Chemogenomics Knowledgebase and Systems Pharmacology for Hallucinogen Target Identification — Salvinorin A as a Case Study

by

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Drug abuse is a serious problem worldwide. Recently, hallucinogens have been reported as a potential therapy for substance abuse. However, the use of hallucinogens as a drug abuse treatment itself has potential risks. The true mechanisms of hallucinogens are not clear. Thus it is necessary to investigate the mechanism of hallucinogens to make sure they are safe to develop as medicine. So far, no scientific database is available for the mechanism research of hallucinogens.

We constructed a hallucinogen-specific chemogenomics database by collecting chemicals, protein targets and pathways closely related to hallucinogens. This information, together with our established computational chemogenomics tools, such as TargetHunter and HTDocking, provided a one-step solution for the mechanism study of hallucinogens.

We chose salvinorin A as an example to demonstrate the usability of our platform. Salvinorin A is a potent hallucinogen extracted from the plant *Salvia divinorum*. It was the first reported non-nitrogenous kappa opioid receptor agonist. Recently, researchers found that oral

administration of salvinorin A can affect drug choice in a monkey model, which suggested a potential use of salvinorin A as an abuse-deterrent formulation. However, some complex effects of salvinorin A were reported, including depersonalization or laughing hysterically. Our aim is to identify the potential targets of salvinorin A to further explore the mechanisms of its complex effects.

With the help of HTDocking program, we predicted four novel targets for salvinorin A, including muscarinic acetylcholine receptor 2, cannabinoid receptor 1, cannabinoid receptor 2 and dopamine receptor 2. We looked into the interactions between salvinorin A and the predicted targets, and compared their binding modes with the known ligands of these proteins. The similar binding modes, interactions and high docking scores indicate that salvinorin A may interact with these four predicted targets. In the future, we will design experiments or find collaborators to validate our predictions. At the same time, we will continuously enrich our hallucinogen-specific chemogenomics database by adding newest data and building more 3D homology models.

Key words: hallucinogen, salvinorin A, drug abuse, chemogenomics database, cloud computation, target identification, systems pharmacology, homology modeling, natural product, drug discovery.

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PREFACE

I would like to thank my advisor Dr. Xiang-Qun (Sean) Xie for his guidance and encouragement. I also appreciated the great instructions from Dr. Lirong Wang, Dr. Zhiwei Feng and Dr. Peng Yang. Thanks to all of our group members for their daily with studies and life in general. I would especially like to thank our lab member Ms. Guanxing Hu and Shifan Ma for their great help with this project. Thanks to all the committee members Dr. Xiang-Qun (Sean) Xie, Dr. Levent Kirisci and Dr. Lirong Wang for the valuable suggestions. This project is funded by NIDA (P30 DA035778A1) from Xiang-Qun (Sean) Xie's lab. We thanks to Bryan L. Roth from NIDA for his enlightenment of this project.

The completion of my master's study couldn't be achieved without the love from my family and my friends. I am grateful for their support every single day. Without my mother's encouragement, I would not have come to the United States to study. With her selfless love, now I have become independent, optimistic and strong. I would especially like to thank my grandmother. The wisdom she gives me is like a beacon lighting the way. I wish my grandmother and grandfather health and happiness every day.

1.0 INTRODUCTION

1.1 DRUG ABUSE

Drug abuse is a serious problem, killing about 200,000 people worldwide each year according to a new United Nations (UN) report. In 2009, alcohol, tobacco and illicit drugs together contributed to 12.6% of all deaths worldwide. Besides the calculation of deaths, the summary of disability-adjusted life-years (DALY) among the population can be considered as a measurement of the gap between the current health status and an ideal health situation. Table 1 shows the deaths and DALY caused by the illicit drugs, alcohol and tobacco in 2009. The percentage of DALY for substance abuse is 9.0, which means about one tenth of a person's healthy life time was lost because of drug abuse (1). Drug abuse threatens health, family, and social life significantly (2). According to a US government report, many people are struggling to overcome an addiction to drugs. The percentage of population who have ever used illicit drugs was rising continuously and arrived at 9.4 in 2013. According to Figure 1, using illicit drugs rose from 2002 to 2013 and seems likely to increase in the future, which means more people will be influenced (3). At this point, the analysis of existed treatments and the exploration of new therapy for drug abuse are necessary and urgent.

Table 1. The deaths and DALY caused by the illicit drugs, alcohol and tobacco in 2009 (Modified from World Drug Report 2012)

	Illicit drugs	Alcohol	Tobacco	Total
Deaths (millions)	0.25	2.3	5.1	7.6
Deaths (percentage)	0.4	3.6	8.7	12.6
DALY (millions)	13.2	69.4	56.9	139.5
DALY (percentage)	0.9	4.4	3.7	9.0

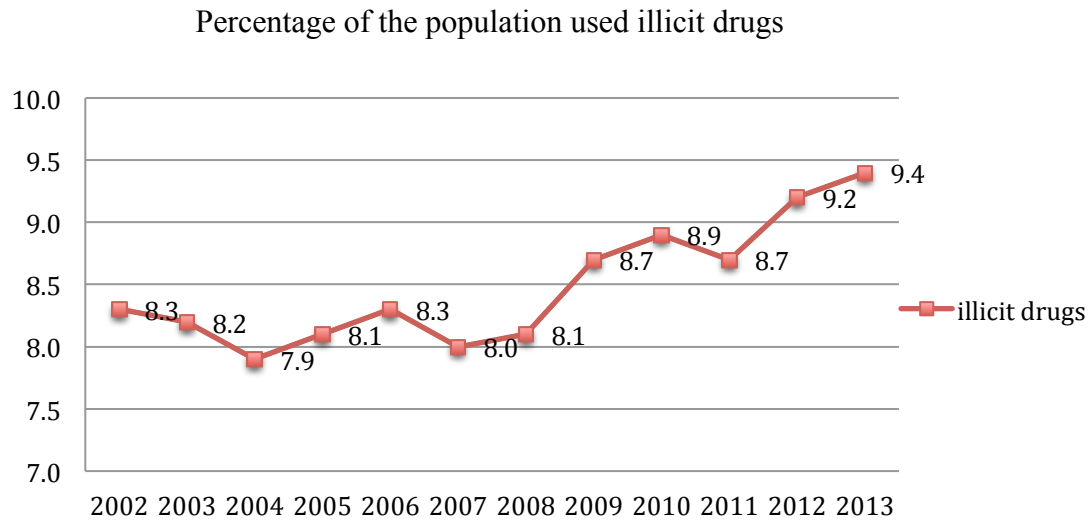


Figure 1. Percentage of the population using illicit drugs. The trend of people who have ever used illicit drugs was summarized from 2002 to 2013. It was continuously rising and arrived at 9.4% in 2013. (Modified from drugabuse.gov)

We collected and analyzed the existing treatments for drug abuse. This data was downloaded from the drug abuse (DA) chemogenomics knowledgebase (DA-KB) (http://www.cbligand.org/CDAR) (4). Sixteen drugs were approved either by FDA or EU for drug abuse treatments like opioid-related disorders, drug overdose, alcoholism and withdrawal

delirium. The approved drugs include Nalorphine, Nikethamide, Lofexidine, Fosenazide, Naltrexone and so on. Nine drugs are withdrawn or discontinued from the market. Many of them have serious side effects like tachycardia, ventricular rhythm disorders, severe depression and so on. Compared to the number of approved drugs, more drugs are in clinical trials, especially on the phase II clinical trial. However, less drugs are investigated in other clinical trials (Figure 2).

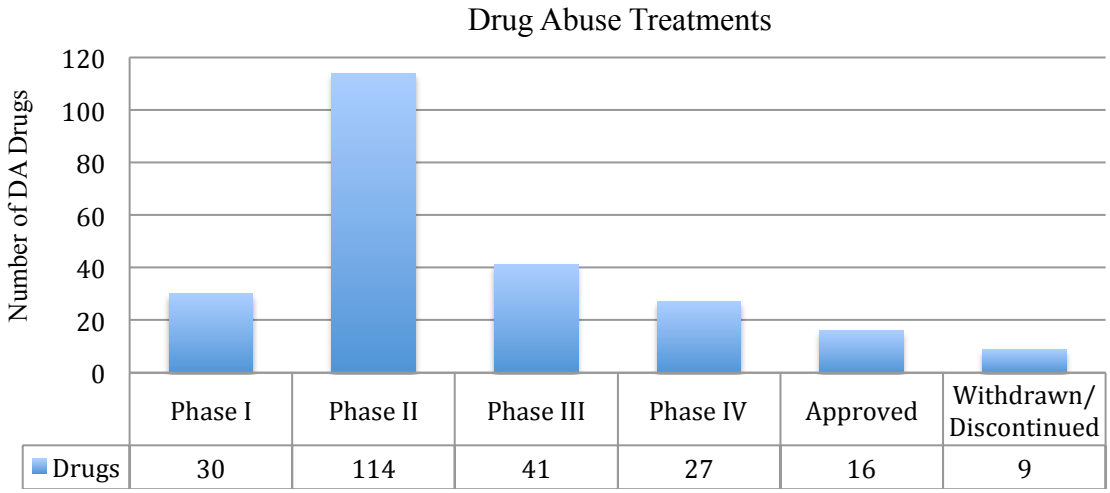
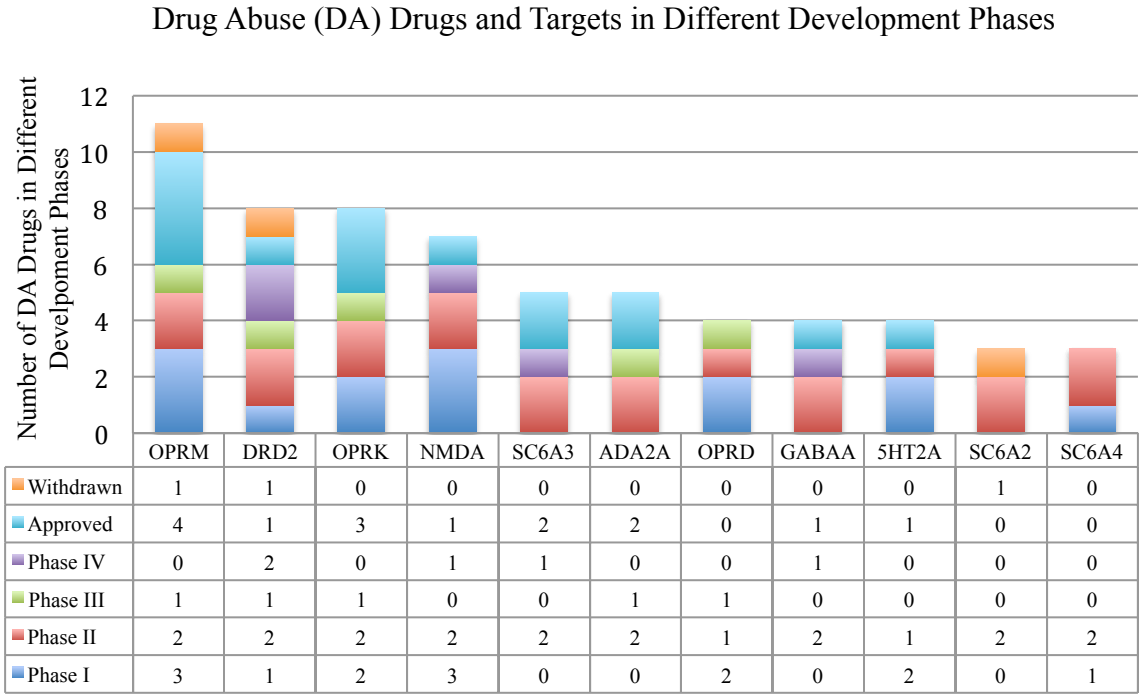


Figure 2. Drugs in different clinical trials that used for drug abuse treatments. Sixteen drugs were approved either by FDA or EU including Nalorphine, Nikethamide, Lofexidine, Fosenazide, Naltrexone and so on. Nine drugs are withdrawn or discontinued from the market for serious side effects like tachycardia, ventricular rhythm disorders, severe depression and so on. Many drugs are under clinical trials, especially on the phase II clinical trial.

