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Screening the receptorome to discover the molecular targets for plant-derived psychoactive compounds: a novel approach for CNS drug discovery

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Abstract

Because psychoactive plants exert profound effects on human perception, emotion, and cognition, discovering the molecular mechanisms responsible for psychoactive plant actions will likely yield insights into the molecular underpinnings of human consciousness. Additionally, it is likely that elucidation of the molecular targets responsible for psychoactive drug actions will yield validated targets for CNS drug discovery. This review article focuses on an unbiased, discovery-based approach aimed at uncovering the molecular targets responsible for psychoactive drug actions wherein the main active ingredients of psychoactive plants are screened at the ''receptorome'' (that portion of the proteome encoding receptors). An overview of the receptorome is given and various in silico, public-domain resources are described. Newly developed tools for the in silico mining of data derived from the National Institute of Mental Health Psychoactive Drug Screening Program's (NIMH-PDSP) K_i Database (K_i DB) are described in detail. Additionally, three case studies aimed at discovering the molecular targets responsible for *Hypericum perforatum*, *Salvia divinorum*, and *Ephedra sinica* actions are presented. Finally, recommendations are made for future studies.

Keywords: Receptorome; Molecular targets; Psychoactive compounds
Abbreviations: NIMH-PDSP, National Institute of Mental Health Psychoactive Drug Screening Program; Ki DB, Ki Database; GPCR, G-protein coupled receptors; hSERT, human serotonin transporter; DMT, dimethyltryptamine; 5-HT, 5-Hydroxytryptamine; KOR, κ opioid receptor

Contents

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1. Introduction: why the study of psychoactive plant actions is important

Psychoactive plants have been used by humans for recreational, spiritual, and therapeutic purposes for millennia (Lewin, 1924). At present, plants and plant-derived substances, such as *Cannabis sativa* (marihuana), *Papaver somniferum* (morphine and heroin), *Coffea arabica* (caffeine), and *Catha edulis* (cathinone), are widely used and abused throughout the world. Because many psychoactive plants exert profound effects on human consciousness, emotion, and cognition, it has long been suggested that detailed studies investigating the molecular mechanisms of action of psychoactive plants will yield clues to the "chemistry of consciousness" (Lewin, 1924; Nichols, 2004). In fact, the isolation of mescaline from *Lophophora williamsii* and the demonstration of its psychoactive properties by Heffter in 1897 and reported in 1898 was the first demonstration that a simple chemical entity could produce a profound alteration of human consciousness. Likewise, the discovery of lysergic acid diethylamide in 1943 (Hoffman, 1979) as a semisynthetic analogue of potent, naturally occurring ergot alkaloids produced by *Claviceps purpurae* and the observations that lysergic acid diethylamide and serotonin shared structural and pharmacological properties led to the suggestion that biogenic amines like serotonin were involved in certain mental disorders, such as schizophrenia (Gaddum & Hameed, 1954; Wooley & Shaw, 1954). Finally, the discovery that reserpine, the active ingredient of *Rauwolfia serpintina*, depleted biogenic amines and induced depression led to the proposal that a lack of serotonin and/or norepinephrine caused depression (Vetulani & Sulser, 1975). Thus, the study of psychoactive plant actions has revolutionized our understanding of the chemical basis of human consciousness and has led to many of the medications used today to treat various mental illnesses. It is our prediction, therefore, that efforts aimed at elucidating the molecular target(s) for psychoactive drug actions will yield validated targets for CNS drug discovery. As we summarize below, however, the completed sequencing of the human genome has revealed a large number of potential molecular targets for psychoactive drug actions necessitating a comprehensive, planned approach to receptorome profiling.

2. The receptorome and receptoromics: an overview

With the recently completed sequencing and partial annotation of the human genome (Lander et al., 2001; Venter et al., 2001), it has become clear that a large portion of the genome is devoted to encoding signal-transduction molecules. Indeed, one estimate is that ~20% of the human genome is devoted to signal transduction (Venter et al., 2001) and that many of the signal-transducing molecules represent receptors of various types. G-protein-coupled receptors (GPCR) represent the largest family of receptors with estimates ranging from a low of 616 (Venter et al., 2001) to a high of 950, of which 500 are putative odorant GPCR (Takeda et al., 2002; Kroeze et al., 2003). Others have recently estimated that there might be at least 367 "endo-GPCR" (GPCR for which endogenous ligands exist) (Vassilatis et al., 2003; Kroeze et al., 2003). Using the upper limit of 950 and the lower estimates of ~26,000 bona fide open-reading frames in the human genome, GPCR represent at most 3.7% of the human genome. Ion channels and transporters, which frequently function as "receptors" for naturally occurring psychoactive compounds, represent another 3% of the genome while non-GPCR receptors represent at least 1.5% of the genome (Venter et al., 2001). Taken together we can estimate that the receptorome, which we have defined as that portion of the proteome encoding "receptors," represents more than 8% of the human genome.

Since GPCR represent the largest single family of "receptors" in the genome and the most common molecular target for psychoactive drugs of all sorts (Kroeze et al., 2003), they will be discussed in some detail. GPCR have a common structural motif of 7-transmembrane domains and, hence, have also been called the heptahelical family of receptors (see Kroeze et al., 2003, for review). Fig. 1A–C shows various renditions of a molecular model for a prototypical GPCR—the 5-HT_{2A} receptor—which serves as the principal molecular target for many plant-derived hallucinogens (e.g., lysergic acid amide, psilocybin, mescaline; Nichols, 2004). Fig. 1A shows a surface rendering of the 5-HT_{2A} receptor, while Fig. 1B shows the residues implicated in receptor activation and Fig. 1C shows the overall arrangement of the helices. This model has previously been validated by a large number of mutagenesis and molecular modeling studies (see Shapiro et al., 2000, 2002; Prioleau et al., 2002; Ebersole et al., 2003, for recent examples and Roth & Shapiro, 2001; Kroeze et al., 2002; Westkaemper & Glennon, 2002, for reviews). The main features of the model, which rely heavily on homology-based modeling using rhodopsin as a template (Palczewski et al., 2000), include (1) "kinks" induced by highly conserved proline residues that distort several of the helices from the canonical α-helical conformation; (2) a tilting of several helices so that they are not perfectly perpendicular to the plane of the plasma membrane; and (3) the presence of an eighth helix that runs approximately parallel to the plane of plasma membrane (Fig. 1C). Other features include the presence of a tight ionic interaction between the cytoplasmic faces of transmembrane domains 3 and 6 (Fig. 1B; Roth & Shapiro, 2001; Shapiro et al., 2002), which is seen in many other, but not all, GPCR (see Ballesteros et al., 2001, for example). Many agonists are thought to cause receptor activation via the agonist-mediated induction of rotations of helices 3 and 6 and the subsequent disruption of this strong ionic interaction (Farrens et al., 1996; Gether & Kobilka, 1998; Dunham & Farrens, 1999; Ballesteros et

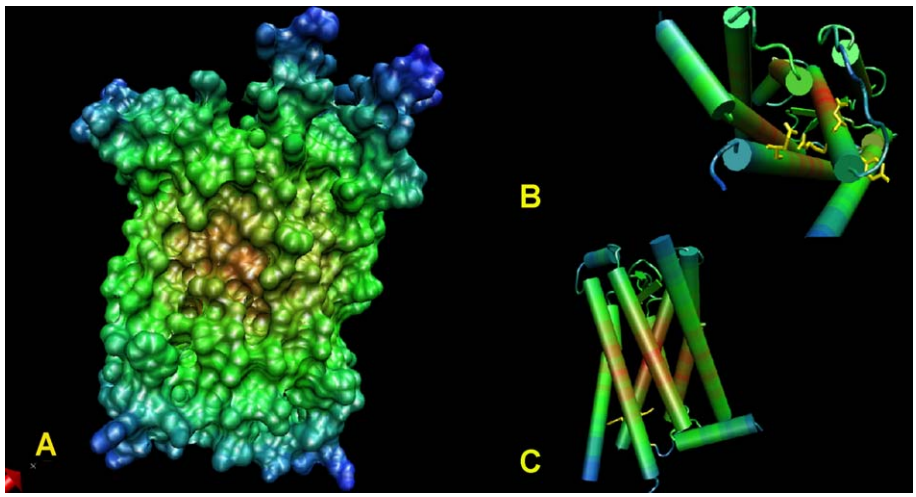


Fig. 1. Molecular model of the 5-HT_{2A} serotonin receptor: a prototypical GPCR. (**A**) Rendering of the 5-HT_{2A} receptor showing "solvent-accessible" regions. (**B**) Residues involved in stabilizing the inactive state of the receptor (Shapiro et al., 2002). (**C**) Rendering of a model of the 5-HT_{2A} receptor wherein the helices have been converted to tubes.

al., 2001; Roth & Shapiro, 2001; Shapiro et al., 2002) (Fig. 1B,C). GPCR as a class have been estimated to represent the proximal molecular target for between 30% and 50% of all currently available pharmaceutical agents (Vassilatis et al., 2003; Kroeze et al., 2003).

As already mentioned, ion channels and transporters also represent proximal molecular targets for drug actions. Ideally, then, we would like to be able to screen the entire receptorome to discover the proximal molecular targets responsible for psychoactive plant actions.

