Supplementary Material

Virtual Screening of Protein Kinase C Inhibitors from Natural Product Library to Modulate General Anesthetic Effects

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Abstract: Protein kinase C (PKC) plays a key role in neurotransmission in the central nervous system, and targeting PKC kinase domain is considered as a strategy to modulate the anesthetic effects. In the current study, we described a synthetic pipeline to perform high-throughput virtual screening against a large library of 3D structural natural products released recently in order to discover those potential PKC modulators. A total of 100 natural products with top scores were raised, from which 12 promising candidates were tested to determine their inhibitory potencies agonist PKC. As might be expected, the promiscuous kinase inhibitor staurosporine showed a high PKC inhibitory activity ($IC_{50} = 64$ nM), and other two tested compounds, *i.e.* fisetin and tetrahydropapaverine, were also highly potent with their activities at nanomolar level ($IC_{50} = 370$ and 190, respectively).

Materials and Methods

Compound data

3D structural natural product library (CamMedNP). A total of 2147 natural products and their derivatives were extracted from the CamMedNP library [Ntie-Kang et al., 2013]. The library is composed of various molecular entities including terpenoids, flavonoids, alkaloids, xanthenes, glycosides and quinines, which had been previously isolated from 224 plant species from 55 plant families. Ntie-Kang and co-workers demonstrated that these compounds are structurally diverse by comparing with the ChemBridge diverse database, and are medicinally interesting by valuating their “drug-likeness” using Lipinski’s “Rule of Five”.

Structure-based data set of kinase-inhibitor affinities (SBKIA). Very recently, Wang et al. [2014] have systematically surveyed the BindingDB database [Liu et al., 2007] to compile a distinct data set of kinase-inhibitor complexes with both known affinities and solved structures. All these complexes have high-resolution structures and high or moderate affinities. The kinases in this set of complexes are diverse in terms of the biological roles they play, with their phosphorylations functioning in immune regulation, cell signaling, disease progression, and differentiation and proliferation. The SBKIA is tabulated in Table S1.

Homology modeling and molecular docking

The three dimensional structure of human PKC δ kinase domain was still unavailable in the PDB database [Berman et al., 2000], and thus we herein employed homology modeling technique to construct the structure from the high-resolution template of human PKC θ complexed with inhibitor staurosporine (PDB: 1XJD). The PKC isoforms δ and θ both belong to the phospholipid-dependent subfamily and they are highly conserved in primary sequence and biological function. The sequence of human PKC δ was retrieved from the UniProt database [Uniprot, 2010] under the accession Q05655, which was modeled into advanced structure based on PKC θ template using the Modeller program [Fiser and Sali, 2003]. In the procedure the inhibitor staurosporine was grafted into the active pocket of the modeled PKC δ kinase

domain to define active-site residues in the pocket that can directly contact ligand molecules. Before the docking the polar hydrogen atoms and Kollman charges were assigned using the ADT program [Morris et al., 2009]. Subsequently, the AutoDock Vina [Trott and Olson 2010] was employed to implement fast docking of natural products into the active pocket of PKC δ kinase domain, which considered flexible effects associated with the ligand molecules and protein active-site residue. The AUDocker LE [Sandeep et al., 2011] was used to facilitate the high-throughput docking of various natural products into the PKC δ active pocket.

Consensus scoring

A number of widely used scoring functions, including force field-based potentials, empirical potentials and knowledge-based potentials, were employed to evaluate binding strength for kinase–natural product complexes. The force field-based potentials are developed based on physical atomic interactions, including van der Waals contacts, electrostatic interactions and bond stretching/bending/torsional forces [Huang et al., 2010]. The empirical potentials are based on counting the number of various types of interactions between the protein receptor and its ligands, and use linear regression fit to determine their weight coefficients. The knowledge-based potentials are derived from the structural information embedded in experimentally determined atomic structures where pairwise potentials are directly obtained from the occurrence frequency of atom pairs in a database using the inverse Boltzmann relation.

Kinase assay

A protocol described in a previous work [Yuan et al., 2009] was modified here to perform kinase activity assay for the inhibited PKC δ . Briefly, the purified PKC δ protein with 5 μ M inhibitor compounds, 50 μ M ATP and 40 μ M PKC δ substrate were added to initiate the reactions in the buffer (50 mM Tris/HCl, 5 mM MgCl₂, 100 μ M Na₃VO₄, 100 μ M Na₄P₂O₇, 1 mM NaF and 100 μ M PMSF). After 30-min incubation at 30 °C, 50 μ l of Kinase-Glo Plus Reagent was added to each well. The plate was mixed and incubated at room temperature for 10 min. The luminescence signal was measured on a Wallac Vector counter. The inhibitory activity IC₅₀ of each sample was

tested for duplicate.