We looked deep into different treatments and their therapeutic targets for drug abuse treatments with multiple targets involved. We summarized the treatments of drug abuse and divided them into different groups based on the specific therapeutic targets of each drug. As shown in Figure 3, the most commonly considered targets for drug abuse treatments were opioid

receptors (OPR), dopamine receptors (DRD), N-methyl-D-aspartate receptors (NMDA) as well as sodium-dependent dopamine transporters (SC6A3).



	OPRM	DRD2	OPRK	NMDA	SC6A3	ADA2A	OPRD	GABAA	5HT2A	SC6A2	SC6A4
Withdrawn	1	1	0	0	0	0	0	0	0	1	0
Approved	4	1	3	1	2	2	0	1	1	0	0
Phase IV	0	2	0	1	1	0	0	1	0	0	0
Phase III	1	1	1	0	0	1	1	0	0	0	0
Phase II	2	2	2	2	2	2	1	2	1	2	2
Phase I	3	1	2	3	0	0	2	0	2	0	1

Figure 3. Treatments of drug abuse in different clinical trials were divided based on specific therapeutic targets. Mu opioid receptor (OPRM), Dopamine receptor 2 (DRD2), Kappa opioid receptor (OPRK) were the top three commonly considered therapeutic targets for drug abuse treatments. N-methyl-D-aspartate receptors (NMDA), sodium-dependent dopamine transporter (SC6A3), sodium-dependent serotonin transporter (SC6A4), other subtypes of DRD, 5HTR, OPR and cannabinoid receptors (CNR) were also considered to be important targets for treatments of drug abuse.

Developing a good drug for addiction treatment is still a big challenge. Although some famous hypotheses tried to explain the mechanisms of addiction, the underlying mechanisms are still complex and unclear. The most commonly accepted reason to explain why people are

continuously trying to seek drugs is the reward effect (5). The sense of euphoria promotes repeated drug use, leading to vulnerable individuals, unable to stop taking drugs over and over again. Common addiction circuitry is in the limbic system of brain. The well-known pathway is the VTA-NAc pathway. Drugs directly or indirectly activate the dopaminergic transmission in the NAc. Some drugs can directly stimulate dopaminergic pathway, causing an increase of dopaminergic transmission. Some directly act on opiates receptors on NAc. Some indirectly act on opiates receptors, inhibit GABAergic interneurons in VTA, which disinhibit dopaminergic transmission (Figure 4) (6, 7). The abuse effect of some drugs was reduced in the opioid and cannabinoid receptors knockout mice, which indicate that these drugs may also activate endogenous opioid and cannabinoid systems (8-10).

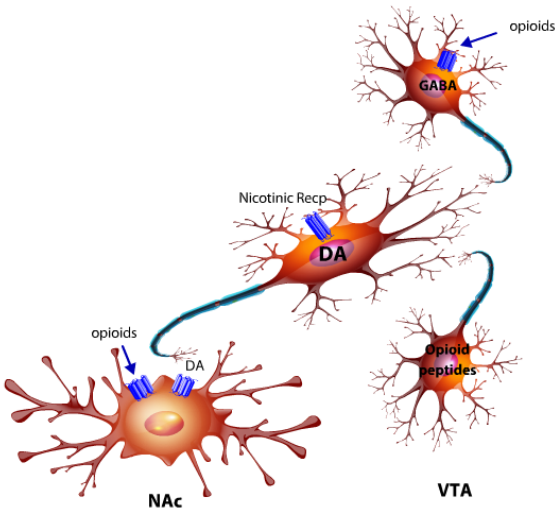


Figure 4. Simple diagram of VAT-NAc pathway. Drugs directly or indirectly activate dopaminergic transmission in the NAc. Some can directly stimulate the dopaminergic pathway, causing an increase of dopaminergic transmission. Some directly act on opiates receptors on NAc. Some indirectly act on opiates receptors, inhibiting GABAergic interneurons in VTA, which disinhibit dopaminergic transmission. (Modified from Nestler EJ. Nature neuroscience. 2005;8(11):1445-9.)

Another point of view is that the anti-reward effect can dramatically impact the addiction progress (11). Different from trying to maintain the happy feeling from taking drugs, the anti-reward effect emphasizes the dysphoria without drugs that force people to use drugs again. Dysregulation of the brain's reward system and anti-reward system cause abnormal emotion that lead to the pathology state (11-13).

Drug abuse is a complex and serious problem that needed to be paid attention to worldwide. Multiple targets contributed to the drug abuse. The exact mechanism of drug abuse is still unclear. The existed therapy of drug abuse faces the challenges including not curable, serious withdrawn symptoms and so on. It is necessary and urgent to study drug abuse and find potential therapy.

1.2 HALLUCINOGENS

Hallucinogens are powerful psychoactive substances that can modify the users' mood, perception, consciousness, cognition and behavior. This class of compounds is considered to be safe physiologically and less possible to form addiction (14).

1.2.1 Therapeutic value and side effects of hallucinogens

Although hallucinogens are in the early stage of study for medical use, some applications already exist in the history. People have tried to use hallucinogens to treat substance abuse. For example, a study showed that lysergic acid diethylamide (LSD) was used to combat alcoholism

in 1950s and LSD, which was recently confirmed to significantly reduce alcohol consumption up to six-months (15). Recently, Dr. Matt Johnson's group was engaged in a program to investigate the effect of psilocybin in long-term smokers and, so far, has gotten promising results (16). Ketamine was used to mitigate alcoholism and heroin addiction in 1985 and showed promising results (17). An observational study documenting the Ayahuasca, in which the major ingredient is dimethyltryptamine (DMT), was used to treat addiction in Canada. Participants showed a decreased use of alcohol, tobacco, and cocaine as well as significant improvements in hopefulness, empowerment, and mindfulness (18).

Table 2. Therapeutic value of hallucinogens

Hallucinogen	Therapeutic value
Psilocybin	Terminal illness, Obsessive-compulsive disorder, Alcoholism, Smoking cessation
LSD	Obsessive-compulsive disorder, Alcoholism, Substance abuse, Terminal illness,Tools for cognitive neuroscience,
Bufotenin	Protection against burn, tourniquet and endotoxin shock
Ketamine	Antidepression, Pain, Mood disorder, Post-traumatic epilepsy, Anesthesia
Salvinorin A	Pain, Mood and personality disorders, Substance abuse, Gastrointestinal disturbances

Hallucinogens have other therapeutic potentials like in the treatment of mood, obsessive-compulsive, gastrointestinal disorders and pain (Table 2). A preclinical study showed mice with pretreatment of bufotenin whom obtained great protection from burn, tourniquet and endotoxin shock (19, 20). Several case studies reported that hallucinogens like LSD, psilocybin or

mescaline improved symptoms of obsessive-compulsive disorder (21, 22). LSD and psilocybin can also dramatically reduce anxiety and stress among patients at the end of the cancer stage. Recently, Heffter's research institute generated a Phase III clinical study to investigate the effects psilocybin in end-stage cancer patients (14, 16, 23). Ketamine is a popular club drug; however, this cannot detract from its great therapeutic value. Many studies pointed out its anti-depressive effect (24). It can also act as an analgesic for its block of N-methyl-D-aspartate receptors (25). New applications of ketamine emerg nowadays, such as the less established treatment for post-traumatic epilepsy, anesthesia in the emergency department and operating theater setting (25, 26).

Table 3. Drugs for pathological laughing

Class	Drug
Serotonin Reuptake Inhibitors (SSRIs)	Fluoxetine
	Paroxetine
	Citalopram
	Escitalopram
	Sertraline
Tricyclic Antidepressant (TCAs）	Nortriptyline
	Amitriptyline
	Imipramine
Other Antidepressants	Reboxetine
	Venlafaxine
	Mirtazapine
	Lamotrigine
Dopaminergic Drugs	Levodopa
	Methylphenidate
	Dexamfetamine
Uncompetitive NMDA Receptor Antagonists	Dextromethorphan/quinidine
	Amantadine

However, using hallucinogens to treat drug abuse has its potential risks, for example, researchers found some patients would be addicted to ketamine itself after treatments (27). Hallucinogens also have different hallucinogenic effects that bother people a lot. For example, salvinorin A has side effects like that people feel loss of control of the body or hysterical laugher (14). In this point of view, we believe that instead of the well-known kappa opioid receptor (OPRK), other off-targets contribute to those side effects. For targets collection purpose and the safe problem of further therapy, we collected the drugs clinically used for pathological laughing (Table 3).

It was our objective to study the mechanism of hallucinogens and then to further make sure that they are safe to develop. Mechanism study is also beneficial for figuring out the strategies to avoid side effects. The more we know about their mechanisms, the more we can know of their possible therapeutic values. So far, among all the targets involved, the serotonin receptors are the most important ones when it refers to the mechanism of hallucinogens. Almost all literature regarding the mechanism hallucinogen mentioned them. For example, serotonin receptor 2A (5HT2A) is the known target of many hallucinogens like LSD, ketamine, 2,5-Dimethoxy-4-methylamphetamine (DOM) and psilocybin (14, 28, 29). However, hallucinogen is a broad class containing nearly fifty natural or synthetic compounds. Every kind of hallucinogen shared little overlap of mechanisms and most of them are unclear. To solve this problem, we applied computational technologies that offer the advantages of approach target identification more quickly and at a lower cost. Our goal is to build a hallucinogen specific chemogenomics knowledgebase and apply computational technologies for mechanisms research.

1.2.2 Salvinorin A

Salvinorin A is the major ingredient of a Mexican plant, *Salvia divinorum*, that is a potent hallucinogen. It has potential therapeutic value for treating pain, mood and personality disorders, substance abuse and gastrointestinal disturbances (30). The Freeman's group has designed an experiment using salvinorin A as a punisher of drug self-administration in monkeys. Compared to the administration of cocaine alone, the average percent of choice for the salvinorin A associated lever decreased dose dependently among all monkeys. It means that moneys were more likely to choose the lever with cocaine alone. This phenomenon was more obvious when cocaine was given associated with higher dose of salvinorin A as punisher. They arrived at the conclusion that salvinorin A can punish drug choice, which suggested a potential use in the development of abuse-deterrent formulations (31).

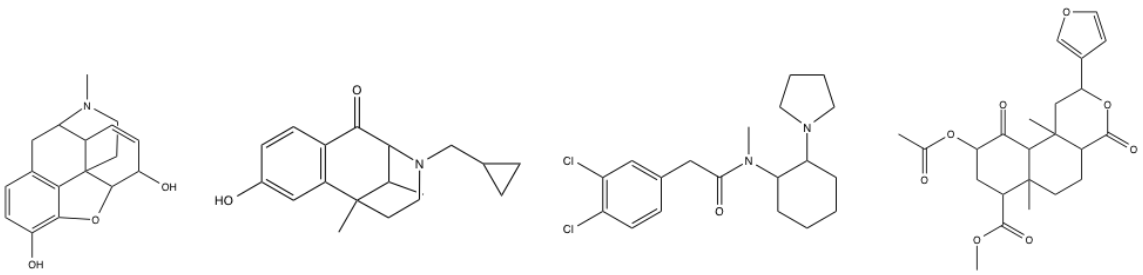


Figure 5. Ligands of opioid receptors. Salvinorin A is a non-nitrogenous opioid receptor agonist that shared little structural similarity with other ligands of opioid receptors.

The well-known target of salvinorin A is OPRK. The structure of salvinorin A is special. Compared to other opioid ligands, salvinorin A shared little structural similarity (Figure 5). It is

the first reported non-nitrogenous opioid receptor agonist. It is also the first known psychoactive diterpenoid. The structure information gave scientists a hint that nonalkaloids, such as salvinorin A, may be potential scaffolds for developing new drugs targeting on G-protein coupled receptors (GPCRs) (32).

However, some reports pointed out that some users experienced a psychic depersonalization condition, which is a unique sensation of being disconnected from one's body (32, 33). A double-blind, placebo-controlled study of vaporized/ inhaled Salvinorin A in eight hallucinogen-experienced adults showed that about half of the participants exhibited a positive affect (audible laughter) during peak drug effects and one described his feeling "I found myself hysterically, almost uncontrollably laughing." (34)

Based on the complex effects of salvinorin A listed above, we believe the concept of "multiple drugs, multiple targets interactions" can provide reasonable explanations. We hypothesized that in addition to the OPRK, other potential targets can also interact with salvinorin A. These interactions may contribute to the complex effects of salvinorin A.