2.1. Informatics resources for identifying molecular targets for psychoactive plant actions: virtual screening of the receptorome

2.1.1. Erowid and related sites

There are currently several open databases for plant-based psychoactive compounds (Table 1). Of the various databases, *The Vaults of Erowid* (http://www.erowid.org/) is the most comprehensive and up-to-date. *The Vaults of Erowid* provides non-reviewed information on the chemistry and molecular targets of the major psychoactive plants. Although not subject to peer review, *The Vaults of Erowid* serve as a handy repository of information and lore regarding psychoactive plants, providing links and summaries of major discoveries relating to psychoactive drug actions. *The Lycaeum* (http://www.lycaeum.org/) is a similar site, providing a comprehensive, searchable database for psychoactive botanicals. Both *The Vaults of Erowid* and *The Lycaeum* are likely to be used frequently by the interested nonscientist due to the format of the sites and not by scientists, since links to published information are not readily accessible and the information is not subjected to any sort of peer review. Nonetheless, both sites are quite useful for providing background information regarding the use of psychoactive botanicals and summaries of their known chemistries.

Table 1
Representative on-line resources for psychoactive botanicals

Name	URL	Type of information
The Vaults of Erowid	http://www.erowid.org/	MT, Anec, Chem, Bot, Link
Entheogen Dot	http://www.entheogen.com/	Anec, Chem, MT
The Lycaeum	http://www.lycaeum.org/	MT, Chem, Anec, Bot
Botanical.com	http://www.botanical.com/	Bot
Multidisciplinary Association for Psychedelic Studies	http://www.maps.org/	Link
Heffter Research Institute	http://www.heffter.org/	Link, MT, Chem
NIMH-PDSP Database	http://kidb.cwru.edu/	MT, Link, Chem

MT = molecular target; Anec = anecdotal user reports; Chem = chemistry; Bot = botanical information; Link = Links to articles.

2.1.2. National Institute of Mental Health's K_i Database

Of greater utility for scientists is the National Institute of Mental Health's Psychoactive Drug Screening Program (NIMH-PDSP) K_i Database (K_i DB; http://kidb.cwru.edu/). This is a large, fully searchable database (currently >25,000 K_i values) that is entirely in the public domain. Like the other databases mentioned, the K_i DB is a curated database that is updated on a daily basis. The main features of the design of K_i DB are summarized in Figs. 2 and 3.

The PDPS K_i DB was designed and developed late in 1999 as part of the NIMH-PDSP. Activity on the database is measured by the number of successful requests; a "successful request" is a transfer of data from the server to the client querying the database. The database has had an accelerating volume of traffic with >1,000,000 successful requests in the past 3 years of which >500,000 came in the first 10 months of 2003. The amount of data transferred each month is also increasing and reflects the growth in the database's total

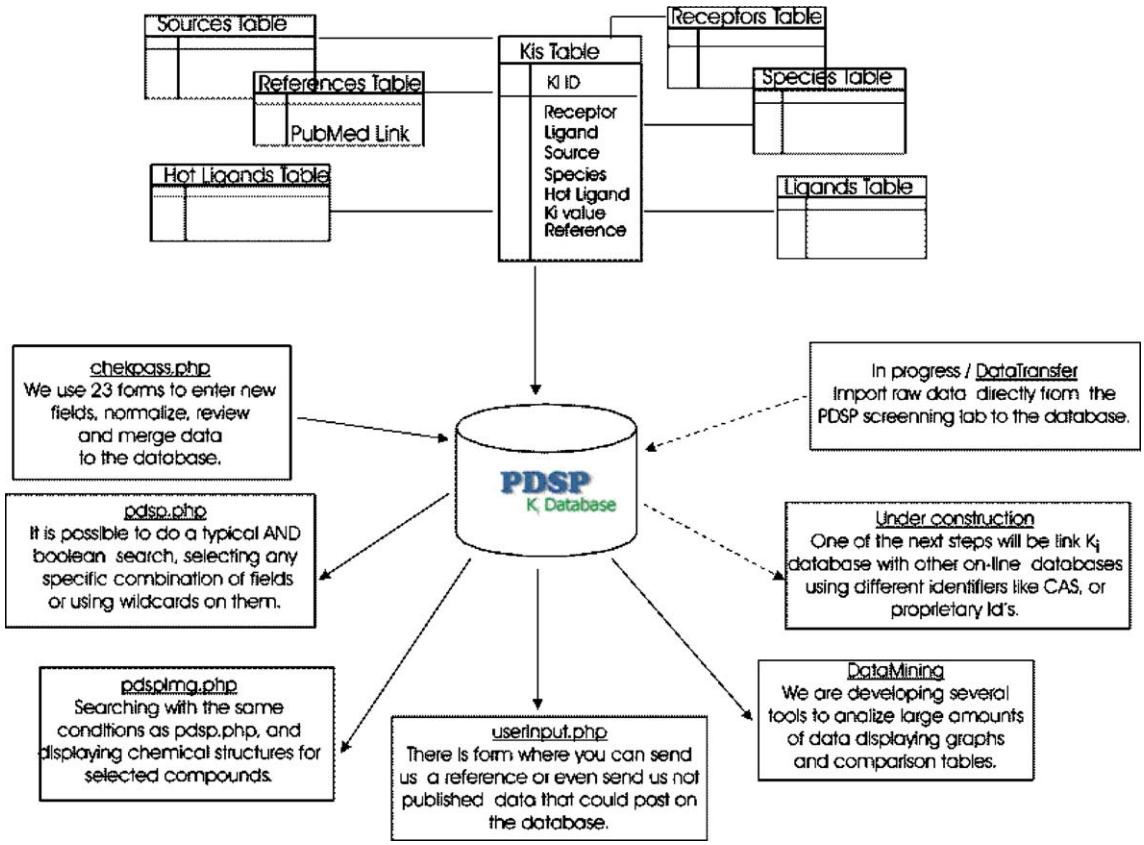


Fig. 2. Organization of K_i DB: a searchable, public-domain database of pharmacological information. Shown is the overall organization scheme for data warehousing and data transfer in K_i DB (http://kidb.cwru.edu/).

amount of information. Between January 2001 and October 2003, almost 30 Gb of information were transferred. Just over 7 Gb were transferred in 2002 with an average of 599 Mb/month. The highest monthly transfer of data was during August 2003 with more than 2.2 Gb transferred.

The K_i DB is a collection of organized tables and programs that are able to store, modify, and present information in various formats; in essence K_i DB is a data warehouse in which K_i (affinity) values for specific compounds screened against a large group of receptors are stored. Additionally, the relevant experimental conditions (e.g., radioligand, species, source of receptor) are also listed. Three different sources of information are used to populate the K_i DB: (1) published data wherein the original publication is linked via PubMed; (2) data sent directly via a user (either in press or unpublished information); (3) internally generated information from the NIMH-PDSP. The data are stored using MySQL, which is an "open source" database management system.

The database can be queried in a highly flexible manner. At the initial search page, pull-down menus appear for each of the six categories including receptor and ligand. Any combination of categories can be used to mine the database; each combination is considered as a Boolean AND operator. One is also able to narrow the search to a particular range of K_i values. If users prefer, they can avoid the pull-down menus and directly enter their search criterion. With direct entry, the use of wildcards for one or more characters is available, thus widening available search options. Some other features of the K_i DB include hot links to referenced material and structures of many compounds that have listed K_i values. Users can also use some data-mining tools to obtain more condensed information from this database.

Several data-mining tools have been implemented (Figs. 3 and 4) including various "receptor mining" (http://kidb.bioc.cwru.edu/dataMining/receptorCross/crossReferenceReceptors.php) and "ligand selectivity" (http://kidb.bioc.cwru.edu/dataMining/pdspCompoundCriteria.php) tools. The receptor mining tool allows one to search for compounds that interact with two different receptors and then to display their averaged K_i values for both receptors (Fig. 4A). The receptor mining tool helps to design and interpret experiments in which drugs with differential selectivity against two receptors are used. The ligand selectivity tool allows one to identify high affinity ligands for a particular molecular target, and then to determine how *selective* those ligands are for a variety of additional molecular targets. Thus, for instance, in the example shown (Fig. 4B), a search was made for all ligands with affinities <10 nM for the human serotonin transporter (hSERT). Once these compounds were identified, they were virtually screened using data in K_i DB to identify other molecular targets with which