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Table S1. The structure-solved, affinity-known kinase–inhibitor systems collected by Wang et al. (2014)

Protein kinase	PDB	Resolution (Å)	K_d (nM)
3-Phosphoinositide-Dependent Protein Kinase 1 (PDK1)	1OKY	2.3	1.7
3-Phosphoinositide-Dependent Protein Kinase 1 (PDK1)	1UU3	1.7	700
3-Phosphoinositide-Dependent Protein Kinase 1 (PDK1)	3HRF	1.9	10000
Mitogen-activated protein kinase kinase 1 (MEK1)	3ORN	2.8	0.24
Mitogen-activated protein kinase kinase 1 (MEK1)	3EQG	2.5	0.4
ABL Kinase	2GQG	2.4	0.3
ABL Kinase	2HZI	1.7	0.7
ABL Kinase	3PYY	1.85	2
ABL Kinase	2F4J	1.91	5
ABL Kinase	3PYY	1.85	10000
Aurora Kinase A	3UO6	2.8	15
Aurora Kinase A	2X81	2.91	6500
Aurora Kinase A	3UOJ	2.9	51
Aurora Kinase A	3UNZ	2.8	16
Aurora Kinase A	3UO5	2.7	39
Aurora Kinase A	3UP2	2.3	40
Aurora Kinase A	3E5A	2.3	4
Aurora Kinase A	2X81	2.91	6.5
Aurora Kinase A	3UOH	2.8	13
Aurora Kinase A	3UO6	2.8	15
Aurora Kinase A	3UNZ	2.8	16
Aurora Kinase A	3UO5	2.7	39
Aurora Kinase A	3UP2	2.3	40
Aurora Kinase A	3UOD	2.5	49
Aurora Kinase A	3E5A	2.3	4100
Aurora Kinase A	2X81	2.91	6500
Aurora Kinase B	4AF3	2.75	7.4
B-Raf Protein Kinase	2FB8	2.9	0.16
B-Raf Protein Kinase	3C4C	2.57	100
B-Raf Protein Kinase	1UWH	2.95	260
Breakpoint cluster region protein/Tyrosine-protein kinase ABL	3K5V	1.74	92
c-Jun N-terminal kinase 3 (JNK3)	3FI2	2.28	25
cAMP-Dependent Protein Kinase (PKA)	3BWJ	2.3	319
cAMP-dependent protein kinase beta-1 catalytic subunit	1STC	2.3	29
cAMP-dependent protein kinase beta-1 catalytic subunit	2UZT	2.1	90
Cyclin-Dependent Kinase 2 (CDK2)	2FVD	1.85	0.53
Cyclin-Dependent Kinase 2 (CDK2)	2VU3	1.85	30
Cyclin-Dependent Kinase 2 (CDK2)	4FKR	1.9	36
Cyclin-Dependent Kinase 2 (CDK2)	4FKW	1.8	47
Cyclin-Dependent Kinase 5 (CDK5)	1UNL	2.2	1900
Cyclin-Dependent Kinase 9 (CDK9)	3BLR	2.8	6.4

Checkpoint Kinase (Chk1)	1NVR	1.8	3.2
Dual specificity protein kinase TTK	2ZMD	2.88	98
EGF-R Tyrosine Kinase	4G5J	2.8	0.1
EGF-R Tyrosine Kinase	4HJO	2.75	0.35
EGF-R Tyrosine Kinase	4I22	1.71	0.52
p38 MAP Kinase	3HV6	1.95	310
p38 MAP Kinase	3O8T	2.0	350
p38 MAP Kinase	2YIS	2.0	0.001
p38 MAP Kinase	1KV2	2.8	0.046
Mitogen-Activated Protein Kinase 10 (JNK3)	1UKI	2.7	22
Mitogen-Activated Protein Kinase 10 (JNK3)	3NPC	2.35	62
Tyrosine-protein kinase JAK3	3LXK	2.0	0.16
Phosphoinositide 3-Kinase (PI3K)	2Y3A	2.9	16
Phosphoinositide 3-Kinase (PI3K)	1E8Z	2.4	1800
Phosphorylase kinase gamma subunit 2	2Y7J	2.5	5.9
PIM-1 Kinase	1YHS	2.15	3.2
PIM-1 Kinase	2J2I	1.9	50
Polo-Like Kinase 1	2RKU	1.95	0.19
Protein kinase B (Akt 1)	3D0E	2.0	2.2
Proto-oncogene tyrosine-protein kinase (cSrc)	3D7T	2.9	8
Proto-oncogene tyrosine-protein kinase (cSrc)	3QLG	2.75	11
Ribosomal S6 Kinase 1 (RSK-1)	2Z7R	2.0	43
p70 Ribosomal S6 Kinase 1	3A60	2.8	1.3
Src Kinase	3QLG	2.75	11
Src Kinase	3F3V	2.6	73
Src Kinase	3QLG	2.75	210
Tyrosine Kinase BMX	3SXR	2.4	1.4
Tyrosine-protein kinase (BTK)	3OCS	1.8	1.5
Tyrosine-protein kinase ITK/TSK	3MIY	1.67	13
Tyrosine-protein kinase JAK1	3EYG	1.9	1.6
Tyrosine-protein kinase LCK	3KMM	2.8	0.2
Tyrosine-protein kinase LCK	2PL0	2.8	40
Tyrosine-protein kinase YES	3QLG	2.75	0.3
Tyrosine-protein kinase TYK2	3LXN	2.5	4.8
Tyrosine-protein kinase FES	4E93	1.84	4.8
Tyrosine-protein kinase (BTK)	3OCS	1.8	1.5

Table S2. A list of the 12 promising natural products

Natural product	Chemical structure	Consensus score	IC ₅₀ (μM)
Bergenin		-1.96	>1000
Honokiol		-1.88	110
Fisetin		-2.06	0.37
Myricitrin		-2.17	12
Rutaecarpine		-2.25	>1000
Staurosporine		-2.14	0.064
Tetrahydropapaverine		-2.07	0.19
Theobromine		-1.89	860

Aloin		-2.34	320
Indirubin		-2.20	>1000
Puerarin		-2.53	57
Chrysophanic acid		-2.18	>1000