2.0 METERIALS AND METHODS

2.1 HALLUCINOGEN SPECIFIC KNOWLEDGEBASE

To provide a comprehensive platform for various kinds of hallucinogens mechanism studies, we did data-mining of all the information. We collected hallucinogenic related compounds, proteins, signaling pathways, therapeutic targets and their associated bioassay data from literature and various public databases. Then, we compiled them into our in-house hallucinogen specific knowledgebase, where stored in a cloud computing server: *www.cbligand.org/hallucinogen*. This method generated by our lab is not a new one but gradually matured. Recently, our lab published two paper based on it, which are the drug abuse knowledgebase (DA-KB) (4) and the Alzheimer's disease knowledgebase (AlzPlatform: http://www.cbligand.org/AD) (35). The features of our Hallucinogen specific knowledgebase are listed below:

- **Database infrastructure and web interface**

Hallucinogen specific knowledgebase is designed with a MySQL database (http://www.mysql.com) and an apache web server (http://www.apache.org) with OpenBabel

(36) at the back end as the search engine for chemical structures. The web interface is written in PHP language (http://www.php.net).

- **Data collection and content**

The hallucinogenic related targets data was collected from literatures and a variety of public databases including the ChEMBL database (37), DrugBank (38), ClinicalTrials.gov (39), BindingDB (40) and SuperTarget (41). The 3D crystal structures of targets were collected from Protein Data Bank (42, 43), while the related signaling pathways were gathered from the KEGG pathway database (44). After the data collection, all these chemical structures, bioactivity values, pathways, bioassays, and references were imported into our hallucinogen specific knowledgebase.

- **Chemo-informatics Tools**

To facilitate the *in silico* mechanism study of hallucinogen and drug design for their potential therapies for drug abuse, the hallucinogen specific knowledgebase cloud computing server also integrates diverse chemo-informatics tools based on state-of-the-art machine learning algorithms developed by our group or from public resources. The functions of our knowledgebase are designed into the following modules: 1) properties explorer (http://www.cbligand.org/hallucinogen/Property_Explorer.php); which can predict the chemical properties of interested molecules based on structures; 2) blood-brain barrier (BBB) predictor (http://www.cbligand.org/BBB), which can analyze the drug-likeness of the interested small

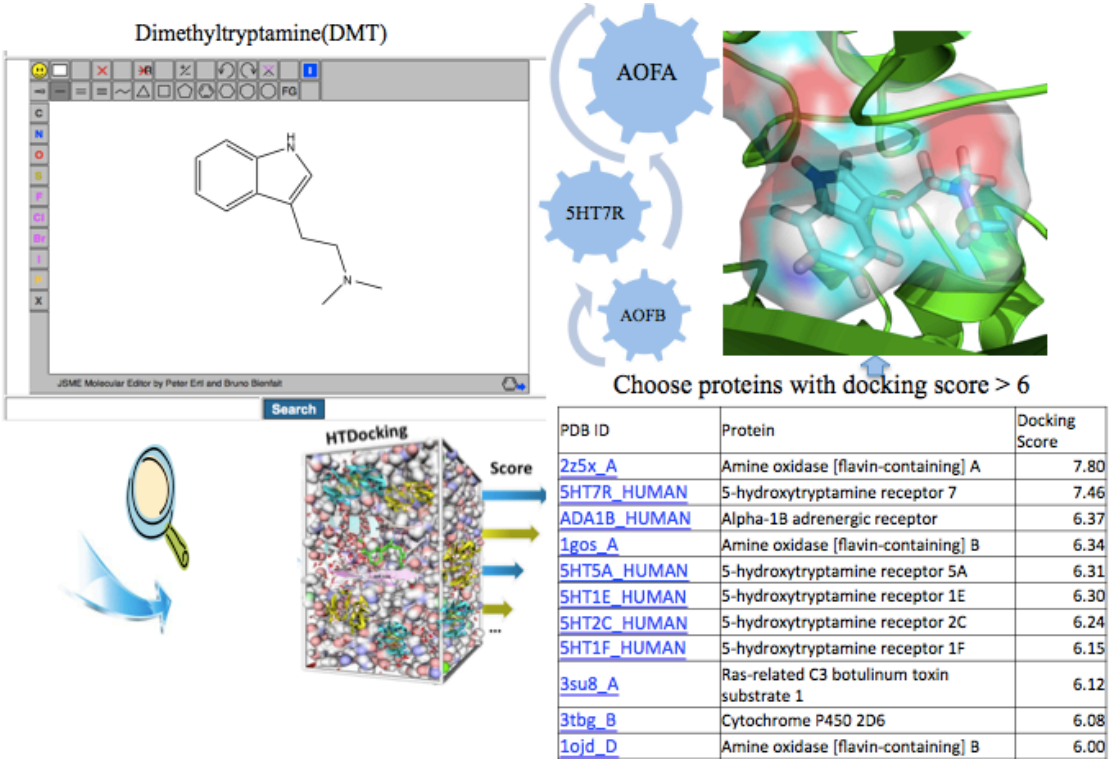
molecules; 3) PAINS predictor (http://www.cbligand.org/PAINS); 4) toxicity predictor (http://www.cbligand.org/Tox), which allows exclusion of molecules with potential safety issue in the early phase of drug discovery. In this thesis, we mainly applied two of our developed systems pharmacological analysis tools, HTDocking and TargetHunter, which core functions in our hallucinogen specific knowledgebase.

2.2 SYSTEMS PHARMACOLOGICAL ANALYSIS TOOLS

- **HTDocking**

Our lab established an online high-throughput docking program, HT-Docking, (http://www.cbligand.org/hallucinogen/docking_search.php) that is a web-interface computing tool that can produce docking procedures automatically (Figure 6). The docking study provided us the information of interactions between compounds and targets. In the current version of hallucinogen specific knowledgebase, crystal structures of hallucinogenic related protein targets have been collected from Protein Data Bank (http://www.rcsb.org/pdb) (43) and for the targets that are lacking crystal structures, homology models are also established by Modeller (45). With all of this information we collected, we built a hallucinogen specific knowledgebase. All of the original ligands are removed from the crystal structures to avoid unnecessary interaction with our interested compounds. Water molecules and ions are removed from the crystal structures if necessary. Pockets are defined according to the co-crystal structure ligands, or automatically predicted by the MOLCAD module implemented in SYBYL-X 1.3 based on our publications

(46-48). After submitting our interested compounds, docking scores are used to assess and rank potential targets. Docking scores are calculated based on the interactions between molecular compounds and targets like hydrogen bonds, van der Waals interactions and hydrophobic interactions. The scores represent the predicted values of $-\log_{10}(K_d)$ of a compound with its predicted targets (49). So we analyzed the results and chose those with docking scores above six, which means the predicted K_d value of this compound with its target should be within the micromole range or even better. We used SYBYL-X 1.3 to further analyze the detailed interactions (50).



PDB ID	Protein	Docking Score
2z5x_A	Amine oxidase [flavin-containing] A	7.80
5HT7R_HUMAN	5-hydroxytryptamine receptor 7	7.46
ADA1B_HUMAN	Alpha-1B adrenergic receptor	6.37
1gos_A	Amine oxidase [flavin-containing] B	6.34
5HT5A_HUMAN	5-hydroxytryptamine receptor 5A	6.31
5HT1E_HUMAN	5-hydroxytryptamine receptor 1E	6.30
5HT2C_HUMAN	5-hydroxytryptamine receptor 2C	6.24
5HT1F_HUMAN	5-hydroxytryptamine receptor 1F	6.15
3su8_A	Ras-related C3 botulinum toxin substrate 1	6.12
3tbg_B	Cytochrome P450 2D6	6.08
1ojd_D	Amine oxidase [flavin-containing] B	6.00

Figure 6. Schematic diagram of HTDocking. Submit structures to the HTDocking website, then the program will automatically define the pockets and do an online docking study. The docking results can be retrieved anytime with the specific website.

- **TargetHunter**

TargetHunter is a ligand-based tool (http://www.cbligand.org/TargetHunter). It was designed based on the principle that structurally similar compounds have great possibility sharing similar targets and biological profiles (51-53). After uploading the interested compounds, TargetHunter can provide not only the targets information, but also structures of the similar compounds and their related bioassays. It can also provide the address information of related bioassays nearby (Figure 7). Detailed descriptions can be found in our published paper (54).

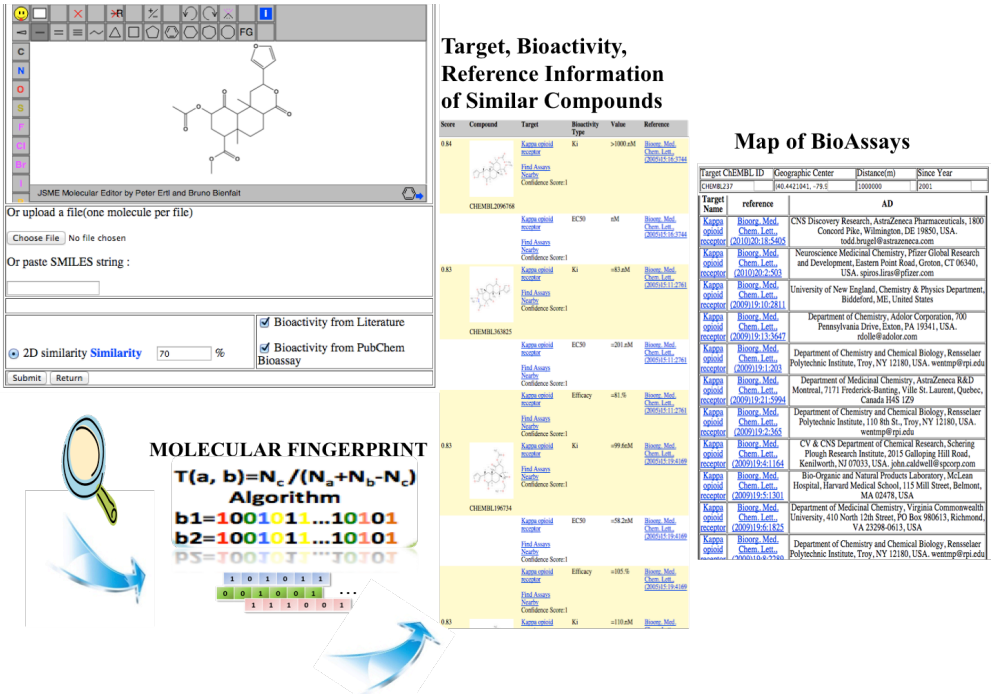


Figure 7. Schematic diagram of TargetHunter. Submit structures to the TargetHunter website and choose the value of similarity, then the program will automatically search similar compounds above that similarity based on the molecular fingerprint. The results will be presented from high to low similarities. TargetHunter will provide information including the structures of similar compounds as well as their targets, bioassays, references and map of the bioassays.

2.3 HOMOLOGY MODELING

For those important targets without crystal structures, we built 3D homology models. In our hallucinogen knowledgebase, 21 GPCR homology models were added. Figure 8 shows a simple schematic diagram of the procedure of how to build 3D homology models. First, the whole sequence of targets were obtained from the UniProtKB / Swiss-Prot (http://www.uniprot.org/uniprot/) (55). The templates were searched from Protein Data Bank according to sequence identities. The 3D homology models were much closer to the natural structures with higher sequence identities. Second, the sequences and templates were aligned using the Modeller 9.12 software (56). In the meantime, we adjusted the alignments according to the literature. Finally, we built homology models. More detailed processes can be found in our published protocol (48). These models can be validated through screening against the known active and inactive compounds of the targets. In our case, this work has been already done previously in our lab (GPCRs database), so we compared our models with those established ones.