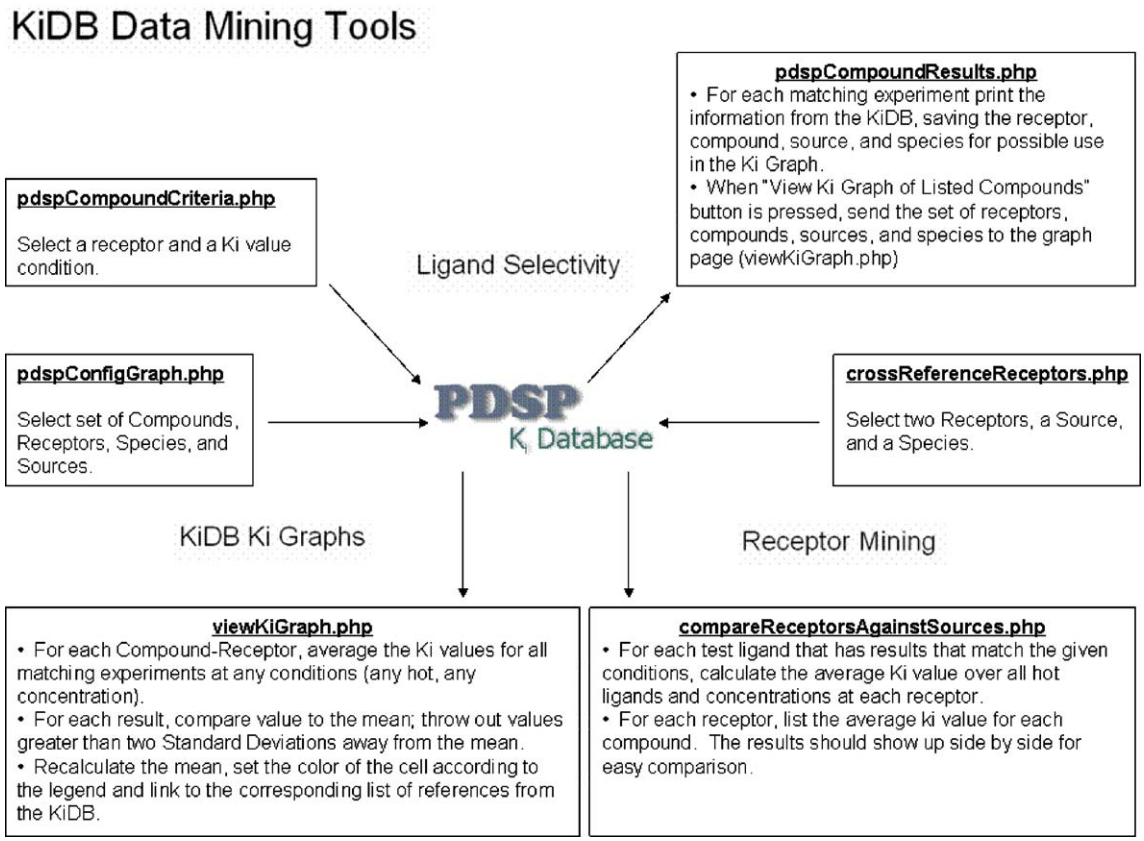


Fig. 3. Data-mining tools for K_i DB. Shown are the various data-mining tools (http://kidb.bioc.cwru.edu/dataMining/) that have been implemented for K_i DB and their overall relationship to the organization of K_i DB.

they interact; the average affinities were then displayed as a color-coded bar (Fig. 4B). The ideal hSERT ligand would be one with high affinity for hSERT (red bar) and low affinity for all other tested molecular targets (purple or blue bars); using this criterion, paroxetine is the most selective hSERT ligand in our current database. Using a mouse, the user can highlight a particular value (in this case, 5-HT_{2A} receptor affinities for amitryptiline) and display the accumulated data from which the numbers were derived.

Because published and internally derived data frequently differ, we developed a tool that allows for the averaging of "good" data and the culling of "bad" data. To accomplish this task, we adapted the sorts of data-mining tools now frequently used to analyze microarray-type studies wherein an algorithm derives the average K_i values. Essentially, this tool calculates a running average K_i value for a particular ligand-receptor pair, and then culls outliers using a well-accepted statistical criterion of ± 2 SD from the mean. The mean is then recalculated and displayed as a color-coded bar (Fig. 4B).

To discover the molecular targets responsible for plant derived psychoactive compounds one can, for instance, simply type in the name of the compound (e.g., dimethyltryptamine; DMT) and see the various molecular targets against which this compound has been screened, as well as literature or internally derived K_i values for this compound (Fig. 5). Such a search reveals that DMT has moderate affinity for several 5-HT receptors, but that a comprehensive pharmacological profile for DMT is not yet available.

3. Physical screening of the receptorome to identify the molecular targets for plant-based psychoactive compounds

3.1. Hypericum perforatum

Hypericum perforatum (also known as St. John's wort) is one of the most widely used psychoactive plants—mainly for its putative antidepressant actions. Although *H. perforatum* extracts are used frequently for the treatment of mild to moderate depression, the worldwide clinical literature is mixed regarding the antidepressant actions of either extracts or purified constituents of *H. perforatum* (e.g., hypericum). Thus, some studies have demonstrated effectiveness in depression (Brenner et al., 2000; Brenner et al., 2001, 2002; Lecrubier et al., 2002), whereas others have found no effect compared with either placebo (Shelton et al., 2001) or sertraline (Hypericum Depression Trial Study Group, 2002). The results of the trial comparing a reference extract of *H. perforatum* (LI-160) against placebo or sertraline are especially problematic since the active comparator (sertra-

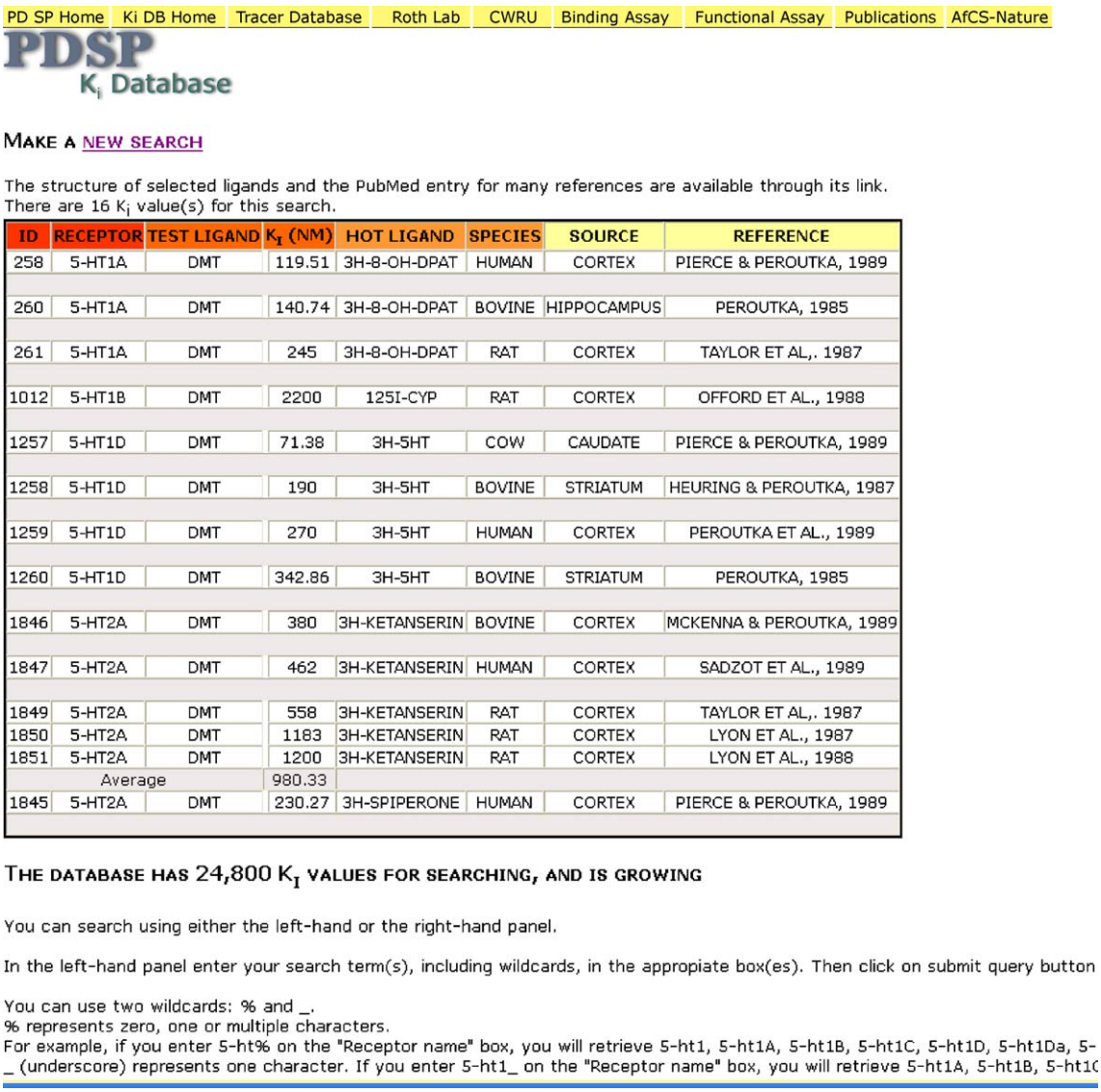
Test Ligand	5-HT2A HUMAN CLONED	5-HT2B HUMAN CLONED
5-HT	180.84	5.044
5-HT,N-ACETYL	89100	6063.3333333333
5-MeOT	61.65	8.455
Agomelatine	5.35	6.59
AMESERGIDE	15.1	1.96
BRL 15572	251.18	39.81
BUSPIRONE	138.03	213.79
CISAPRIDE	10.2	62.3
CLOZAPINE	8.31375	6.665
CYPROHEPTADINE	1.67	1.54
D-367	88.5	521
d-Fenfluramine	2470	3920
DMA	211	1039
DOAc	80.5	313
DOB	0.6	269
DOBz	0.4	35
DOC	1.4	31.8
DOCN	45.7	77.4
DOF	41.7	227
DOHx	0.1	30.3
DOI	10.235	20.033333333333
DON	5.5	166
DOPR	0.9	54.4
DOTB	3.7	24.6
DP-5-CT	512.86	501.18
Duloxetine	504	2100
ERGOTAMINE	0.64	3.27
FLUOXETINE	147.91	7515
GR 113808	10000	870.96
GR 127935	15.84	630.95
HALOPERIDOL	92.73125	1203.9
KETANSERIN	2.1275	799.84
1-Fenfluramine	1430	680
1-nonfenfluramine	267	65
LISURIDE	0.74	1.07
LSD,2-Bromo	0.48	8.04
LY 063518	137	472
LY 193525	09	387
LY 314228	65	1214
LY 320950	248	465
LY 320951	839	2926
LY 320954	96	1642
LY 334359	569	388
LY 334362	179	477
LY 344864	3935	1695
M-154	94.2	341
mCPP	81.155	23.328333333333

