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Receptor screening technologies in the evaluation of Amazonian ethnomedicines with potential applications to cognitive deficits

Dennis J. McKenna[a,*], Juan M. Ruiz[b], Thomas R. Hoye[c], Bryan L. Roth[d], Alan T. Shoemaker[e]

[a] Center for Spirituality and Healing, Academic Health Center, University of Minnesota, MMC505, 420 Delaware St. SE, Minneapolis, MN 55455, USA
[b] Herbarium Amazonense, Faculdad de Ciencias Biológicas, Universidad Nacional de la Amazonía Peruana (UNAP), Iquitos, Peru
[c] Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, USA
[d] Department of Pharmacology, School of Medicine, University of North Carolina Chapel Hill, and NIMH Psychoactive Drug Screeing Program, Chapel Hill, NC 27599, USA
[e] Gracia Ethnobotanicals, Calle Morona 531, Iquitos, Peru

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ABSTRACT

Ethnopharmacological relevance: Amazonian peoples utilize a variety of psychoactive plants that may contain novel biologically active compounds. Efforts to investigate such remedies in terms of neuropharmacology have been limited.
Aim of this study: This study identified Amazonian ethnomedicines with potential for the treatment of cognitive deficits in schizophrenia and dementias, and characterized their interactions with CNS neurotransmitter receptors in vitro.
Materials and methods: Approximately 300 Amazonian species with folk uses or constituents indicative of central nervous system activity were incorporated into a database constructed from literature searches, herbarium surveys, and interviews with traditional practitioners. Approximately 130 of these targeted species were collected in Loreto province, Peru, and 228 fractions derived from them were screened in 31 radioreceptor assays via the resources of the NIMH Psychoactive Drug Screening Program. A subset was also screened in functional assays at selected serotonin, muscarinic, and adrenergic receptors.
Results: Ninety-one samples displayed ≥60% inhibition of radioligand binding activity in receptor assays; 135 samples displayed agonist or antagonist activity (or both) in functional assays.
Conclusions: Potential CNS activity was detected in about 40% of the samples screened, with some correlations to both folk uses and phytochemical constituents. These results may point to novel and potentially therapeutic CNS active compounds.

1. Introduction

Advances in psychopharmacology have resulted in the development of medications that are effective for the treatment of overt psychotic symptoms of schizophrenia, sometimes characterized as positive symptoms of the disease. By contrast, the negative symptoms of schizophrenia are associated with neurocognitive deficits in functions such as attention, executive functions, short- and long-term memory, and verbal ability (Peuskens et al., 2005; Lysaker and Buck, 2007). Treatment of the neurocognitive deficits in schizophrenia is likely key to eventual long-term recovery and productive reintegration of individuals with schizophrenia into social, educational, and employment contexts. While "atypical" antipsychotics such as clozapine have shown some promise for the treatment of neurocognitive deficits in schizophrenia, there remains a continued need for the identification of structurally novel compounds that are more effective, and have more acceptable side-effect profiles (Hill et al., 2010). Similar considerations also inform the search for effective medications to treat neurocognitive deficits in Alzheimer's disease and other dementias (Mangialasche et al., 2010).

Ethnopharmacology – the interdisciplinary investigation of biologically active substances used by indigenous cultures – has repeatedly demonstrated utility for the discovery of natural compounds that eventually find medical applications. In the field of psychopharmacology, ethnopharmacological research has uncovered a wide spectrum of CNS-active compounds ranging from sedatives to anxiolytics to analgesics to hallucinogens. Receptor binding methodologies – in which isotopically labeled compounds are employed to selectively label neurotransmitter or other receptors – have been widely utilized in drug discovery. The technique enables rapid screening of compound "libraries" or crude extracts derived from plants or other natural sources. The application of ethnopharmacology to identify potentially therapeutic psychotropic medicines with a history of human use, combined with

Abbreviation: PDSP, the NIMH Psychoactive Drug Screening Program.

* Corresponding author. Tel.: +1 612 624 9459; fax: +1 612 626 5280.
E-mail address: mcken031@umn.edu (D.J. McKenna).

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receptor binding and functional receptor assays to identify activity in crude extracts, is a productive approach to the discovery and evaluation of psychotropic ethnomedicines that may be suitable for development into clinically applicable psychotherapeutic agents.

1.1. Cognitive deficits in schizophrenia and dementias

Schizophrenia is a psychiatric illness with multiple symptomatologies, including delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behavior, and negative symptoms (Bleuler, 1968)

Negative symptoms reflect a loss or diminution of normal functions and include affective flattening, anhedonia, avolition, and alogia (Andreasen, 1982). Negative symptoms in schizophrenia are difficult to diagnose or evaluate because they occur on a continuum with normality, are non-specific, may occur as a consequence of more overt, psychotic symptoms or manifest as side effects of medication, or result from other types of mental disorders, such as mood disorders or dementias. Negative symptoms typically manifest during the prodromal period of schizophrenia and characteristically involve social withdrawal, loss of interest in formerly pleasurable activities, and diminished verbal and intellectual acuity. Negative symptoms may persist well beyond the resolution of the overtly psychotic symptoms (Kirkpatrick et al., 2006). Recovery of cognitive functions, more than overt symptomatology, has been proposed to be a better predictor of eventual successful psychosocial re-adaptation (Velligan et al., 1997; Prouteau et al., 2005). Accordingly, more recent treatment approaches to schizophrenia have focused on behavioral and pharmacological interventions targeted toward the resolution of neurocognitive deficits (Green et al., 2005).