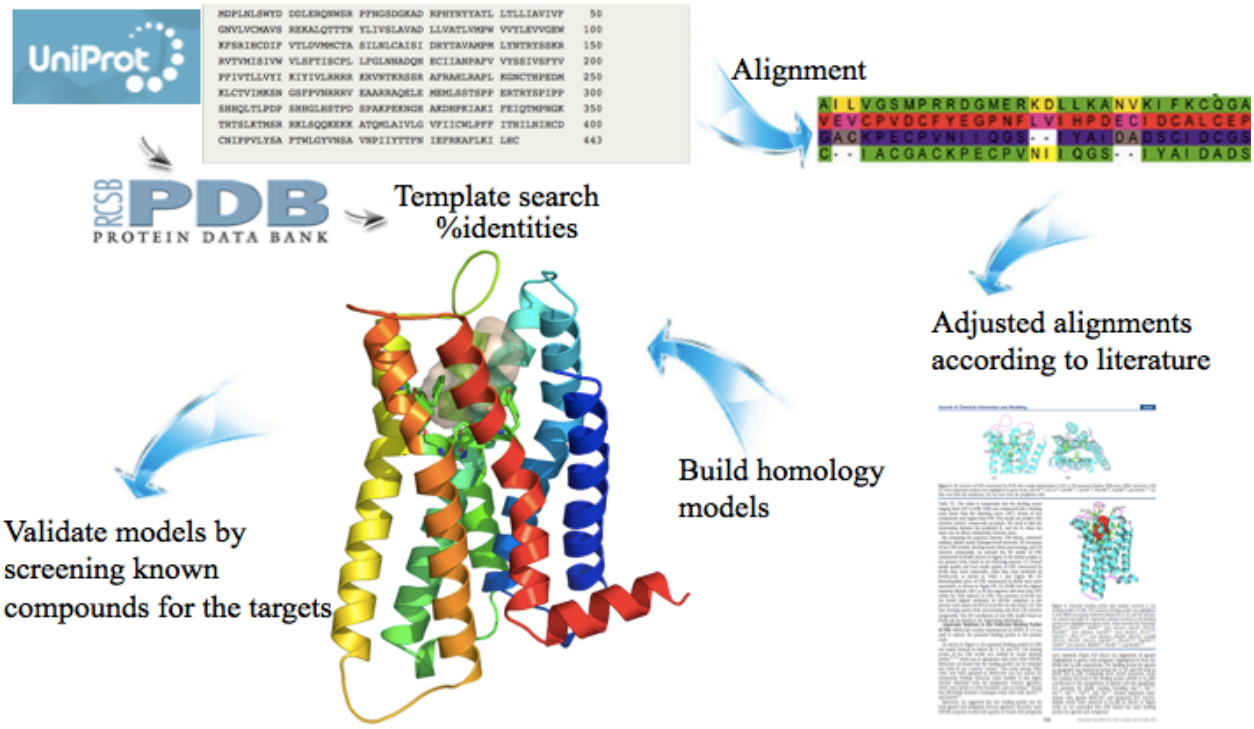


Figure 8. Procedure of 3D homology modeling. First, we searched the whole sequences of proteins from Uniprot. Then we did a template search from the protein data bank according to the sequences identities. Second, we did alignments of the sequences and the templates using the Modeller 9.12 software. Alignments were adjusted according to the literature. Finally, we built homology models. The models were validated by screening known compounds for the targets.

3.0 RESULTS

3.1 GPCR HOMOLOGY MODELS

In our hallucinogen-specific knowledgebase, twenty-one homology models of G-couple protein receptors were established including 5HTR, ACM, OPR, DRD, cannabinoid receptors (CNR) and adrenergic receptors (ADAR). All of them were compared with our in-house data (86 GPCRs Database) to make sure the established models were accurate. For example, Figure 9 shows the alignments between each of five subtypes of ACM with our GPCRs database. The green ones were the models we built in our hallucinogen-specific knowledgebase, where the blue ones were from our 86 GPCRs database. Conserved and important residues within pockets were pointed out for comparison. The positions of the two comparable residues were the same. Most angles of the two comparable residues were almost the same.

Most of GPCRs consisted of two major pockets, the traditional binding site, orthosteric binding pocket and recently discovered allosteric binding site (57). In our situation, we defined the conserved orthosteric binding site of GPCRs for further study.

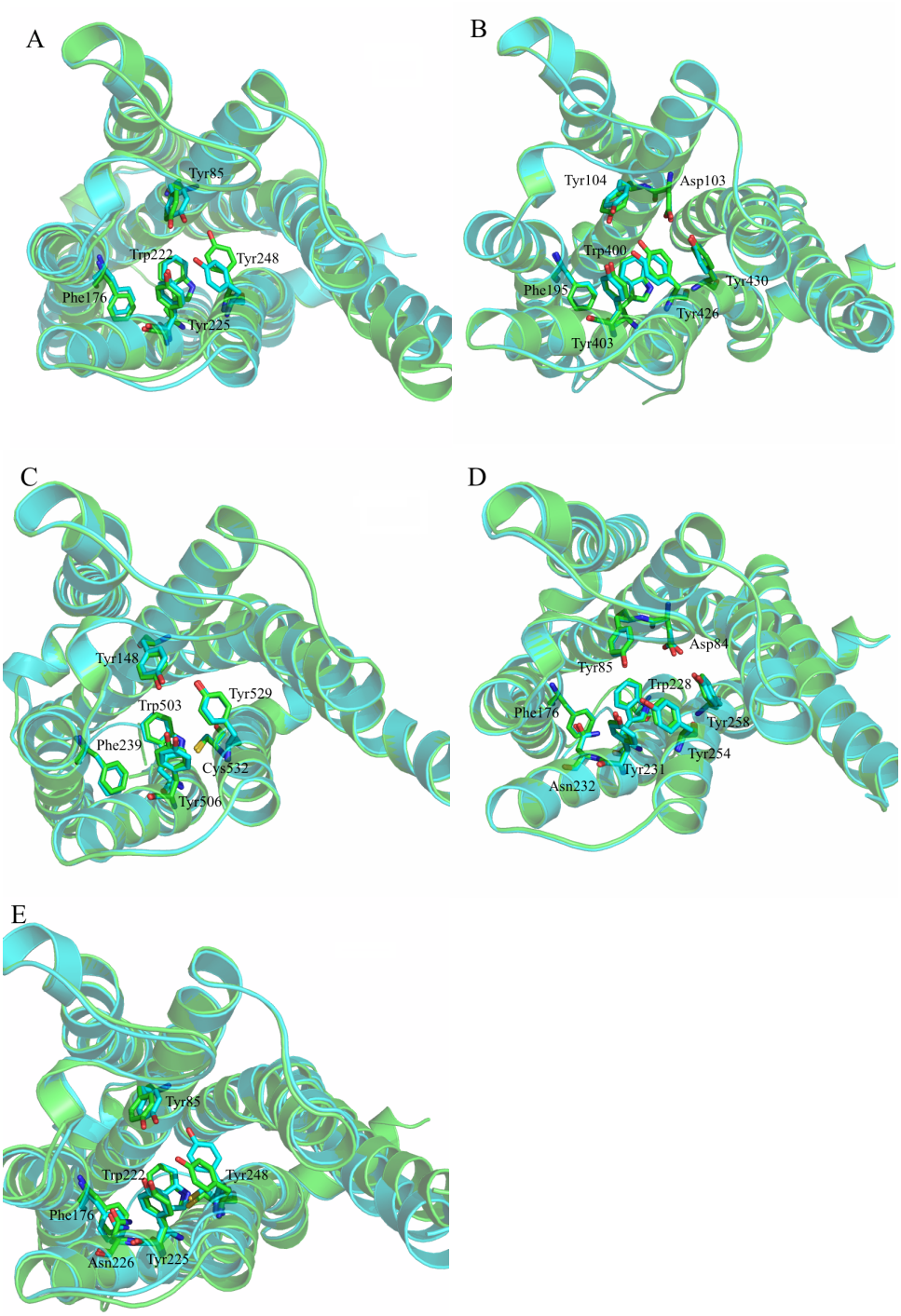


Figure 9. 3D homology models of muscarinic acetylcholine receptor (ACM). The green ones represent the models we built for our hallucinogen-specific knowledgebase. The blue ones represent the models from our 86 GPCRs database. (A) the comparison between the two models of ACM1 and the important residues. Similarly, (B) - (E) the comparisons between the two models of ACM2 to ACM5 and their important residues.

3.2 HALLUCINOGEN RELATED TARGETS AND PATHWAYS

Hallucinogen related chemicals, targets and pathways were collected from literature and databases including PubMed (www.PubMed.org), PubChem (www.Pubchem.org), DrugBank (www.drugbank.ca), ChEMBL (www.ebi.ac.uk/chembl/), KEGG (www.genome.jp/kegg/), PDB (www.pdb.org) and so on. 46 hallucinogenic compounds referring 102 targets and 158 related pathways were collected. The biggest class of targets were the membrane receptors, representing over one third of hallucinogen targets. These membrane receptors included 5HTR, ACM, OPR, DRD, CNR and so on. The second biggest class of targets was enzymes. 30% of hallucinogen related targets were enzymes include amine oxidases (AOF), ras-related C3 botulinum toxin substrates (RAC), surase-isomaltase (SUIS), CYP enzymes and so on. Ion channels occupied a great proportion too. Hallucinogen related targets also included other classes, like transporters, epigenetic regulators, transcription factors and some unclassified proteins (Figure 10).

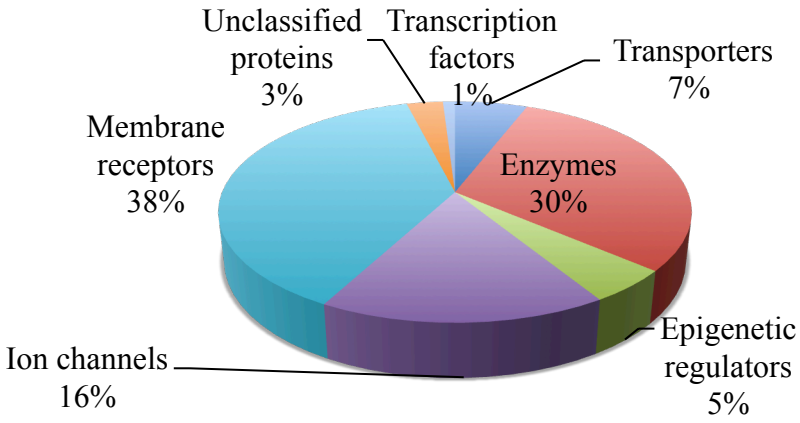


Figure 10. Summary of targets for hallucinogens. 102 hallucinogens related targets were summarized according to 46 hallucinogenic compounds.

Among those hallucinogen related pathways, 5HT2A played an essential role in most of hallucinogen mediated pathways. By now, the two most important and proved pathways were 5HT2A mediated PLC and PLA2 pathways. Figure 11 shows the simple schematic diagram of the 5HT2A mediated PLC pathway, where 5HT2A coupled with Gαq activate PI-specific phospholipase C (PLC). PLC hydrolyzed phosphatidylinositol (PIP2), then generated nositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG). IP3 acted on the IP3 receptor located on the smooth endoplasmic reticulum triggers the release of calcium, where DAG acitivated protein kinase C (PKC) (14, 58).

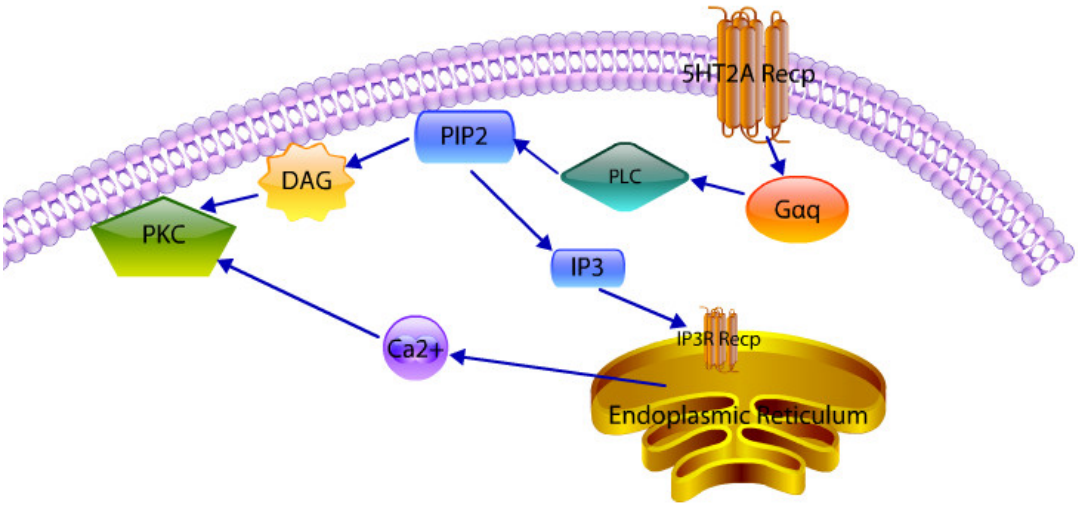


Figure 11. Schematic diagram of the 5HT2A mediated PLC pathway. 5HT2A coupled with Gαq activated PI-specific phospholipase C (PLC). PLC hydrolyzed phosphatidylinositol (PIP2), generated nositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG). IP3 acted on the IP3 receptor triggered the release of calcium, where DAG acitivated protein kinase C (PKC). (Modified from Weinstein H. Drug addiction: springer; 2008. p. 265-86.)