Fig. 4. Representative data-mining session using the receptor-mining tools. (**A**) Representative session wherein the h5-HT_{2A} and h5-HT_{2B} receptors were "virtually screened" to determine which compounds bound to both receptors and the relative averaged affinities. (**B**) Representative session wherein the selectivity of various compounds for the hSERT was calculated (see text for details).

line) failed to show a differential response compared with placebo (Hypericum Depression Trial Study Group, 2002). By contrast, another placebo-controlled trial with a different extract of *H. perforatum* (WS 5570) showed a beneficial effect compared with placebo (Lecrubier et al., 2002). It is likely that the mixed results are in part due to the fact that in one of the pivotal US trials, the comparator medication (in this case, sertraline (Hypericum Depression Trial Study Group, 2002)) failed to show a positive response. As well, different types of extracts have been used for the various trials (e.g., LI-160 in Hypericum Depression Trial Study Group, 2002 vs. WS 5570 in Lecrubier et al., 2002), and it is likely that each extract has a different overall composition.

Preclinical research on *H. perforatum* extracts demonstrate clear-cut effects in various rodent models of depression, including the forced-swim and tail-suspension tests (Butterweck et al., 1997; Nahrstedt & Butterweck, 1997). Additionally, purified constituents from *H. perforatum*, including hypericin and pseudohypericin, have shown antidepressant actions in the forced-swim test (Butterweck et al., 1998), as have the flavinoids hyperoside, isoquercitrin, and miquelianin (Butterweck et al., 2000; Butterweck, 2003). Additionally, in vivo administration of *H. perforatum* constituents leads to down-regulation of β-adrenergic, 5-HT_{1A} and 5-HT_{2A} receptors (see Butterweck, 2003, for a comprehensive review).

Various purified substituents of *H. perforatum* have been screened against a portion of the receptorome (Cott, 1997; Simmen et al., 1999; Gobbi et al., 2001; Simmen et al., 2001; Butterweck et al., 2002; Butterweck, 2003). Amentoflavone had highest affinity for any tested molecular target, with high affinity for the GABA-benzodiazepine receptor complex (K_i = 6 nM) and moderate affinity for δ-opioid receptors (K_i = 37 nM). Several other compounds had affinities in the low nanomolar to micromolar range for several cloned receptors, including various serotonin receptors for amentoflavone (5-HT_{1B}, 5-HT_{1D}, 5-HT_{2C}) and dopamine receptors for hypericin (D_3 and D_4). Another study using fewer receptors (Simmen et al., 1999) disclosed low micromolar affinities for various opioid and 5-HT receptor subtypes. Other studies by the same group found that hypericin was a low affinity CRF-1 antagonist (Simmen et al., 2001, 2003). Finally,



PD SP Home | Ki DB Home | Tracer Database | Roth Lab | CWRU | Binding Assay | Functional Assay | Publications | AfCS-Nature

PDSP
K_i Database

MAKE A NEW SEARCH

The structure of selected ligands and the PubMed entry for many references are available through its link.
There are 16 K_i value(s) for this search.

ID	RECEPTOR	TEST LIGAND	K_I (NM)	HOT LIGAND	SPECIES	SOURCE	REFERENCE
258	5-HT1A	DMT	119.51	3H-8-OH-DPAT	HUMAN	CORTEX	PIERCE & PEROUTKA, 1989
260	5-HT1A	DMT	140.74	3H-8-OH-DPAT	BOVINE	HIPPOCAMPUS	PEROUTKA, 1985
261	5-HT1A	DMT	245	3H-8-OH-DPAT	RAT	CORTEX	TAYLOR ET AL,. 1987
1012	5-HT1B	DMT	2200	125I-CYP	RAT	CORTEX	OFFORD ET AL., 1988
1257	5-HT1D	DMT	71.38	3H-5HT	COW	CAUDATE	PIERCE & PEROUTKA, 1989
1258	5-HT1D	DMT	190	3H-5HT	BOVINE	STRIATUM	HEURING & PEROUTKA, 1987
1259	5-HT1D	DMT	270	3H-5HT	HUMAN	CORTEX	PEROUTKA ET AL., 1989
1260	5-HT1D	DMT	342.86	3H-5HT	BOVINE	STRIATUM	PEROUTKA, 1985
1846	5-HT2A	DMT	380	3H-KETANSERIN	BOVINE	CORTEX	MCKENNA & PEROUTKA, 1989
1847	5-HT2A	DMT	462	3H-KETANSERIN	HUMAN	CORTEX	SADZOT ET AL., 1989
1849	5-HT2A	DMT	558	3H-KETANSERIN	RAT	CORTEX	TAYLOR ET AL,. 1987
1850	5-HT2A	DMT	1183	3H-KETANSERIN	RAT	CORTEX	LYON ET AL., 1987
1851	5-HT2A	DMT	1200	3H-KETANSERIN	RAT	CORTEX	LYON ET AL., 1988
Average			980.33				
1845	5-HT2A	DMT	230.27	3H-SPIPERONE	HUMAN	CORTEX	PIERCE & PEROUTKA, 1989

THE DATABASE HAS 24,800 K_I VALUES FOR SEARCHING, AND IS GROWING

You can search using either the left-hand or the right-hand panel.

In the left-hand panel enter your search term(s), including wildcards, in the appropiate box(es). Then click on submit query button

You can use two wildcards: % and _.
% represents zero, one or multiple characters.
For example, if you enter 5-ht% on the "Receptor name" box, you will retrieve 5-ht1, 5-ht1A, 5-ht1B, 5-ht1C, 5-ht1D, 5-ht1Da, 5-
_ (underscore) represents one character. If you enter 5-ht1_ on the "Receptor name" box, you will retrieve 5-ht1A, 5-ht1B, 5-ht1C

Fig. 5. Representative search results from K_i DB. Shown is a representation of the search results for discovering molecular targets responsible for plant-based psychoactive compound actions. In this case, *N*,*N*-DMT was queried and the receptor-affinity data for this compound were returned using the main K_i DB query page (http://kidb.bioc.cwru.edu/pdsp.php).

Gobbi et al. (2001) found that hypericin and other constitutents had low micromolar affinities for various peptide (NPY-1, NPY-2), serotonin, and δ-opioid receptors. Taken together, these results indicate that certain purified substances obtained from *H. perforatum* can interact with a variety of biogenic amine and peptide receptors with low affinities, generally in the micromolar range. With the exception of amentoflavone, which has high affinity for the GABA-benzodiazepine receptor complex and δ-opioid receptors (Butterweck et al., 2002), and hypericin, which has moderate affinity for CRF-1 receptors, the evidence is not yet persuasive that the main molecular targets responsible for the antidepressant actions of these compounds have been discovered. It is most likely that the putative antidepressant actions are mediated by a mixture of compounds, each of which has a complex pharmacological profile.

3.2. *Salvia divinorum*

Salvia divinorum is a hallucinogenic plant that has been used by *curanderos* in Mexico and other areas for centuries for divination and shamanism and first described by a western observer in 1962 (Wasson, 1962). For many years after its discovery, however, there was considerable controversy regarding psychoactive potential of *S. divinorum* largely because the active ingredient is inactive when taken orally. Additionally, *S. divinorum*'s actions are relatively short-lived and subtle (Siebert, 1994; Valdes, 1994). Nonetheless, *S. divinorum* is a frequently used hallucinogen (Giroud et al., 2000) that is currently nonscheduled (i.e., legal) in the United States.

Phenomenologically, the hallucinatory experience induced by *S. divinorum* is distinct from that induced by

the classical hallucinogenic plants *Psilocybe mexicana* or *Lophophora williamsii*, and is more accurately described as "spatiotemporal dislocation." First-person accounts describe an experience wherein the temporal boundaries among past, present, and future dissolve and the user is transported (frequently instantaneously) to an alternative time and place (Siebert, 1994). Visual hallucinations of the types induced by psilocybin, lysergic acid diethylamide, or mescaline are infrequent, and *S. divinorum*'s actions are frequently barely perceptible (Wasson, 1962; Valdes et al., 1983). The presumed active ingredient, salvinorin-A (Fig. 6) was independently isolated by two groups in the early 1980s (Ortega et al., 1982; Valdes et al., 1984) and shown by Siebert (1994) to be the main active ingredient more than a decade later. Salvinorin-A defines a novel structural family of hallucinogens in that it is a nonnitrogenous neoclerodane diterpene of known absolute stereochemistry. Its structure gives no clue regarding its site of action. Subsequently, a large number of other substituents including salvinorin-B, -C, -D, -E, and -F (Munro & Rizzacasa, 2003), along with divinaturin-A, -B, and -C (Bigham et al., 2003), have been described—all of which are neoclerodane diterpenoids in structure. None of the known diterpenoids, with the exception of salvinorin-A, has any known psychoactive actions and, thus, attention has focused on discovering the mechanism of action of salvinorin-A.