Dementias comprise another set of disorders characterized by multiple cognitive deficits, including memory impairment. The etiologies of dementias are diverse and can result from Alzheimer's disease (dementia of the Alzheimer's type, DAT), cerebrovascular disorders, and dementias arising from other etiologies, such as HIV, Parkinson's disease, head trauma, substance abuse, or combinations of etiologies, e.g. the combination of DAT and cerebrovascular dementia (Ropper and Brown, 2005). Treatment of dementias, especially DAT and cerebrovascular dementias, has been another primary focus in the search for cognition-enhancing medications. Loss of cholinergic neurons and impairment of cholinergic neurotransmission in the basal forebrain cholinergic system is the primary pathology in DAT. Approaches to the pharmacotherapy of DAT and other dementias have emphasized the cholinergic system (Farlow and Evans, 1998) along with interfering with the accumulation of b-amyloid, tau and other protein aggregations (Aguzzi and O'Connor, 2010). Additional research has focused on the development of more potent, less toxic, longer-lasting and more selective cholinergic agents (Lawrence and Sahakian, 1998; Dragunow, 2008). Progressive loss of serotonin and serotonergic innervation has been linked to dementias in the elderly, and cognitive impairment in DAT may result from a combination of serotonergic and cholinergic functional disturbances (Meltzer et al., 1998). Disturbances in serotonin, noradrenergic, and dopaminergic systems have also been linked to behavioral deficits that frequently accompany cognitive dysfunctions in DAT and senile dementias, such as depression, aggressive behaviors, and psychosis (Kirby and Lawlor, 1995). A large number of potential targets have recently emerged for the pharmacotherapy of neurocognitive deficits in schizophrenia (Gray and Roth, 2007b).

More or less successful drug treatment of the psychotic, positive symptoms of schizophrenia has been achieved with the "typical" antipsychotic medications exemplified by haloperidol. The therapeutic actions of the typical antipsychotics are thought to result from the blockade of D_2 receptors in mesolimbic dopamine neurons (Creese et al., 1976). It is now clear that there is enhanced dopaminergic synaptic activity in subcortical regions in patients with schizophrenia (Kegeles et al., 2010). Conversely, the negative/cognitive deficit symptoms are thought to be associated with low dopamine activity in the prefrontal cortex (Kessler et al., 2009). Subcortical dopamine neurons may be inhibited by prefrontal dopamine activity under normal circumstances; in schizophrenia, lower prefrontal activity may contribute to negative/cognitive symptoms, while disinhibiting mesolimbic dopamine activity, leading to positive symptoms (Winterer and Weinberger, 2004). This model may also explain why neuroleptic blockade of D_2 receptors in the prefrontal cortex can sometimes worsen the negative symptoms and cognitive deficits associated with schizophrenia (Meltzer et al., 1991). A newer class of antipsychotic medications, termed the "atypical" antipsychotics, has been developed in recent decades following the observation that the prototype of the class, clozapine, does not induce tardive dyskinesia or extrapyramidal syndrome, two of the most debilitating side effects of the typical antipsychotic drugs. Multiple neuronal systems are implicated in the actions of atypical antipsychotics, including dopamine D_2, D_3, and D_4, $5HT_{2A}$, 5-HT_6, 5-HT_7 adrenergic α_1, histamine H_1, and cholinergic receptors (Roth et al., 2004a; Gray and Roth, 2007b). A commonality among most of these agents, including clozapine, respiridone, olanzapine, and others, is a relatively high affinity and occupancy of $5HT_{2A}$ (and other 5-HT) receptors coupled with an intermediate occupancy of D_2 receptors (Schotte et al., 1996). This combination of actions may explain these agents' ability to reduce the positive symptoms and reduce extrapyramidal side effects, while $5HT_{2A}$, 5-HT_6 and 5-HT_7 blockade may contribute to the amelioration of negative symptoms (Roth et al., 2004b; Hedlund, 2009). The serotonin system mediates a wide variety of functions including perception, emotion, attention, and cognition (Berger et al., 2009). Serotonin receptors putatively involved in the mediation of cognitive functions include $5HT_{1A}$, $5HT_{2A}$, $5HT_4$, and $5HT_6$; not surprisingly these are promising targets for development of cognition-enhancing medications (Roth et al., 2004b; Gray and Roth, 2007b).

