The rational search for PDE10A inhibitors from *Sophora flavescens* roots using pharmacophore- and docking-based virtual screening

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Abstract. Phosphodiesterase 10A (PDE10A) has been confirmed to be an important target for the treatment of central nervous system (CNS) disorders. The purpose of the present study was to identify PDE10A inhibitors from herbs used in traditional Chinese medicine. Pharmacophore and molecular docking techniques were used to virtually screen the chemical molecule database of *Sophora flavescens*, a well-known Chinese herb that has been used for improving mental health and regulating the CNS. The pharmacophore model generated recognized the common functional groups of known PDE10A inhibitors. In addition, molecular docking was used to calculate the binding affinity of ligand-PDE10A interactions and to investigate the possible binding pattern. Virtual screening based on the pharmacophore model and molecular docking was performed to identify potential PDE10A inhibitors from *S. flavescens*. The results demonstrated that nine hits from *S. flavescens* were potential PDE10A inhibitors, and their biological activity was further validated using literature mining. A total of two compounds were reported to inhibit cyclic adenosine monophosphate phosphodiesterase, and one protected against glutamate-induced oxidative stress in the CNS. The remaining six compounds require further bioactivity validation. The results of the present study demonstrated that this method was a time- and cost-saving strategy for the identification of bioactive compounds from traditional Chinese medicine.

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Introduction

Phosphodiesterases (PDEs) are a family of enzymes that are able to lyse phosphodiester bonds, are expressed widely, and have demonstrable clinical significance (1,2). PDE10A has been demonstrated to be a potential target for the treatment of central nervous system (CNS) disorders (3,4). Previous studies have confirmed that PDE10A inhibitors have important biological activity in the treatment of psychosis (5-7). Therefore, screening for PDE10A inhibitors is an effective strategy for the treatment of CNS disorders.

The roots of *Sophora flavescens* (Sophorae radix) have been used as an herbal medicine for thousands of years in East Asian countries. They have been demonstrated to possess diverse pharmacological properties, including antitumor, antioxidant, anti-inflammatory, antiapoptotic, vasodilatory and CNS regulatory functions (8-15). Different types of compounds, including triterpenoids, flavonoids and quinolizidine alkaloids, have been isolated from the roots of *S. flavescens* (16-19). In order to identify natural PDE10A inhibitors, virtual screening based on a pharmacophore model and molecular docking was performed to identify the chemical molecule database of *S. flavescens*. In addition, literature mining was performed to validate the biological activity of the top-ranking hits from the virtual screening. A total of two hits were reported to have inhibitory activity against cyclic adenosine monophosphate (cAMP) phosphodiesterase, while one exhibited protective effects against glutamate-induced oxidative stress in the CNS. The computational methods used in the present study efficiently identified PDE10A inhibitors from the roots of *S. flavescens*. Therefore, this screening method and workflow may be applied to other traditional Chinese medicines in the search for potential bioactive compounds.

Materials and methods

Chemical molecule database of S. flavescens. In order to establish the chemical molecule database of *S. flavescens*, the traditional Chinese medicine database (Chinese Academy

of Sciences, Beijing, China; 2009) was searched using '*Sophora flavescens*' as the key term from the plant sources. A total of 78 molecules that were retrieved from *S. flavescens*, including kushenol B, kushenol E, kosamol A, kosamol Q and kushenol X, were downloaded and saved in Mol2 format. The two-dimensional structures were individually converted to three-dimensional molecular conformations using the CONCORD module in SYBYL X-1.2 (Tripos Inc., St. Louis, MO, USA). All the hydrogen atoms were subsequently added, and the energy optimization was performed using the Tripos force field in SYBYL X-1.2. The chemical molecule database of *S. flavescens* was built as a UNITY hit list file in SYBYL X-1.2.

Pharmacophore-based virtual screening. A diverse dataset of 30 experimentally identified PDE10A inhibitors was retrieved from the published literature (20-24). The molecules were drawn using the ISIS-Draw (version 2.5; MDL Informations Systems, Inc., San Ramon, CA, USA) software and energy-optimized using Tripos force field. The hydrogen atoms were added and Merck Molecular Force Field 94 charges were assigned using SYBYL X-1.2. All the molecules were saved in the mol2 format. From the optimized molecules, six (Fig. 1) were selected to generate pharmacophore models of PDE10A inhibitors, and they all met the following criteria: i) Structural diversity; ii) high inhibitory effect against the PDE10A target; and iii) similar binding mode of inhibition. The other 24 molecules were used to access the generated pharmacophore models.