Initial attempts at discovering the mechanism of action of salvinorin-A were unsuccessful (Siebert, 1994) and a Novascreen, wherein a large number of mainly noncloned receptors, ion channels, and transporters were screened, failed to discover a site of action of salvinorin-A. These initial negative results implied that salvinorin-A likely had selectivity for a single molecular target. In late 2001, Roth's laboratory reinvestigated the pharmacology of salvinorin-A using the resources of the NIMH-PDSP and performed a receptorome profile using mainly cloned, human molecular targets (Roth et al., 2002; Sheffler & Roth, 2003). We discovered that salvinorin-A was a potent and selective κ-opioid receptor agonist (Roth et al., 2002). An initial screen of >50 cloned human receptors, ion channels, and transporters disclosed remarkable selectivity for salvinorin-A with virtually no affinity for any other tested molecular target including μ- and δ-opioid receptors and various GPCR, which have lipids as ligands (e.g., EP3 and EP1 prostaglandin receptors). More recently, the profile has been extended to include ORL-1 opioid receptors, human σ-1 and σ-2, and CB-1 and CB-2 cannibinoid receptors, and it was reported that salvinorin-A had no appreciable affinity for any receptors other than KOR (Chavkin et al., 2004).

In vitro, salvinorin-A is a potent and highly efficacious KOR agonist (Roth et al., 2002; Chavkin et al., 2004). Indeed, salvinorin-A is significantly more efficacious than U69,593 and U50,488H—two prototypical KOR agonists and slightly more effective than dynorphin 1–13—the presumed naturally occurring KOR agonist (Chavkin et al., 2004). Structure-function studies have revealed that the 2′-position of salvinorin-A is crucial for activity as a limited number of substitutions in the 2′-position are tolerated (Chavkin et al., 2004). Indeed, salvinorin-B, which differs from salvinorin-A by loss of the 2′-acetoxy group is inactive (Chavkin et al., 2004). Since salvinorin-A could easily be metabolized to salvinorin-B via esterase activity, the results suggest that the short duration of action of salvinorin-A is due to rapid de-esterification of salvinorin-A, though further studies are needed to test this hypothesis (Fig. 6).

Anecdotal reports that naloxone—a nonselective opioid antagonist—can block the effects of salvinorin-A in humans (D. Siebert, personal communication; Sheffler & Roth, 2003) indicate that salvinorin-A mediates its actions via activating KOR in vivo. In support of this hypothesis, Butelman et al. (2004) have recently reported that salvinorin-A produces psychological effects in nonhuman primates equivalent to those induced by standard KOR agonists. Finally, studies with wildtype and KOR knockout mice have shown that the effects of salvinorin-A on mouse behaviour are mediated by KOR (J. Pintar, personal communication). Taken together, these studies demonstrate that comprehensive receptorome profiling can be used to discover the molecular target(s) for a plant-based psychoactive compounds.

3.3. *Ephedra sinensis* and ephedrine-related compounds

Ephedra sinensis, also known as Ma Huang, has been used for more than 5000 years in China as an herbal remedy for asthma and upper respiratory ailments. More recently, *E. sinensis* and its main active ingredient ephedrine have been used as over-the-counter agents to increase stamina and metabolism. Ephedrine and ephedrine-containing extracts have been used as nonregulated anorectic agents in the United States until quite recently, as the FDA banned the sale and use of ephedra on December 31, 2003—mainly because of adverse cardiovascular consequences (stroke, heart attacks, sudden death; Rothman et al., 2003).

Ephedra extracts contain a complex mixture of phenylpropanolamines (Rothman et al., 2003) with several isomers including (+)- and (−)-ephedrine and (+)- and (−)-pseudoephedrine. A related plant *Catha edulis*, also used for its

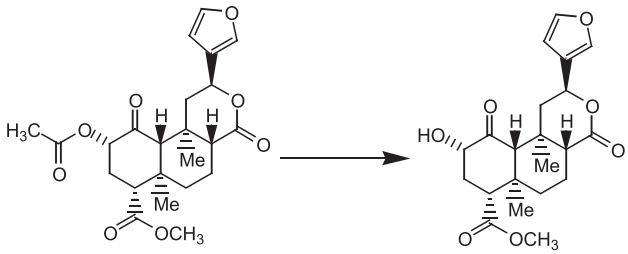


Fig. 6. Structures of salvinorin-A and salvinorin-B. Shown are structures of salvinorin-A (left) and salvinorin-B (right). As can be seen, salvinorin-B could be derived from salvinorin-A via simple ester hydrolysis in vivo via, for example, esterase action.

psychostimulant properties, contains cathine. Until recently, the action of ephedrine and related phenylpropanolamines was presumed to result mainly from a direct action on postsynaptic α_1-adrenergic receptors (see, for instance, Gilman et al., 1992), although this had never been rigorously tested. We thus profiled a large number of ephedrine-like phenylpropanolamines at the receptorome in an effort to discover the main site(s) of action of ephedrine.

To our surprise, we discovered that (−)- and (+)-ephedrine, as well as nearly all other tested phenylpropanol-

Table 2
Main psychoactive botanicals and their principal molecular targets

Plant name	Main active ingredient(s)	Principal molecular target(s)	Class of target (GPCR, ion channel, transporter, other)	Common name
Nicotiana tabacum	Nicotine	Nicotinic cholinergic receptors	Ion channel	Tobacco
Areca catechu	Arecholine	Muscarinic cholinergic receptors	GPCR	Betel nut
Catha edulis	Cathinone	Norepinephrine transporter (Rothman et al., 2003)	Transporter	Kat
Coffee arabica	Caffeine	Adenosine receptors (Snyder et al., 1981)	GPCR	Coffee
Thea viridins	Theophylline	Adenosine receptors (Snyder et al., 1981)	GPCR	Green tea
Piper methysticum	Kava lactones	Multiple ion channels (Singh & Singh, 2002)	Ion channels	Kava kava
Erythroxylum cocoa	Cocaine	Multiple biogenic amine transporters	Transporters	Cocaine
Paulinia cupana, *Yerbe mate*, and others	Caffeine	Adenosine receptors (Snyder et al., 1981)	GPCR	Yerbe
Lophophora williamsii	Mescaline	5-HT_{2A} serotonin receptors (Glennon et al., 1984; Nichols, 2004)	GPCR	Peyote
Psilocybe mexicana	Psilocybin	5-HT_{2A} serotonin receptors (Glennon et al., 1984; Nichols, 2004)	GPCR	Psilocybin mushrooms
Ipomoea violaceae	Lysergic acid amide	5-HT_{2A} serotonin receptors (Glennon et al., 1984; Nichols, 2004)	GPCR	Morning glory seeds
Tabernanthe iboga	Ibogaine	Unknown	Unknown	Ibogaine
Claviceps purpurae	Ergot alkaloids	5-HT receptors (many)	GPCR	Ergot
Myristica fragrens	Myristicin	Unknown	Unknown	Nutmeg
Artemisia absinthium	Thujone	GABA-A receptors and likely other targets	Ion Channels	Absinthe
Hyoscyamus niger	Hyoscamine and other tropanes	Muscarinic receptors	GPCR	Henbane
Atropa belladonna	Atropine	Muscarinic receptors	GPCR	Belladonna
Datura sp.	Scopolamine	Muscarinic receptors	GPCR	Jimson weed
Cannabis sativa	Tetrahydrocannabinol	CB-1 cannabinoid receptors (Howlett et al., 1990)	GPCR	Marihuana
Ephedra sinica	Ephedra	Norepinephrine transporters (Rothman et al., 2003)	Transporters	Ephedra
Salvia divinorum	Salvinorin-A	Kappa opioid receptor (Roth et al., 2002)	GPCR	Salvia
Amanita muscaria	Muscimol, ibotenic acid	Muscarinic and metabotropic glutamate receptors (Nicoletti et al., 1986)	GPCR	Fly agaric
Hypericin perforatum	Hypericin, amentoflavone and many others	Many GPCR, transporters, kinases, and ion channels (Simmen et al., 1999, 2001; Butterweck et al., 2002)	GPCR, ion channels, transporters	St. John's wort
Papaver somniferum	Morphine and many related alkaloids	μ-opioid receptor (Pert & Snyder, 1973)	GPCR	Opium
Psychotria viridis and *Virola* sp.	*N*,*N*-DMT (and related tryptamines)	5-HT_{2A} serotonin receptor (Glennon et al., 1984; Nichols, 2004)	GPCR	Chacruna, ayahuasca
Heimia salicifolia	Cryogenine	Unknown (? prostaglandin synthetase inhibition) (Lema et al., 1986)	Enzyme/GPCR indirectly	Sinicuichi
Vocanga africana	Voacangine	Unknown—related to ibogaine	Unknown	None
Corynanthe yohimbe	Yohimbine	α_2-Adrenergic antagonist	GPCR	Yohimbine

amines, had their highest affinities as norepinephrine transporter substrates with affinities in the 10- to 40-nM range. Most of the compounds also had modest activity as dopamine transporter substrates with affinities in the 200- to 2000-nM range. Cathinone and methcathinone had high affinities as dopamine transporter substrates with affinities in the 14- to 18-nM range. It is likely that the high affinity of cathinone and methcathinone for dopamine transporters is important for their high abuse potential. None of the compounds had appreciable affinities (e.g., K_i values <1000 nM) for any of the other tested receptors, ion channels, or transporters. Indeed, although some derivatives had affinities in the micromolar range for α_1-adrenergic receptors, none of the tested compounds displayed any functional activity at cloned human α_{1A}-adrenergic or cloned human α_2-adrenergic receptors (Rothman et al., 2003). Additionally, drug discrimination studies revealed a direct linear correlation between for their norepinephrine transporter substrate activity and behavioral actions (Rothman et al., 2003). Taken together, these results are consistent with the hypothesis that the main cardiovascular actions of ephedrine and related phenylpropanolamines are due to an indirect sympathomimetic action and not to direct activation of postsynaptic adrenergic receptors. These results thus serve as an additional example showing how comprehensively screening the receptorome reveals the molecular target for psychoactive plant actions.