1.2. Ethnopharmacology applied to CNS drug discovery

The search for new psychotropic medications for the treatment of diseases of the nervous system and mental illnesses has benefited enormously from ethnopharmacology. The history of CNS drug discovery is inextricably intertwined with ethnopharmacology, due to the considerable ingenuity displayed by human societies in identifying and utilizing diverse psychotropic plants. A plethora of psychotropic plant-derived natural substances has resulted. The alkaloid reserpine, for example, from the plant *Rauvolfia serpentina* L. Benth ex Kurz (Apocynaceae), was used in treating psychosis in Ayurvedic medicine, and provided the prototype of modern antipsychotics (Curzon, 1990); the hallucinogens LSD and psilocybin were regarded as possible pharmacological models of psychosis, and basic research with these compounds led to insights into the function of serotonin in the central nervous system (Geyer and Vollenweider, 2008). Some have been a mixed blessing as their pharmacological properties render them prone to abuse; others, even though abused, also have therapeutic properties that have been a boon to modern medicine; almost all, abused or not, have proven to be valuable tools for basic researchers investigating the neuropharmacology of brain functions and dysfunctions (Duke, 1995; Vortherms and Roth, 2006).

1.2.1. Natural products in the treatment of schizophrenia and neurocognitive deficits

Ethnopharmacology has led to the discovery of botanical medicines or natural products useful in psychiatric disorders such

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as anxiety and depression, sleep disorders, and dementias. The identification of natural products effective for the treatment of psychosis has met with less success. The alkaloid reserpine from the Ayurvedic medicine *Rauvolfia serpentina* is the prototype antipsychotic; its discovery resulted from ethnopharmacology, but it was quickly supplanted by synthetic neuroleptics such as chlorpromazine (Marder et al., 1993) although it still finds occasional use in the treatment of tardive syndromes (Fernandez and Friedman, 2003). Recent research has resulted in the identification of traditional medicines with promise for the treatment of a range of psychiatric/neurological disorders including seizures, anxiety, substance abuse, depression, psychosis, and dementias; with few exceptions, most of these studies are in the early stages (Lake, 2000). In a few instances, e.g. *Ginkgo biloba* L. (Ginkgoaceae) for dementia and memory deficits, and St. John's Wort *Hypericum perforatum* L. (Hypericaceae) for depression, these natural medicines have been commercialized as popular dietary supplements (Fugh-Berman and Cott, 1999). There has been considerable interest in recent years in the investigation of botanical remedies for dementia and cognitive disorders (Ott and Owens, 1998; Kidd, 1999; Howes et al., 2003), but almost all of the interest has been focused on cognitive deficits of dementias rather than those associated with schizophrenia. The influence of botanical medicines on schizophrenia, either on the exacerbation of symptoms by patients self-medicating with St. John's Wort in combination with antidepressants (Lal and Iskandar, 2000; Parker et al., 2001), exacerbation of extrapyramidal symptoms by betel nut (*Areca catechu* L. (Arecaceae)) (Deahl, 1989), or the incidental amelioration of symptoms by betel nut (Wilson, 1979; Sullivan et al., 2000) or in adjunct treatment with *Ginkgo biloba* (Zhang et al., 2001) has been noted, but systematic clinical studies are rare. Many of these reports are case studies related to one or at most a few patients (Hanes, 2001). Most published clinical studies have been conducted by Chinese researchers, and are often published in Chinese in journals not readily accessible to Western investigators; moreover, many of these studies have focused on herbal adjunct treatments to conventional antipsychotic therapies and not on the positive or negative symptoms of schizophrenia (Yuan, 1979; Hu, 1984; Wang, 1986; Zhang et al., 1987; Zhu et al., 1996; Yamada et al., 1997). Thus the clinical literature on botanical therapies for schizophrenia is both disappointing and tantalizing; there are promising leads, but the information is sketchy, clinical studies are lacking, and those that do exist are almost all within the context of Chinese Traditional Medicine. Other than the sparse reports on betel nut, there is little published on cognition-enhancing ethnomedicines in schizophrenia. The Amazon basin represents another geographic area with a high biodiversity index and numerous indigenous ethnomedical traditions (Schultes and Raffauff, 1990). Ethnomedical practices in the region incorporate shamanic elements in which the use of psychotropic plants, such as the hallucinogen Ayahuasca (McKenna et al., 1984) is the rule rather than the exception. Amazonian traditional healers are often familiar with the psychotropic properties of many botanical remedies, but ethnopharmacologists have paid disproportionate attention to hallucinogens; those with nootropic or cognition enhancing properties are poorly investigated. Nonetheless, intriguing leads to cognition enhancers have been noted (Schultes, 1993, 1994; McKenna et al., 1995).

1.3. Radioligand binding methodologies in drug discovery

The development of radioligand receptor binding methodologies, pioneered by Solomon Snyder and colleagues in the early 1970s, was a significant breakthrough that has been particularly important for the neurosciences (Pert and Snyder, 1973). The technique gave molecular pharmacologists the means to selectively label specific receptors, enzymes, or other cellular targets using isotope-labeled compounds. Such methodologies have been invaluable for the elucidation of the sites and mechanisms of action of a vast array of drugs and other bioactive substances. Receptor binding methodologies have also been an important tool in drug discovery, enabling the rapid, cost-effective screening of compound libraries for activity against a variety of molecular targets, including neurotransmitter receptors (Phillipson, 1999). The application of these methodologies to the detection and bioassay-directed isolation of psychotropic or neuroactive compounds in plant extracts has been successful in the identification of constituents with analgesic activity (Phillipson, 1999; Sampson et al., 2000), anti-epileptic activity (Jäger et al., 2004) serotonin reuptake inhibition activity (Nielsen et al., 2004) and Ayurvedic medicines with memory-enhancing activities (Misra, 1998). More recently functional assays have emerged as high-through-put approaches to screen for the activities of compounds at CNS targets (Armbruster and Roth, 2005). Using functional assay readouts it is possible to screen compounds in 384, 1536- and higher plate formats allowing for the screening of hundreds of thousands of extracts in a single day (Hodder et al., 2004).