All molecular modeling studies were performed on a Dell Red Hat Linux workstation using the Common Feature Pharmacophore Generation protocol in Discovery Studio (version 3.5; Accelrys, San Diego, CA, USA). The pharmacophore models were generated using the HIPHOP module. All compounds were energy minimized using the CHARMM force field. A principal value of 2 and a maximum omit feature value of 0 was assigned to the six compounds in the training set. Hydrogen-bond donor (HBD), hydrogen-bond acceptor (HBA) and hydrophobic (HY) features were selected during pharmacophore generation. In order to access the generated pharmacophore models, 100 known PDE10A inhibitors obtained from the published literature and 300 non-PDE10A inhibiting compounds were used as a validation set (25-27). A total of four evaluation parameters [A%, Y%, identified effective index (N), and comprehensive appraisal index (CAI)] proposed from previous work were calculated to access the generated pharmacophore models, according to the following formulae (28): A%=Ha/Ax100; Y%=Ha/Htx100; N=HaxD/HtxA; CAI=NxA%.

Where D and A represent the total number of molecules used (n=400) and the total number of known PDE10A inhibitors (n=100) in the validation set, respectively. Ht and Ha represent the number of hits obtained using the pharmacophore-based virtual screening and those from the 100 known PDE10A inhibitors, respectively. N indicates the ability to recognize the known PDE10A inhibitors compared with non-PDE10A inhibiting compounds. CAI expresses the comprehensive ability to discover PDE10A inhibitors from a specific database (28).

The pharmacophore model with the highest CAI value was used to screen the chemical molecule database of *S. flavescens* using the Search 3D Database module in Discovery Studio version 3.5 with the default setting. The fit value was calculated to indicate the pharmacophoric match between the query and the hits, with a higher value implying that a better alignment was obtained between the hit and pharmacophore model (29).

Table I. Assessment of generated pharmacophore models.

Model	Ht	Ha	A, %	Y, %	N	CAI
Model_1	139	69	69.00	49.64	1.99	1.37
Model_2	**171**	**101**	**101.00**	**59.06**	**2.36**	**2.39**
Model_3	141	83	83.00	58.87	2.36	1.95
Model_4	174	92	92.00	52.87	2.12	1.95
Model_5	154	80	80.00	51.95	2.08	1.66
Model_6	160	76	76.00	47.50	1.90	1.44
Model_7	161	84	84.00	52.17	2.09	1.75
Model_8	153	81	81.00	52.94	2.12	1.72
Model_9	160	94	94.00	58.75	2.35	2.21
Model_10	176	88	88.00	50.00	2.00	1.76

Ht, number of hits obtained using pharmacophore-based virtual screening; Ha, number of hits obtained from known PDE10A inhibitors; N, ability to recognize known PDE10A inhibitors compared with non-PDE10A inhibiting compounds; CAI, comprehensive ability to discover PDE10A inhibitors from a specific database. PDE10A, phosphodiesterase 10A; N, identified effective index; CAI, comprehensive appraisal index.

Molecular docking-based virtual screening. The molecular docking-based virtual screening was performed using a Surflex-Dock module, which has been successfully utilized for molecular docking and binding free energy calculations (30-33). The x-ray crystal structure of PDE10A has been resolved (Protein Data Bank no. 2O8H) and, therefore, it was selected as the docking protein (34). The PDE10A protein model was cleaned by removing the co-crystallized water molecules and adding hydrogen atoms. The Gasteiger-Hückel charges were assigned using SYBYL X-1.2. An energy optimization was performed using Tripos force field for 1,000 iterations in SYBYL X-1.2 with the default parameters.

In order to verify the reliability of the docking protocol established in the present study, the co-crystallized PDE10A inhibitor (ligand 227; $C_{24}H_{29}N_5O_4S$) was extracted and re-docked into the active site of PDE10A. The active site of PDE10A was defined as the ProtoMol generated using the steric hydrophobic (CH_4) and hydrogen bond (C=O) groups, and the hydrogen acceptor (N-H) within 0.5 Å of the ligand 227 binding site.

All compounds from the chemical molecule database of *S. flavescens* were docked into the active site of PDE10A in turn using the Surflex-Dock module. The total score was calculated for each compound following running of the Surflex-Dock module. A higher total score implied an increased binding affinity between the protein and the ligand, based on an empirically derived scoring function.