4. Prospects for future studies

Many plants are psychoactive and Table 2 lists the major known psychoactive plants, their presumed active ingredients, and their presumed principal molecular targets. Only a few psychoactive plant-derived chemicals (e.g., *E. sinensis* and *C. eduli*; see Rothman et al., 2003) have been comprehensively profiled, although when receptorome profiles have been done, the results have been highly informative (Roth et al., 2002; Butterweck et al., 2002; Rothman et al., 2003).

Several psychoactive plants have well-described mechanisms of action including *Cannabis sativa* (tetrahydrocannibinol; CB-1 receptors) and *Nicotiana tabacum* (nicotine; nicotinic acetylcholine receptors). Many other psychoactive plants have unknown mechanisms of action including the hallucinogenic plants *Tabernanthe iboga* (ibogaine) and *Myristica fragens* (myristicin). Because many of the known psychoactive plants exert their actions via unknown mechanisms, a comprehensive, discovery-based effort aimed at elucidating the molecular targets for plant-based psychoactive compounds is likely to be highly successful. Using the same technology to discover the molecular targets responsible for marine-based natural products that have CNS actions is also likely to be successful. Additionally, many of the molecular targets of psychoactive drug action have become identified therapeutic targets for many diseases. These include μ-opioid receptors, the main site of action of morphine, for chronic pain conditions (Pert & Snyder, 1973), 5-HT_{2A} receptors, the principal target for hallucinogen actions (Glennon et al., 1984), which have been targeted for antidepressant and antipsychotic drug discovery efforts (Roth & Shapiro, 2001), and the biogenic amine transporters, the principal molecular targets responsible for cocaine actions (Shimada et al., 1991; Sora et al., 2001), which are targeted for antidepressant drug discovery efforts. Our prediction is that discovering the molecular mechanism(s) of action of many psychoactive plants will reveal novel and validated molecular targets for psychotherapeutic drug discovery.

References

Ballesteros, J. A., Jensen, A. D., Liapakis, G., Rasmussen, S. G., Shi, L., Gether, U., & Javitch, J. A. (2001). Activation of the beta 2-adrenergic receptor involves disruption of an ionic lock between the cytoplasmic ends of transmembrane segments 3 and 6. *J Biol Chem 276*, 29171–29177.

Bigham, A. K., Munro, T. A., Rizzacasa, M. A., & Robins-Browne, R. M. (2003). Divinatorins A-C, new neoclerodane diterpenoids from the controlled sage *Salvia divinorum*. *J Nat Prod 66*, 1242–1244.

Brenner, R., Azbel, V., Madhusoodanan, S., & Pawlowska, M. (2000). Comparison of an extract of hypericum (LI 160) and sertraline in the treatment of depression: a double-blind, randomized pilot study. *Clin Ther 22*, 411–419.

Brenner, R., Madhusoodanan, S., Pawlowska, M., & Czobor, P. (2001). St John's wort and major depression. *Jama 286*, 43 (author reply 44–45).

Brenner, R., Madhusoodanan, S., & Pawlowska, M. (2002). Efficacy of continuation treatment with *Hypericum perforatum* in depression. *J Clin Psychiatry 63*, 455.

Butelman, E. R., Harris, T. J., & Kreek, M. J. (2004). The plant-derived hallucinogen, salvinorin A, produces kappa-opioid agonist-like discriminative effects in rhesus monkeys. *Psychopharmacology (Berl) 172, 220–224 (E-pub 2003 Oct 30).*

Butterweck, V. (2003). Mechanism of action of St John's wort in depression: what is known? *CNS Drugs 17*, 539–562.

Butterweck, V., Wall, A., Lieflander-Wulf, U., Winterhoff, H., & Nahrstedt, A. (1997). Effects of the total extract and fractions of *Hypericum perforatum* in animal assays for antidepressant activity. *Pharmacopsychiatry 30*(Suppl. 2), 117–124.

Butterweck, V., Petereit, F., Winterhoff, H., & Nahrstedt, A. (1998). Solubilized hypericin and pseudohypericin from *Hypericum perforatum* exert antidepressant activity in the forced swimming test. *Planta Med 64*, 291–294.

Butterweck, V., Jurgenliemk, G., Nahrstedt, A., & Winterhoff, H. (2000). Flavonoids from *Hypericum perforatum* show antidepressant activity in the forced swimming test. *Planta Med 66*, 3–6.

Butterweck, V., Nahrstedt, A., Evans, J., Hufeisen, S., Rauser, L., Savage, J., Popadak, B., Ernsberger, P., & Roth, B. L. (2002). In vitro receptor screening of pure constituents of St. John's wort reveals novel interactions with a number of GPCRs. *Psychopharmacology (Berl) 162*, 193–202.

Chavkin, C., Sud, S., Jin, W., Stewart, J., Zjawiony, J. K., Siebert, D. J., Toth, B. A., Hufeisen, S. J., & Roth, B. L. (2004). Salvinorin A, an active component of the hallucinogenic sage *Salvia divinorum*, is a highly efficacious kappa opioid receptor agonist: structural and functional considerations. *J Pharmacol Exp Ther 308, 1197–1203 (E-pub 2004 Jan 8).*

Cott, J. M. (1997). In vitro receptor binding and enzyme inhibition by *Hypericum perforatum* extract. *Pharmacopsychiatry 30*(Suppl. 2), 108–112.

Dunham, T. D., & Farrens, D. L. (1999). Conformational changes in rho-

dopsin. Movement of helix f detected by site-specific chemical labeling and fluorescence spectroscopy. *J Biol Chem 274*, 1683–1690.

Ebersole, B. J., Visiers, I., Weinstein, H., & Sealfon, S. C. (2003). Molecular basis of partial agonism: orientation of indoleamine ligands in the binding pocket of the human serotonin 5-HT2A receptor determines relative efficacy. *Mol Pharmacol 63*, 36–43.

Farrens, D. A. C., Yang, K., Hubbell, W. L., & Khorana, H. G. (1996). Requirement of rigid-body motion of transmembrane helices for light activation of rhodopsin. *Science 274*, 768–770.

Gaddum, J. H., & Hameed, K. A. (1954). Drugs which antagonize 5-hydroxytryptamine. *Br J Pharmacol 9*, 240–248.

Gether, U., & Kobilka, B. K. (1998). G protein-coupled receptors: II. Mechanism of agonist activation. *J Biol Chem 273*, 17979–17982.

Gilman, A. G., Goodman, L. S., & Gilman, A. (1992). *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. New York: Pergamon Press.

Giroud, C., Felber, F., Augsburger, M., Horisberger, B., Rivier, L., & Mangin, P. (2000). *Salvia divinorum*: an hallucinogenic mint which might become a new recreational drug in Switzerland. *Forensic Sci Int 112*, 143–150.

Glennon, R. A., Titler, M., & McKenney, J. D. (1984). Evidence for 5-HT2 involvement in the mechanism of action of hallucinogenic agents. *Life Sciences 35*, 2505–2511.

Gobbi, M., Moia, M., Pirona, L., Morizzoni, P., & Mennini, T. (2001). In vitro binding studies with two *Hypericum perforatum* extracts—hyperforin, hypericin and biapigenin—on 5-HT6, 5-HT7, GABA(A)/benzodiazepine, sigma, NPY-Y1/Y2 receptors and dopamine transporters. *Pharmacopsychiatry 34*(Suppl. 1), S45–S48.

Heffter, A. (1898). Uber peyote. *Naunyn Schmiedebergs Arch Exp Pathol Pharmacol 40*, 385–429.

Hoffman, A. (1979). How LSD originated. *J Psychedelic Drugs 11*, 53–60.

Howlett, A. C., Bidaut-Russell, M., Devane, W. A., Melvin, L. S., Johnson, M. R., & Herkenham, M. (1990). The cannabinoid receptor: biochemical, anatomical and behavioral characterization. *Trends Neurosci 13*, 420–423.

Hypericum Depression Trial Study Group (2002). Effect of *Hypericum perforatum* (St John's wort) in major depressive disorder: a randomized controlled trial. *JAMA 287*, 1807–1814.

Kroeze, W. K., Kristiansen, K., & Roth, B. L. (2002). Molecular biology of serotonin receptors structure and function at the molecular level. *Curr Top Med Chem 2*, 507–528.