1.4. Objectives of the present study

In the present study we utilized a combination of screening approaches to evaluate a selected sample of Amazonian ethnomedicines for indications of CNS activities that may have therapeutic applications for the treatment of cognitive deficits. We used literature reviews, databases, surveys of herbarium collections, and field interviews with traditional healers to compile a database of approximately 311 candidate species. Approximately 130 species from this original list were collected, and crude extracts and fractions were screened in a broad spectrum of in vitro radioligand receptor binding and functional assays.

2. Materials and methods

2.1. Identification and pre-selection of targeted species

We relied on a variety of resources to partially pre-select candidate species for collection and follow-up investigation. Because of the lack of extensive published data on the use of Amazonian ethnomedicines specifically for schizophrenia or cognitive deficits, and due to the lack of exact correspondence between Western diagnostic categories and cultural conceptualizations of mental disease, we elected to develop a list of species targeted for collection that conformed to a broad set of inclusion criteria. The rationale for this approach was that initial, broadly defined inclusion criteria would be less likely to overlook candidates of potential interest, compared to inclusion criteria that were more narrowly defined. Our reasoning was that fractionation and in vitro screening of a set defined using broad criteria would rapidly result in the identification of a subset of collections inviting more extensive evaluation.

2.1.1. Literature survey

We initially relied on literature searches in Pubmed, supplemented by published ethnobotanical references on Amazonian ethnomedical species and on searches in the NAPRALERT[sm] database to identify targeted species. Four published volumes were key to our literature survey, viz. Duke and Vasquez (1994), Schultes and Raffauff (1990), Von Reis and Lipp (1982) and Von Reis (1973).

In addition to published volumes, targeted species were selected based on peer-reviewed journal articles accessed through Pubmed. Three of these were key references for the identification of promising leads (Schultes, 1981; Russo, 1992; Schultes, 1993).

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Table 1
Selected NAPRALERT[sm] activity codes relevant to neuropsychiatry (modified from Lake, 2000).

CNS activity (NAPRALERT code)	Number of cumulative citations as of 2009
Anticonvulsant activity (11006)	783
Narcotic antagonist activity (11020)	45
Antipsychotic activity (11081)	24
Tranquilizing effect (11041)	225
Memory-enhancing effect (11044)	263
Antiaggressive effect (11052)	24
Antidepressant activity (11062)	254
Antianxiety activity (11094)	29
Psychotropic activity (11032)	45
Hallucinogenic activity (11012)	162
Monoamine oxidase inhibition (16005)	127

2.1.2. NAPRALERT surveys

NAPRALERT[sm] and Pubmed were primary online resources used in conducting the literature survey. The NAPRALERT[sm] database (http://www.napralert.org) is a natural products database maintained and administered by the Program for Collaborative Research in the Pharmaceutical Sciences in the College of Pharmacy, University of Illinois at Chicago (Loub et al., 1985; Farnsworth, 1993). It contains information on the ethnomedical uses, chemical constituents and pharmacological and biological activities of natural products from plant, animal, microbial, and marine sources. The information is compiled from a variety of sources including published abstracts, journals, government reports, newsletters, patents, and books. Approximately 50% of the data is derived from a systematic survey of the literature from 1975 to the present, but includes some data from older sources, some as old as 1650. NAPRALERT[sm] is the most comprehensive collection of data on natural products and ethnomedicine in existence. Of particular relevance to this project, NAPRALERT[sm] contains over 3600 biological/pharmacological activity codes related to compounds and extracts. For example, there are approximately 98 codes related to central nervous system activity; more than 50 codes related to autonomic nervous system activity; over 126 codes related to receptor binding or receptor-mediated activity. Initially, NAPRALERT[sm] was searched for references to plants or extracts having one or more pharmacological codes related to CNS activity, with the additional constraint that the plants were native to South America (Table 1). Plants indigenous to South America that were identified in searches of the NAPRALERT[sm] pharmacological activity codes were parsed for occurrence in Peru, then the genus and species (or the genus if the species was not listed) was searched again in NAPRALERT[sm] using its "3-part" search protocol, which retrieves information on ethnomedical uses, biological activities detected in extracts evaluated in vitro and in animal models (including humans) and lists secondary compounds isolated, and presents a consolidated citation summary. Genera and species retrieved from the NAPRALERT[sm] searches were further parsed to omit well-known and well-studied species (e.g. *Nicotiana tabacum* L. (Solanaceae), *Banisteriopsis caapi* Spruce ex. Griseb Morton (Malpighiaceae)). Additionally, species with relatively well-studied secondary chemistry (as evidenced by the existence of extensive phytochemical studies in published literature) were not included as candidates or were assigned a lower priority than species with relatively unstudied phytochemistry, on the rationale that species with limited phytochemical data were more likely to yield novel compounds.