Compared to PLC-mediated signaling, the PLA2 pathway was more complicated. Figure 12 shows the simple schematic diagram of the 5HT2A mediated PLA2 pathway, where 5HT2A

coupled with Gαi/o stimulate phospholipase A2 (PLA2). PLA2 hydrolyzed AA-containing phospholipids and then generated arachidonic acid (AA) and lysophospholipid (14, 59). It was also proposed that the simulation of PLA2 is through a Gαi/o-Ras-Raf-MEK-ERK signaling cascade (60).

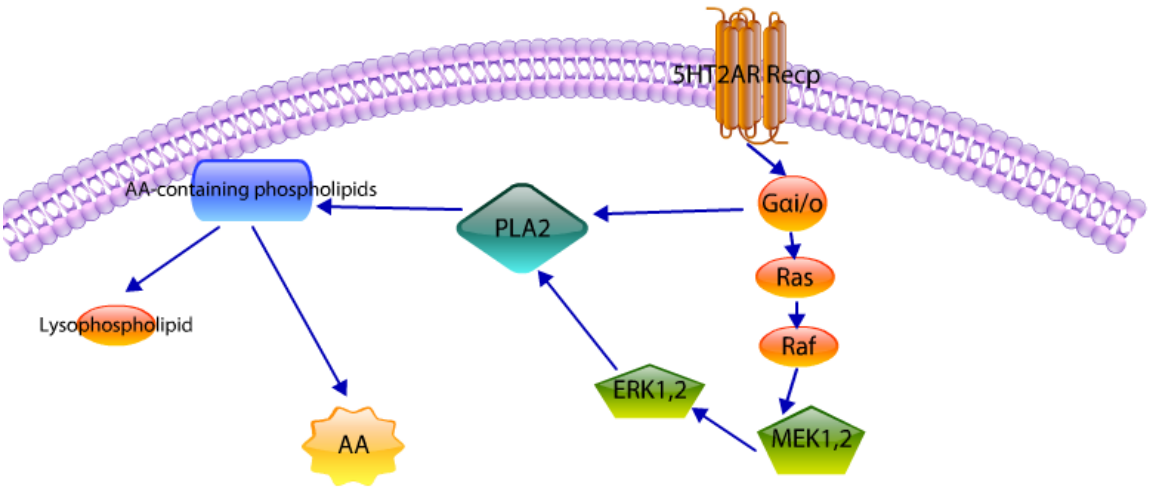


Figure 12. Schematic diagram of the 5HT2A mediated PLA2 pathway. 5HT2A coupled with Gαi/o stimulated phospholipase A2 (PLA2). PLA2 hydrolyzed AA-containing phospholipids, generated arachidonic acid (AA) and lysophospholipid. (Modified from Kurrasch-Orbaugh DM, et al. Journal of pharmacology and experimental therapeutics. 2003;304(1):229-37.)

Recently, the 5HT2A mediated phospholipase D (PLD) pathway was proposed (61). PLD can hydrolyze the terminal diester bond of phosphatidyl choline and then generates phosphatidic acid and choline. It was also involved in a phospatidyl transfer reaction. However, this signaling still needs to be further proved (14).

3.3 SYSTEMS PHARMACOLOGICAL ANALYSIS OF HALLUCINOGENS

Systems pharmacology is the study of drugs, drug-target interactions and drug effects in a macroscopic view, combining large-scale biological experiments data with computational analysis (62). Systems pharmacology focuses on big data analysis, which tends to believe in "multiple drugs, multiple targets interactions", unlike the traditional concept "one drug, one target interaction" (63). In this way, it has obvious advantages of rapid identification of the networks and connections among different drugs and targets. It is a very useful method for quick analysis of drug repurposing. It is also beneficial to analyzing the complex drug adverse effects (62-65).

As described in the method section, we put several hallucinogenic compounds into HTdocking program of Hallucinogen Knowledgebase (*www.cbligand.org/hallucinogen/*). In order to validate our database, we selected five representative hallucinogenic compounds that share little structural similarity to perform the docking results. Based on the docking scores, we drew a map that showed the relationship between compounds and their targets. As shown in Figure 13, five compounds were listed including ketamine, LSD, mescaline, DMT and salvinorin A. All of the targets listed in the map were with docking scores above six, indicated that the K_d values should be within micromole range or even better. For example, the docking score between salvinorin A and the kappa opioid receptor was 7.40. This was congruent with the fact that the kappa opioid receptor is a known target of salvinorin A (*Ki* value of 16nM at OPRK) (66, 67). Similarly, as Figure 13 illustrates, 5HT2A, 5HT6R and 5HT7R were the known targets of DMT. 5HT2A was also the target of mescaline and LSD. 5HT1A was the target of Ketamine,

Mescaline, DMT and LSD. Our docking results matched the information we collected either from literature or databases, which meant this method is reliable.

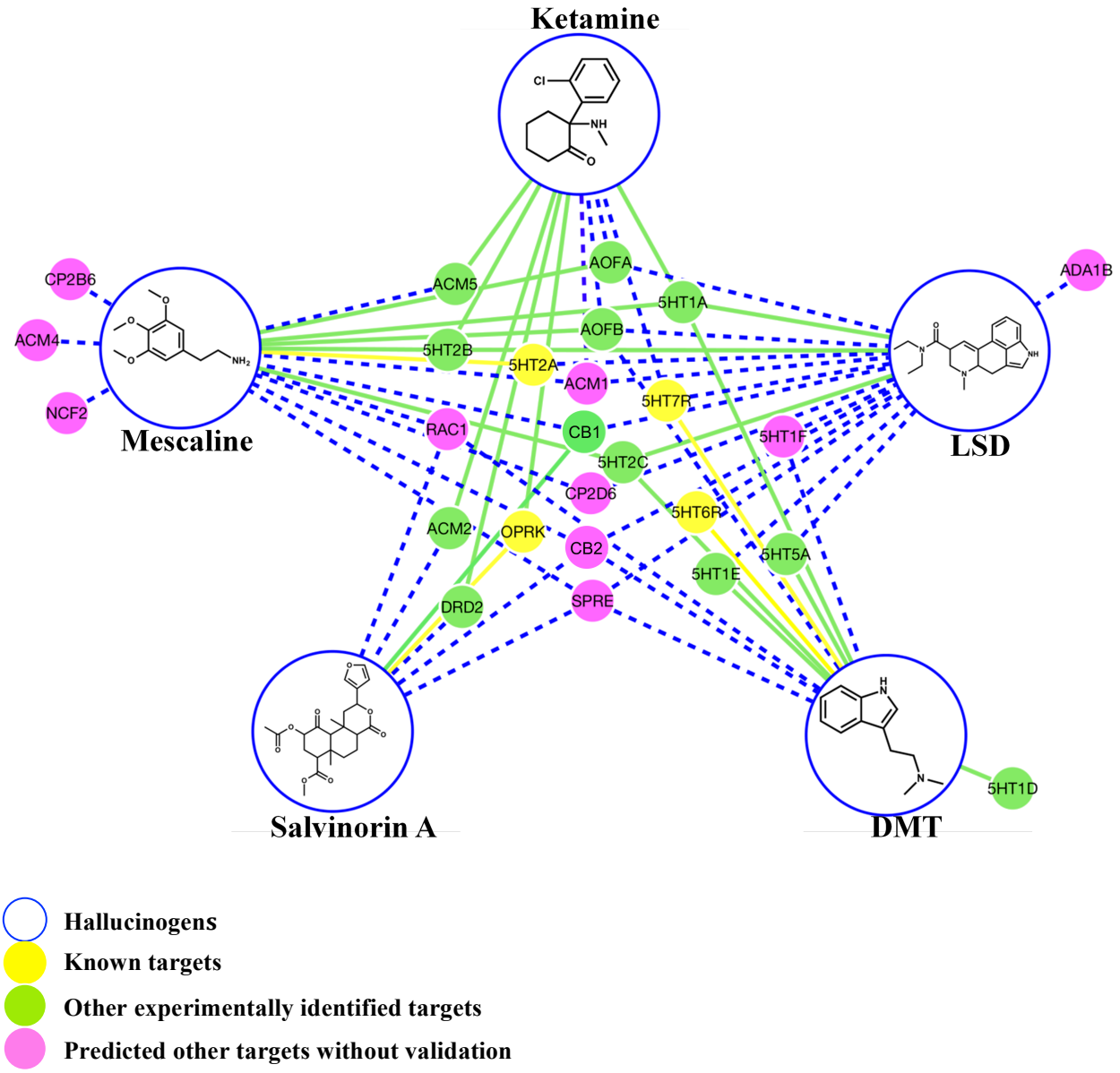


Figure 13. Systems pharmacological analysis of known hallucinogens.

ABBREVIATIONS, 5HT=5-hydroxytryptamine receptor, ACM=Muscarinic acetylcholine receptor, ADA1A=Alpha-1A adrenergic receptor, AOFA=Amine oxidase [flavin-containing] A, AOFB=Amine oxidase [flavin-containing] B, CNR=Cannabinoid receptor, OPRK=Kappa opioid receptor, DRD2=Dopamine receptor 2, CP2B6=Cytochrome P450 2B6, CP2D6=Cytochrome P450 2D6, NCF2=Neutrophil cytosol factor 2, RAC1=Ras-related C3 botulinum toxin substrate 1, SPRE=Sepiapterin reductase, SUIS=Sucrase-isomaltase

However, this method has its own limitations. As we discussed before, all of these validations and predictions were structure-based investigations. Since several targets didn't have crystal structures, we tried to build reliable homology models for the future study. So far, we have built homology models for GPCRs. Compared to other receptors, the structures of GPCRs are well studied and characterized. They are very important for drug developments, especially for CNS drugs. By now, about 40% of drugs currently on the market are targeting GPCRs (68). In our case, 5HTR were very classic targets of hallucinogens. The reliable structures were a footstone for the future study of hallucinogens in depth. But there were still large amounts of structures that need to be established, especially like ion channels and kinase for their complex structures and less characterized features. In our case, the known target of ketamine was glutamate receptor ionotropic, N-methyl-D-aspartate receptor 3A (NMDA 3A). Since lack of its crystal structures, NMDA 3A was not in our map. To enrich our knowledgebase, besides GPCRs, we will build more homology models for the further study.

3.4 SYSTEMS PHARMACOLOGICAL ANALYSIS OF SALVINORIN A

After our hallucinogen knowledgebase validation, we mapped out the interactions between salvinorin A and its related targets. At the same time, we coupled HTdocking results with the results from TargetHunter (Figure 12). Because of the principle, structurally similar compounds had great possibility sharing similar targets and biological profiles (51-53). We further mapped out to see if salvinorin A and similar compounds may share the same predicted targets. In Figure 14, we chose three compounds from TargetHunter that are structurally similar

to salvinorin A, which all the similarities were above 0.70 and targeting the OPRK (CHEMBL196670, *Ki* = 28.7nM; CHEMBL371079, *Ki* = 3.2nM; CHEMBL363825, *Ki* = 83.2nM) (69). The similar compounds tended to interact with CB2 instead of CB1. It might depend on whether they had the amino group or not. From our docking results, we found that ligands with the ester bond instead of the amino group tend to form both hydrogen bonds and charge-charge interactions with Ile150 in extracellular loop 2 region of CB1 (Figure 18). It may explain the possible selection of CB2 instead of CB1 of these two similar compounds (CHEMBL371079 and CHEMBL363825) with the amino group. This idea was supported by literature, from which we noticed that the extracellular loop 2 of CB1 is important for binding. The Bertalovitz's group farther pointed out Ile150 was an important residue of CB1 (23, 70). After the systems pharmacological study, we continued looking in depth into each one and discussed the detailed interactions.

