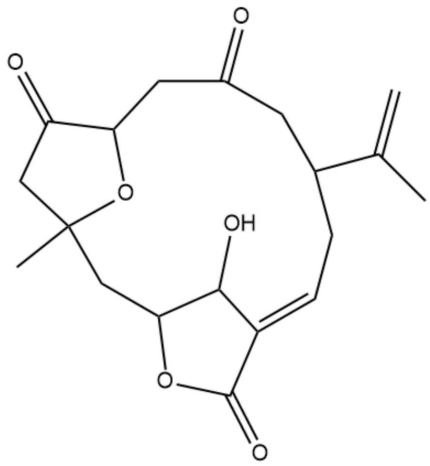

Figure 5: Structure of UNPD192494 (6E)-17-hydroxy-1-methyl-9-prop-1-en-2-yl-4,16-dioxatricyclo [11.2.1.1] heptadec-6-ene-5,11,14-trione

Table 4: Key interactions between UNPD192494 (dark grey) and PDE 5 protein structure (PDB: 1UDT)

Compound	No. of Interaction	Residues
UNPD192494	3	Pi-interactions: Leu804, Phe820 Carbon Hydrogen: His613

binding affinity after each modification (Table 5). Overall, compound 9 proved to be the best among the 14 newly designed compounds. Although the binding affinity towards PDE 4 enzyme had decreased from -9.7 kcal/mol to -9 kcal/mol, the binding affinity of PDE 5 enzyme had slightly increased from -10.1 kcal/mol to -10.2 kcal/mol. The binding affinity of compound 10 towards PDE 4 enzyme had decreased from -9.7 kcal/mol to -8.8 kcal/mol however, the binding affinity of PDE 5 enzyme had increased from -10.1 kcal/mol to -10.9 kcal/mol (Table 5).

Table 5: structural modifications of UNPD192494 with its binding affinity to PDE 4 and PDE 5 enzymes

Compound	R_1	R_2	Binding affinity, ΔG (kcal/mol)	
			PDE 4	PDE 5
UNPD192494	OH	=O	-9.7	-10.1
1	F	=O	-8.8	-9.8
2	COOH	=O	-8.3	-9.6
3	$CONH_2$	=O	-8.5	-9.6
4	NH_2	=O	-8.3	-9.5
5	OH	OH	-8.5	-9
6	OH	NH_2	-8.5	-9.6
7	OH	COOH	-8.6	-9.6
8	OH	$CONH_2$	-8.6	-9
9	=O	OH	-9	-10.2
10	=O	COOH	-8.8	-10.9
11	=O	$CONH_2$	-8.3	-9.9
12	=O	NH_2	-8.9	-10.1
13	=O	F	-8.3	-9.7
14	$NHCH_3$	=O	-8.2	-8.7

Conclusion

Computational studies are a cost-effective and reliable approach to search for lead compounds. Ligand-based pharmacophore modelling was applied since it is a very efficient approach for virtual screening and lead identification. The pharmacophore models generated consisted of hydrophobic and hydrogen bonding features, the models were further validated with good sensitivity, specificity, and ROC. The validated pharmacophore models were used for virtual screening of UNPD, and this led to identification of UNPD192494 as lead for the development of dual PDE4/5 inhibitor. The interaction between target protein and ligands were visualised and analysed to guide the optimisation of the structure of UNPD192494. Compound 9 demonstrated good affinity for both PDE4 and PDE5 and it can be a good candidate compound for further investigations and development.

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