2.1.3. Collections database

The information acquired through NAPRALERT[sm], Pubmed, published books, and later through herbarium surveys and field interviews with local informants was incorporated into a database using the program Filemaker Pro[TM] (Filemaker, Inc., Santa Clara, CA). Filemaker is a relational database that accommodates the incorporation of large text blocks into data fields, and that permits simultaneous searches on numerous text and numerical parameters. It is easily customized for specific uses, is cross-platform compatible (Macintosh[TM] and Microsoft Windows[TM] PCs) and can be published on the World Wide Web using HTML formats. Filemaker Pro[TM] was thus ideal for the purposes of this project as it enables data to be shared among all investigators and is suitable for eventual publication of the data on the Internet. The initial database was constructed using Filemaker Pro 5[TM], but the software was periodically upgraded over the course of the project and the current version now runs under Filemaker Pro 9[TM]. The Filemaker database was initially constructed as a repository for the data collected on targeted species in the literature surveys, but over the course of the project lifetime, this database was expanded to include the collection data on the acquired specimens (including herbarium voucher labels), digitized scans of targeted species and associated herbarium labels from the Herbarium Amazonense at UNAP, and records of the fractions generated by chemical fractionation protocols and the summarized results of radioligand binding and functional receptor assays.

Based on the data extracted from NAPRALERT[sm], Pubmed, and other data sources, searchable database fields were defined for probable CNS activities (including all of the searched NAPRALERT[sm] activity codes, plus additional activity definitions based on folk uses). Additional fields included information on the plant parts used, modes of preparation, routes of administration, presence/absence of classes of secondary compounds, and results of radioligand receptor assays and receptor mediated functional assays (Table 2).

2.1.4. Herbarium survey

Species that were targeted for collection based on the data collected from NAPRALERT[sm] and the other literature searches specified were cross-referenced with the genera on file in the Herbarium Amazonense at the Universidad Nacional de la Amazonía Peruana (UNAP) in Iquitos. In some cases, the identical species were found in the herbarium, while in others, only related species were found, and in still others, there were no specimens on deposit. If the genus and species of interest was found in the Herbarium, or if related species belonging to the same genus were found, the specimens were digitally photographed and these images, along with the data recorded on the herbarium labels, were incorporated into the database. The herbarium labels in most cases contained information on the location of the collection, the collector(s), date of collection, and, rarely, information on ethnobotanical and/or ethnomedical uses. All of this information was also incorporated into the database.

2.2. Specimen collections

Specimen collections were carried out in the Loreto province of Peru on several different expeditions between November 2004 and July 2006. The initial focus of the collections was on the acquisition of targeted species that had been identified in the literature surveys and for which location data was available. In addition, other species, not originally on the target acquisition list, came to our attention in the course of fieldwork, usually as a result of information shared by local informants, and these were also collected when possible. Other targeted species were not collected either because no location data was available, the location of the populations was inaccessible, or the species were not known from the area of collections. Herbarium voucher specimens for each collection were prepared and assigned a unique collection number.

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Table 2
Searchable categories defined in the Filemaker™ collections database.

CNS activities: Analgesic; anxiolytic; stimulant; sedative; sudorific; antipyretic; tranquilizer; smoked[a]; snuff[a]; epilepsy; tremorogenic; paralytic; memory; geriatric; dementia; depressant; intoxicant; hallucinogen; anticonvulsant; convulsant; headache; narcotic; antitussive; hysteria; insomnia; insanity; nervousness; "susto"[b]; tremors; vertigo; depression; magical[c]; tonic; spasmolytic; aphrodisiac; nervous disorders
Preparation methods: Decoction; Infusion; Poultice; Topical Application; Baths; Ayahuasca admixture; Not specified; Not processed; Squeezed juice; Macerate; Powder; Alcoholic extract
Plant parts utilized: Wp – whole plant; Ap – aerial parts; Lv – leaves; Bk – bark; Rt – roots; Br – branches; St – stems; Wd – wood; Sd – seeds; Fl – flowers; Ft – fruits; Sp – sap; Lx – latex; Rz – rhizomes; Co – corms; Eo – essential oil; Ns – not specified
Secondary compound occurrence: Acetogenins; acyclics; alkaloids (any type); benzenoids; betaines; cardenolide glycosides; chromones; coumarins; diterpenes; essential oil; flavonoids; glycosides; indole alkaloids; iridoids; isoflavonoids; isoquinoline alkaloids; lactones; lignans; lipids; monoterpenes; nitrogen heterocycles; non-protein amino acids; phenylpropanoids; polyacetylenes; pyrrolizidine alkaloids; quinoids; quinoline alkaloids; saponins; sesquiterpenes; β-carbolines; steroids; triterpenes; unknown; xanthones
Binding profiles[d]: $5HT_{1A}$; $5HT_{1B}$; $5HT_{1D}$; $5HT_{1E}$; $5HT_{2A}$; $5HT_{2C}$; $5HT_3$; $5HT_{5A}$; $5HT_6$; $5HT_7$; α_{1A}; α_{1B}; α_{2A}; α_{2B}; α_{2C}; D_1; D_2; D_3; D_4; D_5; DOR; MOR; H_2; M_1; M_2; M_3; M_4; M_5; DAT; NET; SERT
Functional assay profiles[d]: $5HT_{2A}$; $5HT_{2B}$; $5HT_{2C}$; M_1; M_3; M_5; α1A