Kroeze, W. K., Sheffler, D. J., & Roth, B. L. (2003). G-protein-coupled receptors at a glance. *J Cell Sci 116*, 4867–4869.

Lander, E. S., Linton, L. M., Birren, B., Nusbaum, C., Zody, M. C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., Funke, R., Gage, D., Harris, K., Heaford, A., Howland, J., Kann, L., Lehoczky, J., LeVine, R., McEwan, P., McKernan, K., Meldrim, J., Mesirov, J. P., Miranda, C., Morris, W., Naylor, J., Raymond, C., Rosetti, M., Santos, R., Sheridan, A., Sougnez, C., Stange-Thomann, N., Stojanovic, N., Subramanian, A., Wyman, D., Rogers, J., Sulston, J., Ainscough, R., Beck, S., Bentley, D., Burton, J., Clee, C., Carter, N., Coulson, A., Deadman, R., Deloukas, P., Dunham, A., Dunham, I., Durbin, R., French, L., Grafham, D., Gregory, S., Hubbard, T., Humphray, S., Hunt, A., Jones, M., Lloyd, C., McMurray, A., Matthews, L., Mercer, S., Milne, S., Mullikin, J. C., Mungall, A., Plumb, R., Ross, M., Shownkeen, R., Sims, S., Waterston, R. H., Wilson, R. K., Hillier, L. W., McPherson, J. D., Marra, M. A., Mardis, E. R., Fulton, L. A., Chinwalla, A. T., Pepin, K. H., Gish, W. R., Chissoe, S. L., Wendl, M. C., Delehaunty, K. D., Miner, T. L., Delehaunty, A., Kramer, J. B., Cook, L. L., Fulton, R. S., Johnson, D. L., Minx, P. J., Clifton, S. W., Hawkins, T., Branscomb, E., Predki, P., Richardson, P., Wenning, S., Slezak, T., Doggett, N., Cheng, J. F., Olsen, A., Lucas, S., Elkin, C., Uberbacher, E., Frazier, M., Gibbs, R. A., Muzny, D. M., Scherer, S. E., Bouck, J. B., Sodergren, E. J., Worley, K. C., Rives, C. M., Gorrell, J. H., Metzker, M. L., Naylor, S. L., Kucherlapati, R. S., Nelson, D. L., Weinstock, G. M., Sakaki, Y., Fujiyama, A., Hattori, M., Yada, T., Toyoda, A., Itoh, T., Kawagoe, C., Watanabe, H., Totoki, Y., Taylor, T., Weissenbach, J., Heilig, R., Saurin, W., Artiguenave, F., Brottier, P., Bruls, T., Pelletier, E., Robert, C., Wincker, P., Smith, D. R., Doucette-Stamm, L., Rubenfield, M., Weinstock, K., Lee, H. M., Dubois, J., Rosenthal, A., Platzer, M., Nyakatura, G., Taudien, S., Rump, A., Yang, H., Yu, J., Wang, J., Huang, G., Gu, J., Hood, L., Rowen, L., Madan, A., Qin, S., Davis, R. W., Federspiel, N. A., Abola, A. P., Proctor, M. J., Myers, R. M., Schmutz, J., Dickson, M., Grimwood, J., Cox, D. R., Olson, M. V., Kaul, R., Raymond, C., Shimizu, N., Kawasaki, K., Minoshima, S., Evans, G. A., Athanasiou, M., Schultz, R., Roe, B. A., Chen, F., Pan, H., Ramser, J., Lehrach, H., Reinhardt, R., McCombie, W. R., de la Bastide, M., Dedhia, N., Blocker, H., Hornischer, K., Nordsiek, G., Agarwala, R., Aravind, L., Bailey, J. A., Bateman, A., Batzoglou, S., Birney, E., Bork, P., Brown, D. G., Burge, C. B., Cerutti, L., Chen, H. C., Church, D., Clamp, M., Copley, R. R., Doerks, T., Eddy, S. R., Eichler, E. E., Furey, T. S., Galagan, J., Gilbert, J. G., Harmon, C., Hayashizaki, Y., Haussler, D., Hermjakob, H., Hokamp, K., Jang, W., Johnson, L. S., Jones, T. A., Kasif, S., Kaspryzk, A., Kennedy, S., Kent, W. J., Kitts, P., Koonin, E. V., Korf, I., Kulp, D., Lancet, D., Lowe, T. M., McLysaght, A., Mikkelsen, T., Moran, J. V., Mulder, N., Pollara, V. J., Ponting, C. P., Schuler, G., Schultz, J., Slater, G., Smit, A. F., Stupka, E., Szustakowski, J., Thierry-Mieg, D., Thierry-Mieg, J., Wagner, L., Wallis, J., Wheeler, R., Williams, A., Wolf, Y. I., Wolfe, K. H., Yang, S. P., Yeh, R. F., Collins, F., Guyer, M. S., Peterson, J., Felsenfeld, A., Wetterstrand, K. A., Patrinos, A., Morgan, M. J., Szustakowki, J., de Jong, P., Catanese, J. J., Osoegawa, K., Shizuya, H., Choi, S., & Chen, Y. J. (2001). International Human Genome Sequencing Consortium. *Nature 409*, 860–921.

Lecrubier, Y., Clerc, G., Didi, R., & Kieser, M. (2002). Efficacy of St. John's wort extract WS 5570 in major depression: a double-blind, placebo-controlled trial. *Am J Psychiatry 159*, 1361–1366.

Lema, W. J., Blankenship, J. W., & Malone, M. H. (1986). Prostaglandin synthetase inhibition by alkaloids of *Heimia salicifolia*. *J Ethnopharmacol 15*, 161–167.

Lewin, L. (1924). *Phantastica: Narcotic and Stimulating Drugs*. Rochester: Park Street Press.

Munro, T. A., & Rizzacasa, M. A. (2003). Salvinorins D-F, new neoclerodane diterpenoids from *Salvia divinorum*, and an improved method for the isolation of salvinorin A. *J Nat Prod 66*, 703–705.

Nahrstedt, A., & Butterweck, V. (1997). Biologically active and other chemical constituents of the herb of *Hypericum perforatum* L. *Pharmacopsychiatry 30*(Suppl. 2), 129–134.

Nichols, D. (2004). Hallucinogens. *J Pharmacol Ther 101*, 131-181.

Nicoletti, F., Meek, J., Iadarola, M. J., Chuang, D. -M., Roth, B. L., & Costa, E. (1986). Coupling of inositol phospholipid metabolism with excitatory amino acid recognition sites in rat hippocampus. *J Neurochem 46*, 40–46.

Ortega, A., Blount, J. F., & Manchand, P. S. (1982). Salvinorin, a new trans-neoclerodane diterpene from *Salvia divinorum* (Labiatae). *J Chem Soc Perkins Trans*, 2505–2508.

Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., Le Trong, I., Teller, D. C., Okada, T., Stenkamp, R. E., Yamamoto, M., & Miyano, M. (2000). Crystal structure of rhodopsin: A G protein-coupled receptor. [see comments] *Science 289*, 739–745.

Pert, C. B., & Snyder, S. H. (1973). Opiate receptor: demonstration in nervous tissue. *Science 179*, 1011–1014.

Prioleau, C., Visiers, I., Ebersole, B. J., Weinstein, H., & Sealfon, S. C. (2002). Conserved helix 7 tyrosine acts as a multistate conformational switch in the 5HT2C receptor. Identification of a novel "locked-on" phenotype and double revertant mutations. *J Biol Chem 277*, 36577–36584.

Roth, B. L., & Shapiro, D. A. (2001). Insights into the structure and function of 5-HT2 family serotonin receptors reveal novel strategies for therapeutic target development. *Expert Opin Ther Targets 5*, 685–695.

Roth, B. L., Baner, K., Westkaemper, R., Siebert, D., Rice, K. C., Steinberg, S., Ernsberger, P., & Rothman, R. B. (2002). Salvinorin A: a potent naturally occurring nonnitrogenous kappa opioid selective agonist. *Proc Natl Acad Sci USA 99*, 11934–11939.

Rothman, R. B., Vu, N., Partilla, J. S., Roth, B. L., Hufeisen, S. J., Compton-Toth, B. A., Birkes, J., Young, R., & Glennon, R. A. (2003). In vitro

characterization of ephedrine-related stereoisomers at biogenic amine transporters and the receptorome reveals selective actions as norepinephrine transporter substrates. *J Pharmacol Exp Ther 307*, 138–145.

Shapiro, D. A., Kristiansen, K., Kroeze, W. K., & Roth, B. L. (2000). Differential modes of agonist binding to 5-hydroxytryptamine(2A) serotonin receptors revealed by mutation and molecular modeling of conserved residues in transmembrane region 5. *Mol Pharmacol 58*, 877–886.

Shapiro, D. A., Kristiansen, K., Weiner, D. M., Kroeze, W. K., & Roth, B. L. (2002). Evidence for a model of agonist-induced activation of 5-HT2A serotonin receptors which involves the disruption of a strong ionic interaction between helices 3 and 6. *J Biol Chem 277*, 11441–11449 (E-pub 2002 Jan 18).

Sheffler, D. J., & Roth, B. L. (2003). Salvinorin A: the "magic mint" hallucinogen finds a molecular target in the kappa opioid receptor. *Trends Pharmacol Sci 24*, 107–109.