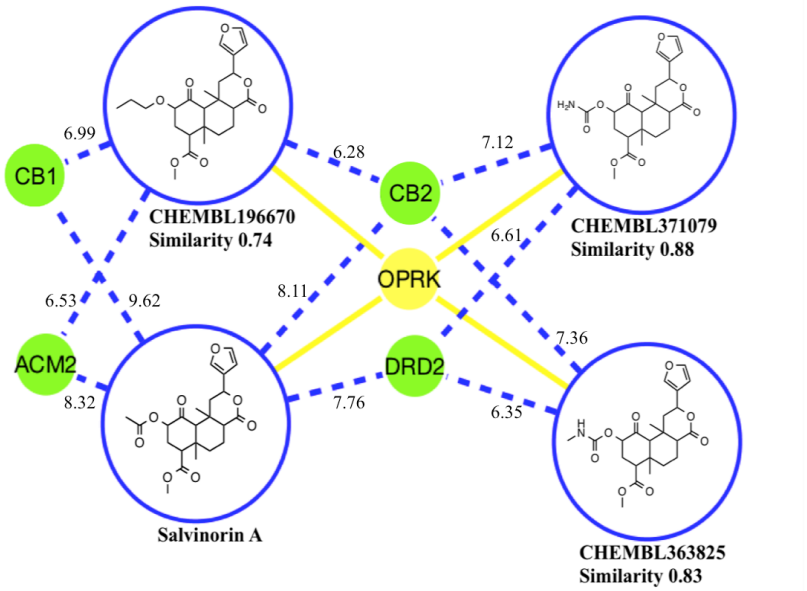


Figure 14. Systems pharmacological analysis of salvinorin A and its similar compounds. Three other similar compounds of salvinorin A were chosen from TargetHunter. Kappa opioid receptor is the known target for all of them. Four other targets including CB1, CB2, ACM2, DRD2 were predicted according to TargetHunter and HTDocking results.

First, the OPRK was the well-known target of salvinorin A. Salvinorin A had high affinity with both cloned OPRK (*Ki* = 16nM) and guinea-pig brain OPRK (*Ki* = 4.3nM) according to radioligand binding assays (66, 67). We used the co-crystal structure of OPRK (PDB entry: 4DJH, resolution 2.90 Å) to do a docking study for salvinorin A. The original ligand, JKTic, was a selective OPRK antagonist with a high affinity (*Ki* = 0.32nM) (71). Figure 15A shows the original interaction within the co-crystal structure. JDTic fitted tightly within the pocket forming ionic, hydrogen bonds and hydrophobic interactions with helix II, III, V, VI and VII. Asp138 formed salt bridges with amino in both piperidine and isoquinoline moieties of JDTic. Tyr139 formed a hydrogen bond with the amide linkage of JDTic. According to the modeling study and residues' mutations, Asp138 was a key residue (71, 72). A literature study showed that the Asp138 residue was conserved and important in all aminergic GPCRs (73). The residues listed in the Figure 15 included Thr111, Gln115, Val118, Trp124, Val134, Leu135, Asp138, Tyr139, Met142, Lys227, Val230, Trp287, Ile290, His291, Ile294, Tyr312, Ile316, Tyr320 were proven essential for ligands binding with OPRK (71).

We docked salvinorin A into the crystal structure, OPRK (PDB entry: 4DJH, resolution 2.90 Å). The pocket was defined according to the original ligand of the co-crystal, JDTic. The docking score was 7.40. Figure 15B shows the interactions between salvinorin A and OPRK. Compared to the original ligand, we found that salvinorin A was closer to helix II, III, VI and VII. Tyr312 and Gln115 formed strong hydrogen bonds with the ester bonds of salvinorin A. In the original co-crystal structure, Tyr312 was reported to be an important residue for selectivity of JDTic with the kappa opioid receptor (71). These may explain the high selectivity of salvinorin A with the OPRK. From our docking results, we found the moiety of the pocket that is closer to helix II, III, VI and VII are more important for ligands binding to OPRK.

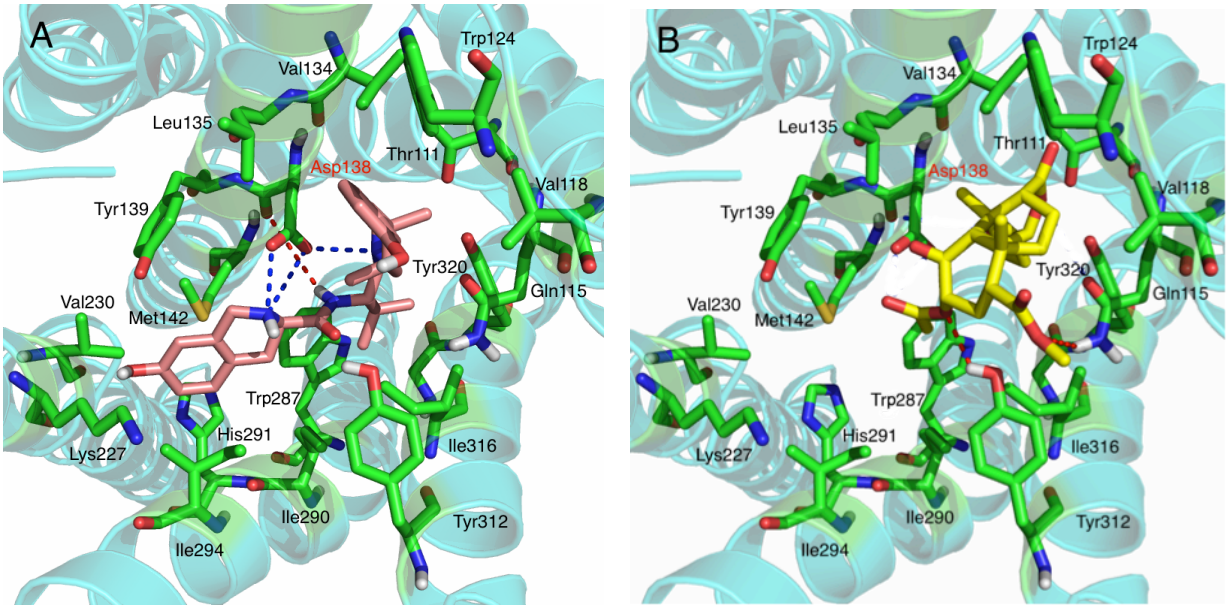


Figure 15. Interactions between salvinorin A and kappa opioid receptor (OPRK). The red dash lines represent the hydrogen bonds between the ligands and targets. The blue dash lines represent charge-charge interactions between the ligands and targets. (A) The co-crystal structure of OPRK (PDB entry: 4DJH, resolution 2.90 Å). The original ligand, JDTic, fitted tightly within the pocket forming ionic, hydrogen bond and hydrophobic interactions with helix II, III, V, VI and VII. The important residues were labeled. (B) The docking results between salvinorin A and OPRK. The salvinorin A interacted closer to the moiety of the pocket, helix II, III, VI and VII.

According to the prescreening HTDocking study, we found four promising targets of salvinorin A (Figure 13). The most potential one was the ACM2, which had the docking score of 8.32 (Figure 16). The molecular docking study was based on the co-crystal structure of ACM2 (PDB entry: 3UON, resolution 3.00 Å) published by Haga's group in 2012 (74, 75). The original ligand was QNB, an antagonist of ACM2 (K_d = 0.2nM). Figure 16A shows the interactions between QNB and helix III, IV, V, VI and VII of ACM2. Asp103, Tyr104, Ser107, Trp155, Phe181, Trp400, Tyr403, Asn404 and Cys429 listed in Figure 16 were the important residues

within the binding pocket. The amine moiety of QNB formed a salt bridge with the side chain of Asp103 in TM3, which was proven to be important by modeling study, mutagenesis and covalent-labeling experiments (74, 75). Asn404 formed hydrogen bonds with both hydroxyl and carbonyl group of QNB. However, according to the residues' mutation result, Asn404 was not important for the interactions between endogenous acetylcholine and the receptor (74, 75). Phe181 and the benzene ring formed a weak π-π interaction. Indeed, Phe181 was the unique residue for ACM2, in which this position for other ACM was leucine (74). These might explain the reason why QNB was not a highly selective ligand of ACM2.

Salvinorin A was located in the same pocket as QNB at ACM2. The positions and poses of QNB and salvinorin A in ACM2 were highly similar. Some similar interactions of salvinorin A could be found. Asn404 formed a hydrogen bond with the ester bond of salvinorin A. Ser107 formed a hydrogen bond with another part of the ester bond of salvinorin A. Trp400 and the ternary heterocyclic ring of salvinorin A formed hydrophobic contact. Similarly, Trp400 had hydrophobic contact with the nitrogen heterocyclic ring of QNB from the original co-crystal (74). According to the residues mutations study, mutation of Tyr104, Trp155, or Trp400 reduced both agonist and antagonist binding affinity over 10 fold. Based on these similar interactions, we could predict that ACM2 was a potential target of salvinorin A.

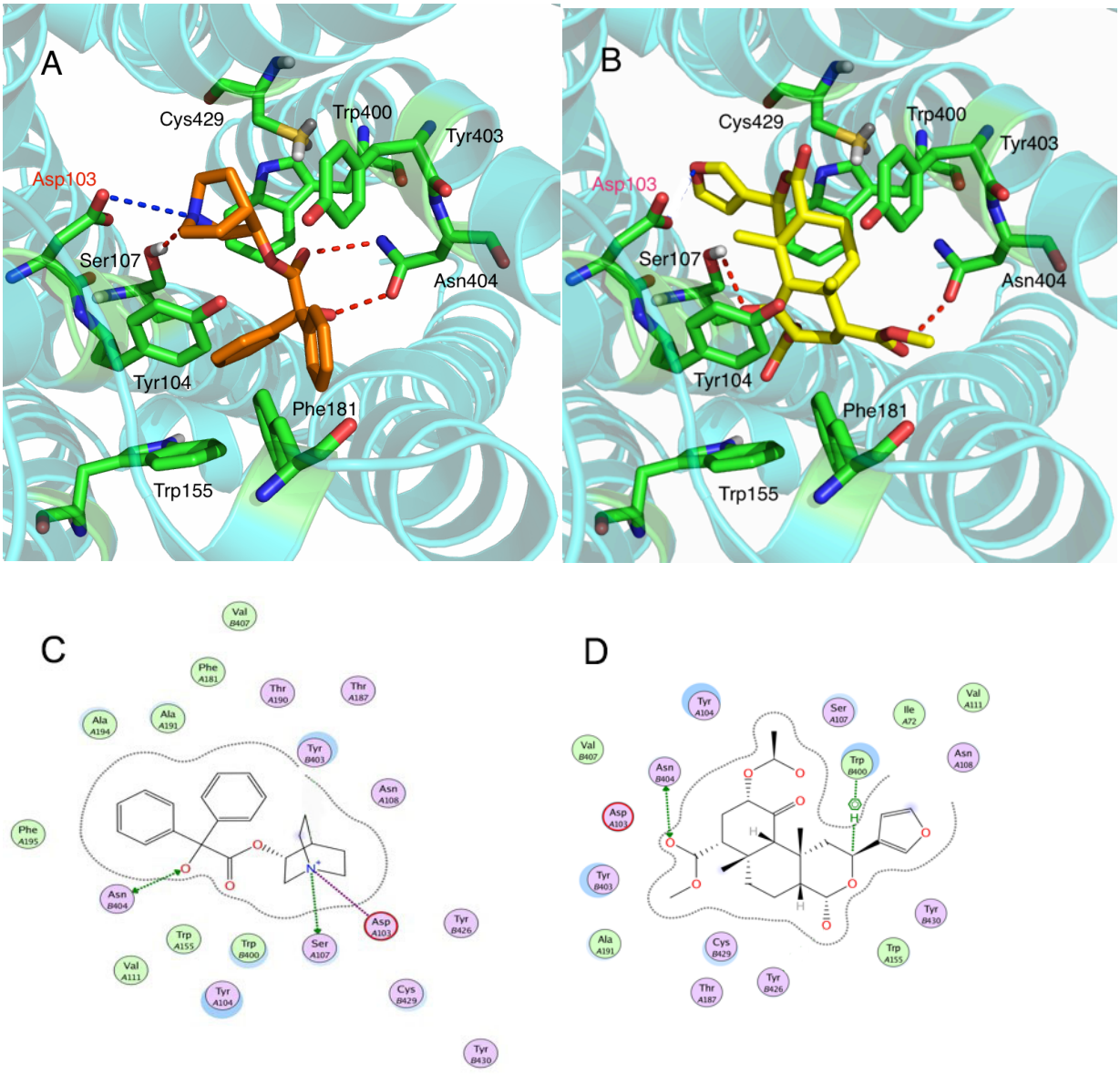


Figure 16. Interactions between salvinorin A and muscarinic acetylcholine receptor 2 (ACM2). (A) The co-crystal structure of ACM2 (PDB entry: 3UON, resolution 3.00 Å). (B) The docking results between salvinorin A and ACM2. QNB, the original ligand of ACM2, and salvinorin A have similar position and pose within binding pocket of ACM2. (C) 2D version of the interactions between QNB and ACM2. (D) 2D version of the interactions between salvinorin A and ACM2. The important residues were listed.