[a] Preparations that were commonly smoked or snuffed were interpreted as likely to display psychoactive effects.
[b] "susto" is a folk disease commonly recognized in Amazonian ethnomedicine that is similar to generalized anxiety disorder (cf. Logan, 1993).
[c] "magical" indicates the plant is used in the context of ritual, witchcraft, or sorcery rather than for a specific pharmacological action. It is included here because plants used in this context are often psychoactive or have other CNS activities.
[d] 5HT – 5-hydroxytryptamine, (serotonin) receptor subtypes; α – alpha adrenergic receptor subtypes; D – dopamine receptor subtypes; DOR, MOR – delta opiate and mu opiate receptors; H_2 – histamine-2 receptor; M – muscarinic acetylcholine receptor subtypes; DAT – dopamine reuptake transporter; NET – norepinephrine reuptake transporter; SERT – serotonin reuptake transporter.

Duplicate vouchers were deposited in the Herbarium Amazonense and in the Herbarium of the Bell Museum of Natural History, University of Minnesota. In addition to the vouchers, small samples (~100–500 g) of plant materials were also collected for each specimen to provide material for chemical analysis and bioassay. Initially, the material was extracted in organic solvent in the laboratory of Gracia Ethnobotanicals, a local producer/exporter of herbal supplements in Iquitos. This proved impractical, however, due to difficulties in maintaining proper storage conditions, and also due to regulatory prohibitions pertaining to the export of crude extracts. For subsequent collections, the bulk collections were ground in a ball mill, dried at ~60 °C in a forced-convection plant drying room at Gracia Ethnobotanicals, and stored in heat-sealed polyethylene-lined storage pouches (4.5 mil, 10 × 12″ or 9.5 × 16″, Fisher Scientific catalog # 01-812F series) until shipment.

2.2.1. Collection data and herbarium labels

The Filemaker™ database program was used to design collection labels, and each collection was assigned a unique collection number. Collection data included the family, genus, species and taxonomic authority of the collected specimen, the GPS coordinates and other location data, the date of collection, the name of the collectors, the elevation in meters (where known), common names, the plant parts collected, any pertinent data on the specimen derived from the literature and NAPRALERT℠ searches (and incorporated from the targeted species table in the database) and a cross-reference to NAPRALERT℠ profiles, if they exist (Fig. 1). By designing the labels as a layout in the Filemaker™ database, it enabled collection data to be easily revised, updated, and selectively searched and sorted. See Appendix I for a complete list of all collected specimens. Appendix I contains only collection numbers, family, genus, species, and authority; other collection details are not included in this appendix.

2.2.2. Collection and export authorizations

Collections in the Loreto region and the export of dried plant biomass and herbarium voucher specimens were carried out under joint authorization from UNAP, Department of Biosciences, and INRENA (Instituto Nacional Recursos Natureles) the Peruvian Department of Natural Resources that has jurisdiction over bioprospecting, export of plant specimens, and scientific investigations of Peruvian biota. Securing permission to collect, and then export, plant specimens were subject to repeated delays even though the application process was initiated immediately on first entry into Peru at the start of the project. Although final permission was not secured until May 2007, when field collections were for the most part already completed, in the interim it was possible to export specimens under a provisional letter of authorization from the Directora of the Herbarium Amazonense at UNAP, Blga. Maria Nancy Arevalo Garcia. As a result laboratory evaluations of the collections were only moderately delayed. Bulk dried samples were shipped to the Department of Chemistry at the University of Minnesota and stored at room temperature until extractions could be carried out. Herbarium voucher specimens were hand-carried to the University of Minnesota and released into the care of Dr. George Weiblen, vascular plant curator of the Herbarium at the Bell Museum of Natural History. These specimens were sub-

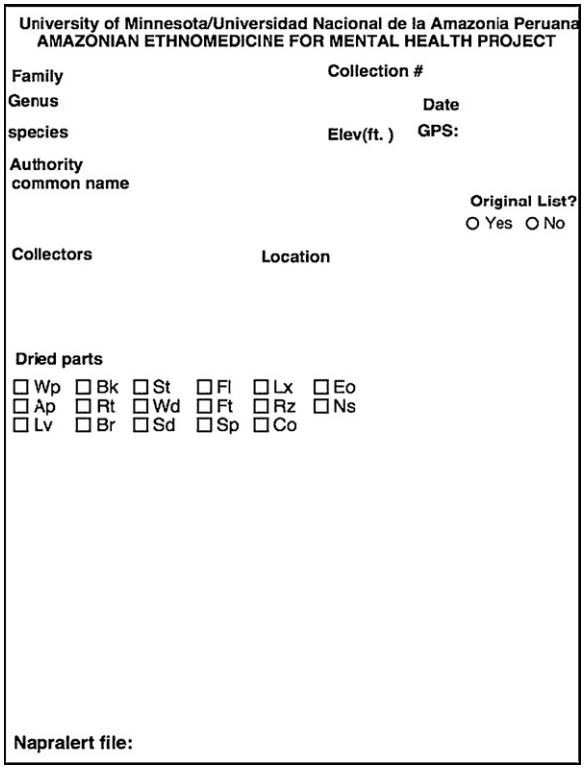

Fig. 1. Herbarium voucher collection label showing categories of information recorded.