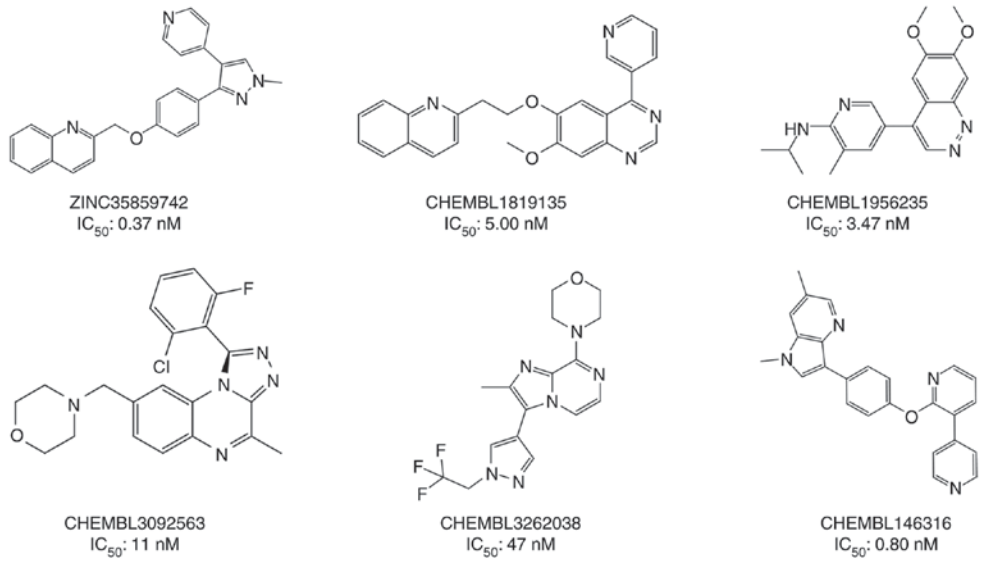

Figure 1. Molecular structures of known phosphodiesterase 10A inhibitors used in pharmacophore model generation. IC_{50}, half-maximal inhibitory concentration.

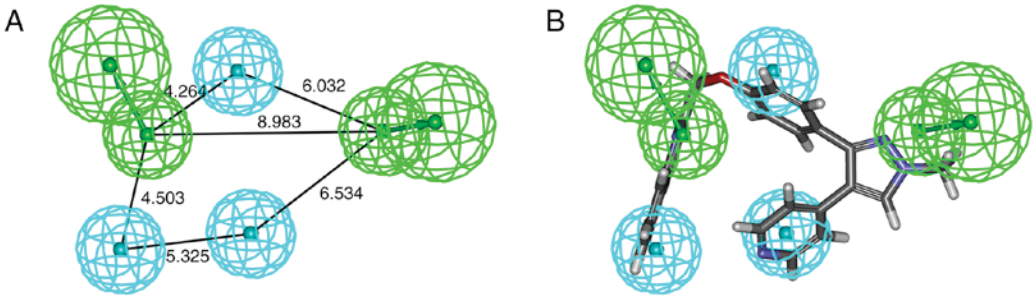

Figure 2. Pharmacophore-based virtual screening. (A) The pharmacophore Model_2 of phosphodiesterase 10A inhibitors; numbers represent the distances between two pharmacophore features. (B) The matching pattern between pharmacophore model_2 and ZINC35859742. Arrows represent the direction of the hydrogen bond groups. Grey, red, blue and yellow atoms represent carbon, oxygen, nitrogen and sulfur atoms, respectively.

Results

Pharmacophore-based virtual screening. Ten HIPHOP models were generated and model assessment studies (Table I) indicated that Model_2 (Fig. 2A) had the highest CAI and N values (Table I). Therefore, Model_2 was used to screen the chemical molecular database of *S. flavescens*. Model_2 contained two HBA (marked in green) and three HY (marked in cyan). The best active compound (ZINC35859742) was able to map all features of Model_2 with a fit value of 2.23 (Fig. 2B). A total of 32 hits were obtained following the pharmacophore-based virtual screening against the chemical molecule database of *S. flavescens*. The top 20 hits ranked by fit value are presented in Table II.

Molecular docking-based virtual screening. The root-mean-square deviation (RMSD) of the conformations between the re-docked and co-crystallized ligand 227 (Fig. 3) was calculated as 2.6 Å, which indicated that the difference between the co-crystal and re-docked conformation of ligand 227 was very small. The docking protocol established in the present study has a strong ability to reproduce the co-crystal conformation and binding mode of PDE10A inhibitors. The molecular docking analysis of the chemical molecule database of *S. flavescens* and the PDE10A protein resulted in a hit list of 14 molecules with total scores >6.0 (Table III).

Ligand-protein binding mode analysis is important for the study of molecular interactions, binding affinity and active ingredient identification from traditional Chinese medicine (35,36). The binding mode between PDE10A and kosamol Q and kosamol A (Fig. 4A and B, respectively) was investigated.

Discussion

The combination of three-dimensional pharmacophore modeling and molecular docking in the present study demonstrated marked advantages for identifying direct PDE10A inhibitors from the chemical molecule database of *S. flavescens*. The three-dimensional pharmacophore model focused on quick generation of the common characteristics of the known PDE10A inhibitors, while the molecular docking method was able to rapidly calculate the binding force between the small-molecule ligands and the target protein. In

Table II. Hits obtained using pharmacophore-based virtual screening.