Besides ACM2, our HTDocking results showed that CB1 and CB2 had highly possibility to interact with salvinorin A. Since there were no crystal structures of cannabinoid receptors, we built the homology models to do the docking studies. (The detail methods have been mentioned in Chapter 2.0). The binding pocket of CB2 mainly located in helix III, V, VI and VII (Purple region in Figure 17B). The important residues of CB2 were Phe87, Phe91, Phe94, Asp101, Phe106, Lys109, Ile110, Val113, Phe117, Glu181, Leu182, Phe183, Trp194, Phe197, Trp258, Val261, Met265, Lys278, Lys279, Phe281 and Ser285 (Highlighted with green in Figure 17B). This model was validated by virtual screening, molecular dynamics simulation, and residues' mutations coupled with experiments radio-ligand binding results (46).

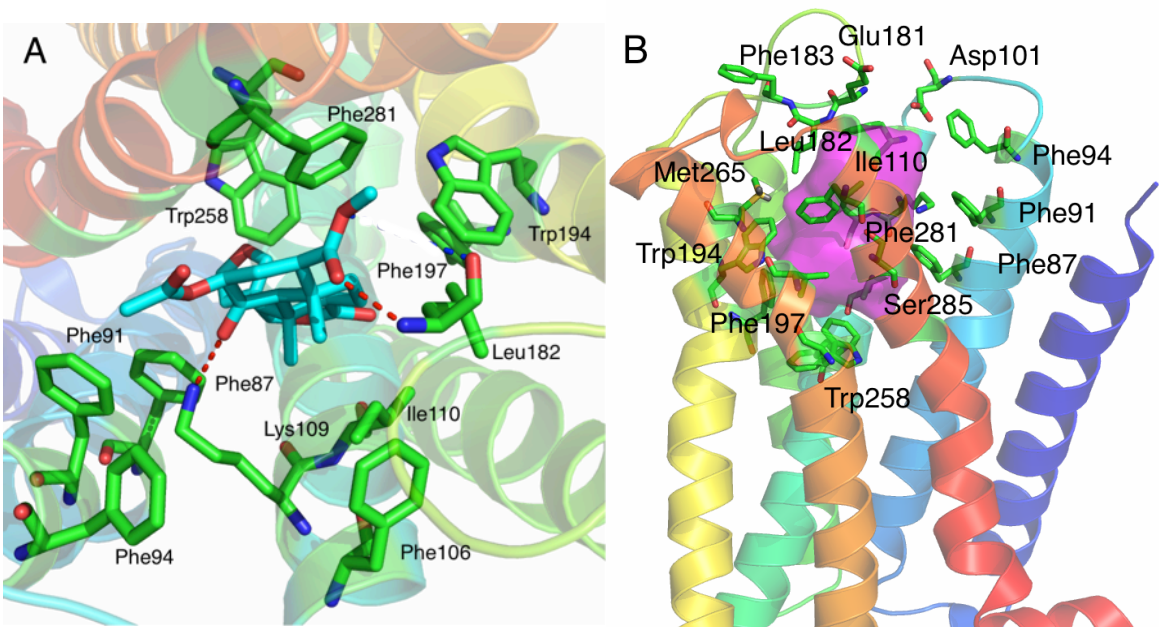


Figure 17. Interactions between salvinorin A and cannabinoid receptor 2 (CB2). (A) The interactions between salvinorin A and CB2. (B) The 3D homology model of CB2. The pocket was emphasized in purple. The important residues within pocket were highlighted with green.

The docking result between salvinoin A and CB2 was 8.11. In extracellular loop 2 region, Leu182 formed a hydrogen bond with the ester bond moiety of salvinoin A. As literature reported, extracellular loop 2 region of CB2 was proved essential for ligand bindings (76, 77). Besides, the amine part of Lys109 in helix III formed a hydrogen bond with the oxygen of salvinorin A. Lys109 is a conserved residue in GPCRs. Phe281 had hydrophobic contact with the ternary heterocyclic ring of salvinorin A. In summary, salvinorin A preferred hydrophobic interactions within CB2 binding pocket with residues including Phe91, Phe94, Phe106, Ile110, Trp194, Phe197, Trp258 and Phe281.

CB1 shared 48% of the whole sequence identity and 68% of the trans-membrane domains sequence identity with CB2 (46). We used the same method to construct the CB1 homology model. We used the MOLCAD module implemented in SYBYL-X1.3 to explore its potential binding pocket. Based on the model reported by literature, our pocket was reasonable (Figure 18B) (46, 78). The binding site of CB1 mainly focused on helix III, V, VI and VII, which was similar to CB2. The important residues listed in Figure 18B included Ile150, Phe57, Phe60, His61, Lys75, Val79, Asp149, Leu216, Met220, Phe236, and Ser240 (78).

We got a relatively high docking score of 9.62 when salvinorin A interacted with CB1. Amino part of His61 formed a hydrogen bond with the furan moiety of salvinoin A. The furan also had a π-π interaction with Phe57. Both the hydrogen bond and the π-π interaction contributed to the slightly extend of the furan moiety of salvinorin A to helix II. In the extracellular loop 2 region, Ile150 formed a hydrogen bond with salvinorin A. The insight interactions between salvinorin A and CB1, CB2 indicated that these two targets might also contribute to the pharmacological effects of salvinorin A.

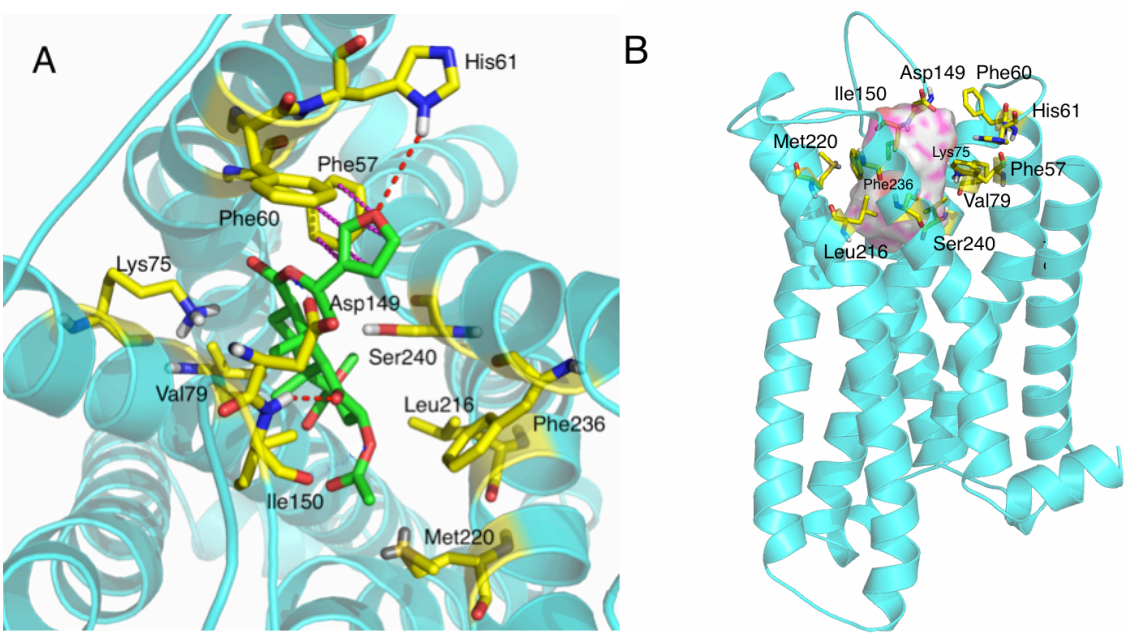


Figure 18. Interactions between salvinorin A and cannabinoid receptor 1 (CB1). (A) The interactions between salvinorin A and CB1. The purple dash lines represent the π-π interactions between Phe57 and the furan moiety of the salvinorin A. (B) The 3D homology model of CB1. The pocket was emphasized in pink. The important residues within pocket were highlighted with yellow.

DRD2 played a central role in pathophysiology of psychosis, movement disorders such as Parkinson's disease or multiple system atrophy, and addiction (79-81). It was also reported associative with pathological laughing (82). This indicated that DRD2 might contribute to the hysterically laughing side effect of salvniorin A. We got the docking score of 7.76 for salvinoin A within the DRD2 homology model. The homology model of DRD2 was established according to the template of DRD3 (83, 84). Figure19 shows that Ser157 and Ser161 formed a strong hydrogen bond (with 2.8 Å) with the ester moiety of salvinorin A. Tyr240 formed a hydrogen bond with the oxygen. Salvinorin A fitted well within the binding pocket that formed by helix III, V, VI and VII. The important residues in the binding pocket were Asp78, Cys82, Ser157,

Ser161, Trp210, Phe213, His217, Thr236 and Tyr240 (83, 84). Based on these similarities, we suggested salvinorin A might bind to DRD2.

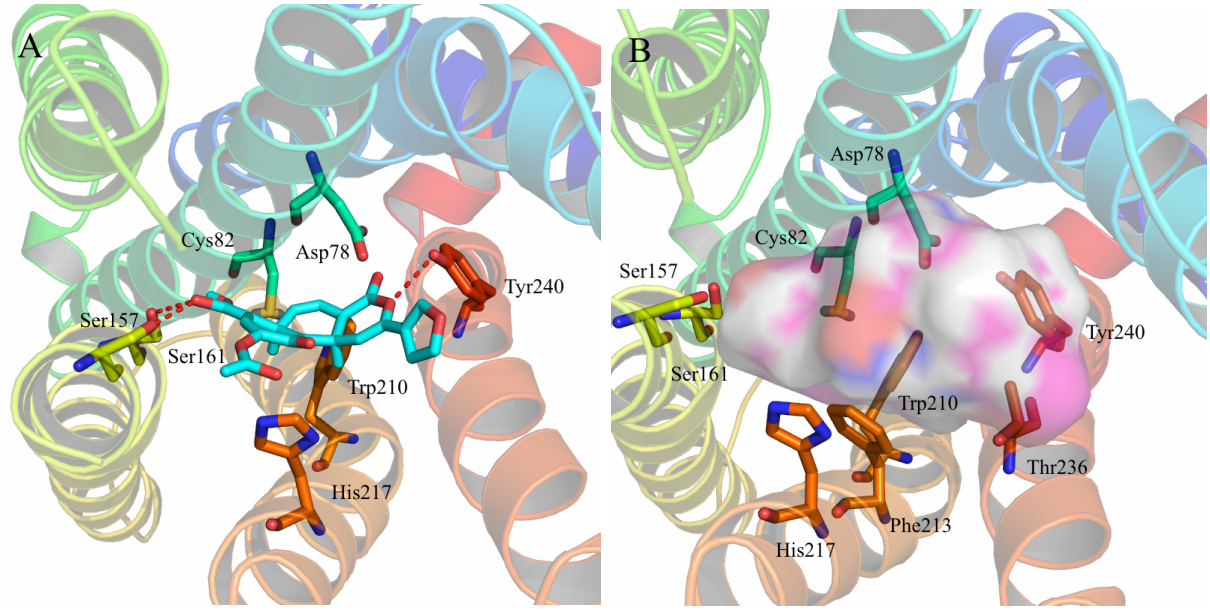


Figure 19. Interactions between salvinorin A and dopamine receptor 2 (DRD2). (A) The interactions between salvinorin A and DRD2. (B) The 3D homology model of DRD2. The pocket was emphasized in pink. The important residues within pocket were highlighted with green.