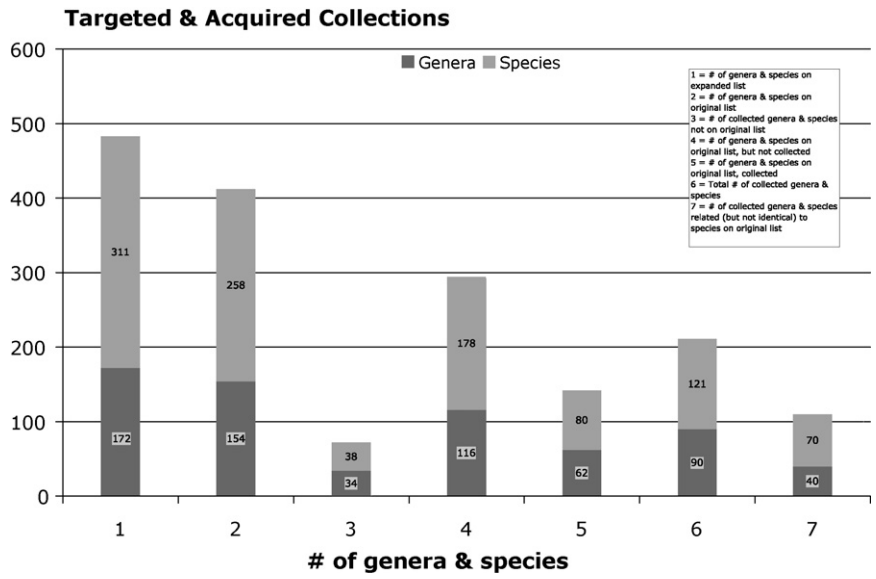

Fig. 2. Distribution of genera and species targeted for collection.

sequently mounted and assigned Herbarium access numbers. The database-generated collection labels were affixed to the specimens.

2.3. Screening of extracts and fractions in radioligand assays

Bulk dried plant specimens were processed into crude extracts in preparation for screening. Twenty to 50 g of the powdered, dried plant material was placed in a 250 mL screw-capped rotary shaker flask, and covered with ca. 150 mL of 1:1 CH_2Cl_2:MeOH. The flask was gently agitated on a rotary shaker table for 24 h. The solvent was decanted, additional solvent was added, and the extraction procedure was repeated for an additional 24 h. The extracts were combined, and the solvent was removed under vacuum by rotary evaporation. Other investigators have reported yields ranging from 2 to 16% of the dry weight using a similar strategy (Zhu et al., 1996), so extraction of 50 g of plant material yields between 1 and 8 g of crude extract. The combined extracts were reduced in volume to ca. 5% of the original volume by rotary evaporation. The concentrated extracts from each collection were transferred to 30 mL screw-capped Nalgene™ vials for storage. Vials were labeled with a collection number keyed to the collection data in the project database, plant part, weight of plant material extracted, and final volume of the concentrated extract. In instances where multiple plant parts were collected (e.g. bark and leaves), each part was extracted and processed separately. Excess unextracted dried plant material was resealed in the heat-sealable plastic pouches, labeled, and stored until needed for further extractions.

2.3.1. Preparation of crude extracts for screening

Samples were screened using the resources of the NIMH Psychoactive Drug Screening Program (PDSP), first at Case Western University Medical School and, later, after the program relocated at the Department of Pharmacology in the School of Medicine at the University of North Carolina at Chapel Hill. Samples were submitted to the program in two batches, and preparation procedures were modified for the second batch to address some of the difficulties encountered in screening of the initial batch, in order to improve the reliability of the results. The samples were prepared as follows: The concentrated crude extracts were resolubilized to a concentration of 10 mg/mL in methanol/ethyl acetate/methyl-t-butyl ether (6:3:1). Samples were allowed to stand over night at RT, then sonicated if necessary to improve solubility. Insoluble solids were removed by centrifugation. For some samples, reagent tests (precipitation and TLC) had indicated the presence of alkaloids. For these samples, the crude extracts were fractionated into acid/base fractions (A/B fractions) and alkaloids were partially purified using preparative TLC. These procedures are described below. To prepare the master plates, 100 μL of the resolubilized crude extracts (10 mg/mL) was added to 900 μL DMSO yielding a final concentration of 1.0 mg/mL; Base fractions were first diluted to 100 μg/mL (10 μL of the 10 mg/mL stock solution was added to 990 μL DMSO). For semi-purified alkaloids, stock solutions (10 mg/mL) were first diluted 1:10 with methanol/ethyl acetate/methyl-t-butyl ether (6:3:1) and then 10 μL of this diluted stock solution (1.0 mg/mL) was diluted in 990 μL DMSO to yield a final solution of 10 μg/mL. 25 μL of this solution, equivalent to 25 μg crude extract was added to 1 mL 96-well polypropylene assay plates (Fisher Scientific catalog # 12565505) and then 25 μL, equivalent to 2.5 μg, was added to the assay plates. Twenty-five μL of this solution containing 0.25 μg semipurified alkaloid, was transferred to the 96-well assay plates. Following distribution of the extracts and fractions, the plates were sealed with silicon plate seals (Fisher Scientific catalog # 03-396-49), frozen and shipped on dry ice to the program for screening.