ID	Name	QFIT
1	Isokurarinone	4.03
2	Leachianone A	4.03
3	Kushenol X	3.80
4	Kosamol Q	3.73
5	Kushenol C	3.67
6	Kuraridinol	3.57
7	2'-Methoxykurarinone	3.51
8	Kuraridin	3.50
9	Kushenol P	3.48
10	Norkurarinol	3.36
11	Kurarinone	3.32
12	Kushenol G	3.31
13	Neokurarinol	3.09
14	Sophoraflavanone G	3.05
15	Kushenol E	2.60
16	Kosamol V	2.47
17	Kosamol A	2.45
18	Kushenol B	2.42
19	Kushenol L	2.35
20	Kushenol K	2.11

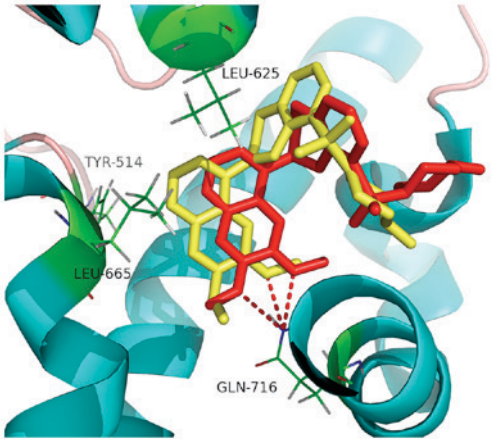

Figure 3. Binding conformation of the co-crystallized (yellow) and re-docked (red) ligand 227 at the active site of phosphodiesterase 10A.

addition, molecular docking was able to provide the binding conformations between the ligands and the PDE10A protein, which is important for the identification of active compounds from traditional Chinese herbs, in addition to the modification and optimization of molecular structures.

According to the virtual screening based on the pharmacophore model and molecular docking, nine compounds were obtained as hits. These nine hits were kushenol B, kurarinone, sophoraflavanone G, kosamol Q, kosamol A, kushenol X, neokurarinol, kushenol E and kuraridinol. It has been reported that kushenol B and kurarinone have *in vitro* inhibitory activity against cAMP phosphodiesterase, and their

Table III. Hits obtained through docking-based virtual screening.

Compound	Name	Total score
1	Kosamol Q	8.82
2	Kosamol A	8.39
3	Kushenol X	8.08
4	Kurarinol	7.34
5	Neokurarinol	7.21
6	Kurarinone	7.21
7	Norkurarinone	7.09
8	Kushenol E	7.02
9	Kosamol S	6.92
10	Sophoraflavanone G	6.91
11	Kuraridinol	6.90
12	Kushenol O	6.89
13	Kushenol B	6.72
14	5-O-Methyl kushenol C	6.69

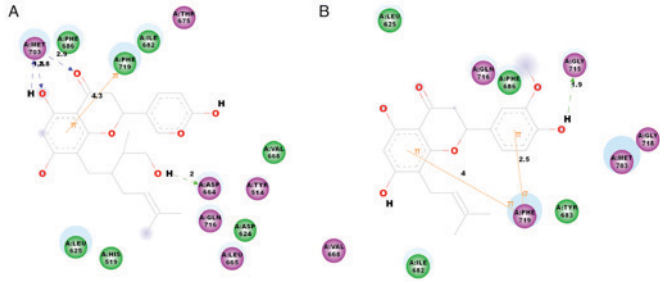

Figure 4. Ligand-protein binding mode analysis. Phosphodiesterase 10A docking interactions with (A) kosamol Q and (B) kosamol A.

half-maximal inhibitory concentrations were determined as 31 and 25 μmol/l, respectively (37). The CNS protective effect of four lavandulyl flavanones isolated from *S. flavescens* was examined and sophoraflavanone G was observed to exhibit the function of protecting HT22 immortalized hippocampal cells against glutamate-induced oxidative stress (38). Therefore, the published data confirmed, to a certain extent, the predictions of the computational model approach used in the present study. The models additionally elucidated the recognition mode of the intermolecular actions between the compounds and PDE10A.

In conclusion, pharmacophore- and molecular docking-based virtual screening provided an effective approach to identify PDE10A inhibitors from Chinese medical herbs, and nine molecules were determined to be potential inhibitors. In addition, molecular docking is a feasible strategy to characterize the interactions of the natural ingredients from *S. flavescens* with the PDE10A target. In particular, kosamol compounds, including kosamol Q and kosamol A, which exhibit pharmacological effects, including CNS protectant activities, may be promising PDE10A inhibitors. These compounds required further investigation, including additional bioactivity evaluation.

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