Shelton, R. C., Keller, M. B., Gelenberg, A., Dunner, D. L., Hirschfeld, R., Thase, M. E., Russell, J., Lydiard, R. B., Crits-Cristoph, P., Gallop, R., Todd, L., Hellerstein, D., Goodnick, P., Keitner, G., Stahl, S. M., & Halbreich, U. (2001). Effectiveness of St John's wort in major depression: a randomized controlled trial. *JAMA 285*, 1978–1986.

Shimada, S., Kitayama, S., Lin, C. L., Patel, A., Nanthakumar, E., Gregor, P., Kuhar, M., & Uhl, G. (1991). Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA. *Science 254*, 576–578.

Siebert, D. J. (1994). *Salvia divinorum* and salvinorin A: New pharmacologic findings. *J Ethnopharmacol 43*, 53–56.

Simmen, U., Burkard, W., Berger, K., Schaffner, W., & Lundstrom, K. (1999). Extracts and constituents of *Hypericum perforatum* inhibit the binding of various ligands to recombinant receptors expressed with the Semliki Forest virus system. *J Recept Signal Transduct Res 19*, 59–74.

Simmen, U., Higelin, J., Berger-Buter, K., Schaffner, W., & Lundstrom, K. (2001). Neurochemical studies with St. John's wort in vitro. *Pharmacopsychiatry 34*(Suppl. 1), S137–S142.

Simmen, U., Bobirnac, I., Ullmer, C., Lubbert, H., Berger Buter, K., Schaffner, W., & Schoeffter, P. (2003). Antagonist effect of pseudohypericin at CRF1 receptors. *Eur J Pharmacol 458*, 251–256.

Singh, Y. N., & Singh, N. N. (2002). Therapeutic potential of kava in the treatment of anxiety disorders. *CNS Drugs 16*, 731–743.

Snyder, S. H., Katims, J. J., Annau, Z., Bruns, R. F., & Daly, J. W. (1981). Adenosine receptors and behavioral actions of methylxanthines. *Proc Natl Acad Sci USA 78*, 3260–3264.

Sora, I., Hall, F. S., Andrews, A. M., Itokawa, M., Li, X. F., Wei, H. B., Wichems, C., Lesch, K. P., Murphy, D. L., & Uhl, G. R. (2001). Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. *Proc Natl Acad Sci USA 98*, 5300–5305.

Takeda, S., Kadowaki, S., Haga, T., Takaesu, H., & Mitaku, S. (2002). Identification of G protein-coupled receptor genes from the human genome sequence. *FEBS Lett 520*, 97–101.

Valdes III, L. J. (1994). *Salvia divinorum* and the unique diterpene hallucinogen, salvinorin (divinorin) A. *J Psychoact Drugs 26*, 277–283.

Valdes III, L. J., Diaz, J. L., & Paul, A. G. (1983). Ethnopharmacology of Ska Maria Pastora (*Salvia divinorum*, Epling and Jativa-M.). *J Ethnopharmacol 7*, 287–312.

Valdes, L. J., Butler, W. M., Hatfield, G. M., Paul, A. G., & Koreeda, M. (1984). Divinorin A: a psychotropic terpenoid and divnorin B from the hallucinogenic Mexican mint *Salvia divinorum*. *J Org Chem 49*, 4716–7720.

Vassilatis, D. K., Hohmann, J. G., Zeng, H., Li, F., Ranchalis, J. E., Mortrud, M. T., Brown, A., Rodriguez, S. S., Weller, J. R., Wright, A. C., Bergmann, J. E., & Gaitanaris, G. A. (2003). The G protein-coupled receptor repertoires of human and mouse. *Proc Natl Acad Sci USA 100*, 4903–4908.

Venter, J. C., Adams, M. D., Myers, E. W., Li, P. W., Mural, R. J., Sutton, G. G., Smith, H. O., Yandell, M., Evans, C. A., Holt, R. A., Gocayne, J. D., Amanatides, P., Ballew, R. M., Huson, D. H., Wortman, J. R., Zhang, Q., Kodira, C. D., Zheng, X. H., Chen, L., Skupski, M., Subramanian, G., Thomas, P. D., Zhang, J., Gabor Miklos, G. L., Nelson, C., Broder, S., Clark, A. G., Nadeau, J., McKusick, V. A., Zinder, N., Levine, A. J., Roberts, R. J., Simon, M., Slayman, C., Hunkapiller, M., Bolanos, R., Delcher, A., Dew, I., Fasulo, D., Flanigan, M., Florea, L., Halpern, A., Hannenhalli, S., Kravitz, S., Levy, S., Mobarry, C., Reinert, K., Remington, K., Abu-Threideh, J., Beasley, E., Biddick, K., Bonazzi, V., Brandon, R., Cargill, M., Chandramouliswaran, I., Charlab, R., Chaturvedi, K., Deng, Z., Di Francesco, V., Dunn, P., Eilbeck, K., Evangelista, C., Gabrielian, A. E., Gan, W., Ge, W., Gong, F., Gu, Z., Guan, P., Heiman, T. J., Higgins, M. E., Ji, R. R., Ke, Z., Ketchum, K. A., Lai, Z., Lei, Y., Li, Z., Li, J., Liang, Y., Lin, X., Lu, F., Merkulov, G. V., Milshina, N., Moore, H. M., Naik, A. K., Narayan, V. A., Neelam, B., Nusskern, D., Rusch, D. B., Salzberg, S., Shao, W., Shue, B., Sun, J., Wang, Z., Wang, A., Wang, X., Wang, J., Wei, M., Wides, R., Xiao, C., Yan, C., Yao, A., Ye, J., Zhan, M., Zhang, W., Zhang, H., Zhao, Q., Zheng, L., Zhong, F., Zhong, W., Zhu, S., Zhao, S., Gilbert, D., Baumhueter, S., Spier, G., Carter, C., Cravchik, A., Woodage, T., Ali, F., An, H., Awe, A., Baldwin, D., Baden, H., Barnstead, M., Barrow, I., Beeson, K., Busam, D., Carver, A., Center, A., Cheng, M. L., Curry, L., Danaher, S., Davenport, L., Desilets, R., Dietz, S., Dodson, K., Doup, L., Ferriera, S., Garg, N., Gluecksmann, A., Hart, B., Haynes, J., Haynes, C., Heiner, C., Hladun, S., Hostin, D., Houck, J., Howland, T., Ibegwam, C., Johnson, J., Kalush, F., Kline, L., Koduru, S., Love, A., Mann, F., May, D., McCawley, S., McIntosh, T., McMullen, I., Moy, M., Moy, L., Murphy, B., Nelson, K., Pfannkoch, C., Pratts, E., Puri, V., Qureshi, H., Reardon, M., Rodriguez, R., Rogers, Y. H., Romblad, D., Ruhfel, B., Scott, R., Sitter, C., Smallwood, M., Stewart, E., Strong, R., Suh, E., Thomas, R., Tint, N. N., Tse, S., Vech, C., Wang, G., Wetter, J., Williams, S., Williams, M., Windsor, S., Winn-Deen, E., Wolfe, K., Zaveri, J., Zaveri, K., Abril, J. F., Guigo, R., Campbell, M. J., Sjolander, K. V., Karlak, B., Kejariwal, A., Mi, H., Lazareva, B., Hatton, T., Narechania, A., Diemer, K., Muruganujan, A., Guo, N., Sato, S., Bafna, V., Istrail, S., Lippert, R., Schwartz, R., Walenz, B., Yooseph, S., Allen, D., Basu, A., Baxendale, J., Blick, L., Caminha, M., Carnes-Stine, J., Caulk, P., Chiang, Y. H., Coyne, M., Dahlke, C., Mays, A., Dombroski, M., Donnelly, M., Ely, D., Esparham, S., Fosler, C., Gire, H., Glanowski, S., Glasser, K., Glodek, A., Gorokhov, M., Graham, K., Gropman, B., Harris, M., Heil, J., Henderson, S., Hoover, J., Jennings, D., Jordan, C., Jordan, J., Kasha, J., Kagan, L., Kraft, C., Levitsky, A., Lewis, M., Liu, X., Lopez, J., Ma, D., Majoros, W., McDaniel, J., Murphy, S., Newman, M., Nguyen, T., Nguyen, N., Nodell, M., Pan, S., Peck, J., Peterson, M., Rowe, W., Sanders, R., Scott, J., Simpson, M., Smith, T., Sprague, A., Stockwell, T., Turner, R., Venter, E., Wang, M., Wen, M., Wu, D., Wu, M., Xia, A., Zandieh, A., & Zhu, X. (2001). The sequence of the human genome. *Science 291*, 1304–1351.

Vetulani, J., & Sulser, F. (1975). Action of various antidepressant treatments reduces reactivity of noradrenergic cyclic AMP-generating system in limbic forebrain. *Nature 257*, 495–496.

Wasson, R. G. (1962). A new Mexican psychotropic drug from the mint family. *Bot Mus Leaf Harv Univ 20*, 77–84.

Westkaemper, R. B., & Glennon, R. A. (2002). Application of ligand SAR, receptor modeling and receptor mutagenesis to the discovery and development of a new class of 5-HT(2A) ligands. *Curr Top Med Chem 2*, 575–598.

Wooley, D. W., & Shaw, E. (1954). A biochemical and pharmacological suggestion about certain mental disorders. *Proc Natl Acad Sci USA 40*, 228–231.