4.0 DISCUSSION

The hallucinogen specific knowledgebase constructed in this study is essential for the mechanism study and off-target identifications. Some other open source websites only provide a part of hallucinogen information. For example, some related websites, General Hallucinogen Information (www.pearltrees.com), CPSG (www.cpsg.org.uk/Drug-Database.aspx), Drugs Encyclopedia Bolg (drugsencyclopedia.net/) and Paid Clinical Trials (www.paidclinicaltrials.org), they all only provide some general information about hallucinogens, either including fancy articles or pictures for the purpose of popularization of science. And none of them is a scientific database that can be used for the research of hallucinogens. Our hallucinogen specific knowledgebase established in this study is the first comprehensive database for hallucinogenic drug research, which also includes several analysis tools (HTDocking, TargetHunter, BBB Predictor, properties explorer, the pains predictor, toxicity predictor). It could provide a quicker, less costly and relatively easier way for researchers to do systems pharmacological analysis, off-target identifications and mechanism studies.

Benefited from our hallucinogen specific knowledgebase, we applied it to explore possible targets of a special hallucinogen – salvinorin A. We found that besides OPRK, CB1, CB2, ACM2 and DRD2 might also be involved in the pharmacological effects of salvinorin A.

Actually, there was already some literature that could support our predictions. Seeman's group designed an *in vitro* experiment testing the binding affinity of salvinorin A at D2High receptor. D2High receptor is an agonist high-affinity state of DRD2. They found salvinorin A had EC_{50} values of 89 nM at the D2High receptor and were blocked by 10 μM S-sulpiride, an antagonist of DRD2. They claimed that the D2High receptors may be more pathophysiologically important than the overall expression of dopamine 2 receptors (85). However, the overall effects of salvinorin A on DRD2 still need to be further tested. Since it was proven that DRD2 contributed a lot to the pathological laughing (82), we assumed that the interaction with DRD2 should be blamed for the serious side effect, hysterical laughter of salvinorin A.

As we discussed in the introduction section, the dopaminergic system and the cannabinoid system are very important for the mechanism of addiction. Based on our similarity search results and docking results, we predicted that targeting on CB1, CB2 or DRD2 could explain the potential therapeutic profile of salvinorin A to treat drug abuse. Some related literature supported our idea. The Capasso's group reported the inhibitory effect of salvinorin A on ileitis-induced hypermotility. They believed that the cross-talk between the kappa opioid receptor and CB1 contributed to this effect. All their experiments were designed based on pathological conditions that in the inflamed gut (86). However, we believed that the effects of salvinorin A on the normal condition should also be considered. At this point, whether salvinorin A acts on CB1 or CB2 under health conditions should be tested in the future. Later, Fichna's group got interesting results of the effects of salvinorin A on colonic. They found differences between *in vivo* and *in vitro* phenomena, where the effects of salvinorin A on colonic motility *in vitro* were inhibited by antagonists of OPRK, CB1 and CB2, but i*n vivo* the effects were largely mediated by OPRK (87). The reasons for these different results still need to be discussed.

ACM2 is expressed both in the central nervous system (CNS) and the periphery system, especially in heart and smooth muscle tissues. They are involved in the smooth muscle contractility through both direct and indirect mechanisms (88). We assumed that the effects of salvinorin A on colonic motility is partly because of its interactions with ACM2. Besides, the Capasso's group also found that salvinorin A depressed enteric cholinergic transmission in the guinea-pig ileum. They concluded that this effect was triggered through OPRK. However, we can't exclude the possibility of the function of ACM.

5.0 CONCLUSIONS AND FUTURE SPECULATION

In our study, we established a hallucinogen specific knowledgebase by collecting hallucinogenic related data and constructing 3D homology models. We used our knowledgebase to study the mechanism of hallucinogens, a class of compounds that have recently been considered of its emerging potential drug abuse therapy. One case, salvinorin A was discussed in this thesis. The results showed that besides OPRK, four new receptors were predicted for the complex effects of salvinorin A, including ACM2, CB1, CB2 and DRD2 (Table 4). In the future, we are going to validate our predictions by experiments. Once we validate the predicted targets and the effects they contributed, we would combine the chemistry experiment methods with our computational techniques to process the structur-activity relationship (SAR) study. And finally, if possible, we will be able to modify the structure of salvinorin A to reduce its side effects and to increase its drug-likeness properties.

Table 4. Targets that needed to be validated for salvinorin A

Targets	Docking Score	Related reference
Muscarinic acetylcholine receptor 2	8.32	
Cannabinoid receptor 1	9.62	(86) (87)
Cannabinoid receptor 2	8.11	(86) (87)
Dopamine receptor 2	7.76	(82) (85)

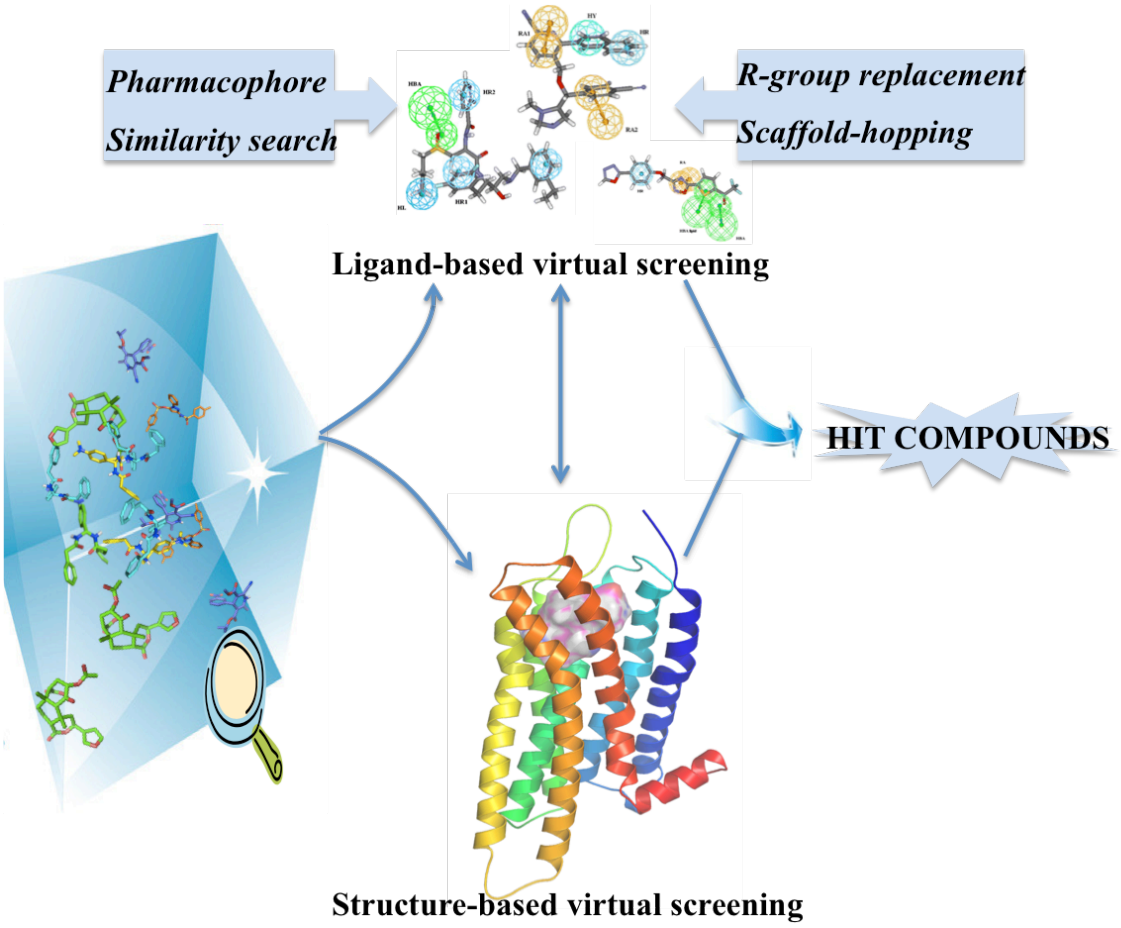


Figure 20. Schematic diagram of lead compounds exploring by computational approach. Structure-based and ligand-based virtual screening were used to extract lead compounds from compounds library. During structure-based virtual screening, the accurate crystal structures or 3D homology models are needed, in which identifying the pockets was critical. For ligand-based exploration, several strategies could be used, such as pharmacophore studies, similarity search, R-group replacement and scaffold-hopping.

Furthermore, for those promising therapeutic targets, we could do further study for drug discovery. Figure 18 shows our basic computational strategies for drug discovery and development. (89) As shown in this schematic diagram, the two major methods for extracting lead compounds from larger compound libraries were structure-based and ligand-based virtual screening, in which they can work independently or complementarily. During structure-based virtual screening, the accurate crystal structures or 3D homology models are needed, in which identifying the pockets was critical. For ligand-based exploration, several strategies could be used, such as pharmacophore studies, similarity search, R-group replacement and scaffold-hopping. In a word, ligand-based screening was much quicker, convenient and beneficial for structure modifications, while structure-based screening had big advantages of exploring novel ligands for the therapeutic targets. For our hallucinogen specific knowledgebase, we will keep updating hallucinogenic related information and establishing more 3D homology models if necessary. Meanwhile, we will start more promising case studies in this area.

APPENDIX ABBREVIATION

DALY	Disability-adjusted life-years
5HT	Serotonin receptors
5HT2B	Serotonin receptors 1B
5HT1B	Serotonin receptors 2B
5HT2A	Serotonin receptors 2A
5HT6R	Serotonin receptors 6
5HT7R	Serotonin receptors 7
AA	Arachidonic acid
ACM	Muscarinic acetylcholine receptors
ACM2	Muscarinic acetylcholine receptor 2
ADA1A	Alpha-1A adrenergic receptor
ADAR	Adrenergic receptors
AOF	Amine oxidases
AOFA	Amine oxidase [flavin-containing] A
AOFB	Amine oxidase [flavin-containing] B
BBB	Blood-brain barrier
CB1	Cannabinoid receptor 1
CB2	Cannabinoid receptor 2
CNR	Cannabinoid receptors
CNS	Central nervous system
CP2B6	Cytochrome P450 2B6
CP2D6	Cytochrome P450 2D6
DA	Drug abuse
DA-KB	Drug abuse chemogenomics knowledgebase
DAG	Diacylglycerol

DMT	Dimethyltryptamine
DOM	2,5-Dimethoxy-4-methylamphetamine
DRD	Dopamine receptors
DRD1	Dopamine receptor 1
DRD2	Dopamine receptor 2
DRD3	Dopamine receptor 3
DRD4	Dopamine receptor 4
DRD5	Dopamine receptor 5
GPCRs	G-protein coupled receptors
IP3	Nositol-1,4,5-triphosphate
LSD	Lysergic acid diethylamide
NCF2	Neutrophil cytosol factor 2
NMDA	N-methyl-D-aspartate receptor
NMDA 3A	N-methyl-D-aspartate receptor 3A
OPR	Opioid receptors
OPRM	Muopioid receptors
OPRK	Kappa opioid receptor
PIP2	Hydrolyzes phosphatidylinositol
PKC	Protein kinase C
PLA2	Phospholipase A2
PLC	PI-specific phospholipase C
RAC	Ras-related C3 botulinum toxin substrates
RAC1	Ras-related C3 botulinum toxin substrate 1
SAR	Structur-activity relationship
SPRE	Sepiapterin reductase
SSRIs	Serotonin reuptake inhibitors
SUIS	Surase-isomaltase
TCAs	Tricyclic antidepressant
SC6A3	Sodium-dependent dopamine transporter
SC6A4	Sodium-dependent serotonin transporter

ACE	Angiotensin-converting enzyme
NEP	Neprilysin
AA2AR	Adenosine receptor A2a
OPRD	Delta opioid receptors
COMT	Catechol *O*-methyltransferase

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