2.3.2. Radioligand receptor binding and functional assays

The NIMH Psychoactive Drug Screening Program (PDSP) has published standardized methods for radioligand binding assays and functional assays (for example see Roth et al., 2002; Shapiro et al., 2003; Keiser et al., 2009). Assays are conducted according to standardized methods, but the details of each assay vary according to the receptor being analyzed. Full details of the methods used in the radioligand receptor assays and the functional assays are described in the PDSP Assay Protocol book (http://pdsp.med.unc.edu/).

3. Results

3.1. Ethnobotanical characteristics

3.1.1. Targeted vs. acquired collections

Initial literature and database surveys resulted in the compilation of 258 species targeted for acquisition. Subsequent herbarium surveys and interviews with traditional practitioners resulted in an expanded list of 311 species. Eighty species and 62 genera on the original list were collected, and a total of 121 species and 90 gen-

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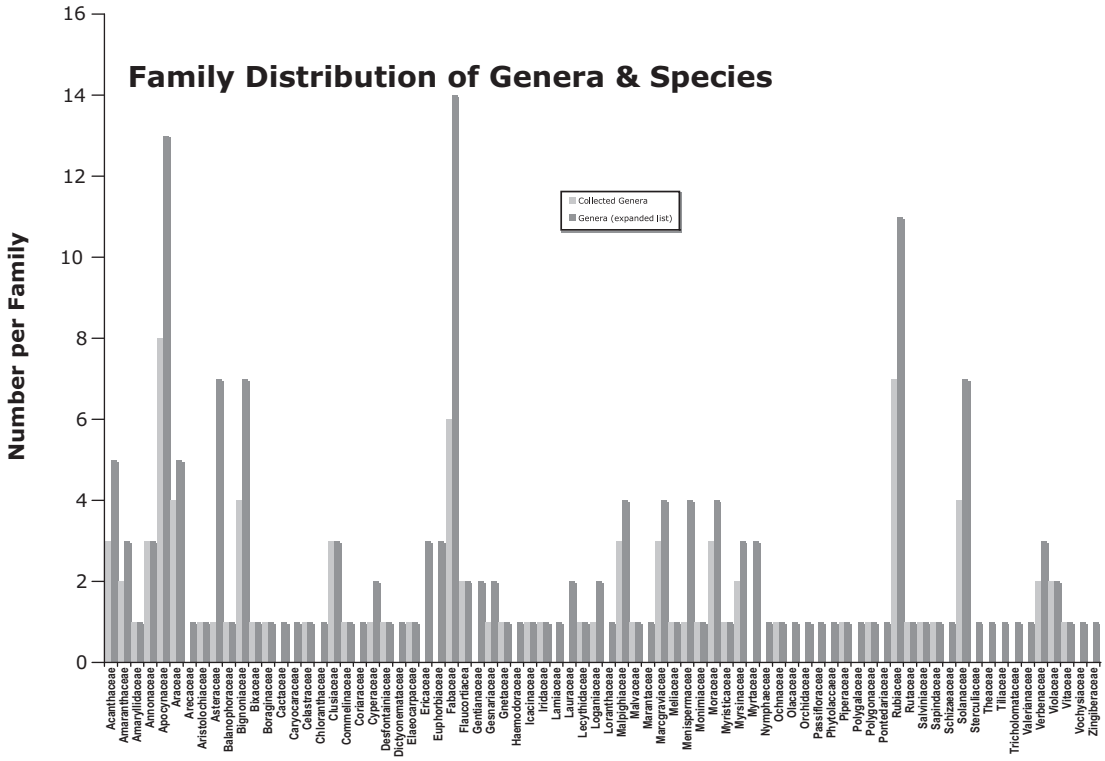

Fig. 3. Family distribution of targeted genera, compared to family distribution of collected genera.

era were collected (Fig. 2). The family distribution of the targeted and acquired collections is shown in Fig. 3. In general, the most represented families in the targeted list were also the most represented in the acquired collections, with the Apocynaceae, Fabaceae, Rubiaceae, and Solanaceae being the most frequently represented families on both lists.

3.1.2. Folk use categories

Thirty-six categories of folk use, deemed to be indicative of CNS activity, were defined in the Filemaker database (Table 2). Of these, the five most frequently represented categories in both the targeted collections and the acquired collections were intoxicants, hallucinogens, analgesics, stimulants, and those used for geriatric purposes (Table 3).

3.1.3. Phytochemical distribution

Based on published literature, the phytochemical distribution of targeted and acquired collections (Fig. 4), show a parallel distribution, with the most frequently represented phytochemical categories being (1) unknown constituents; (2) alkaloids of any type; (3) triterpenes; (4) sesquiterpenes; (5) flavonoids; (6) isoquinoline alkaloids; and (7) indole alkaloids. The distribution of collections in these categories shows approximately the same frequencies with some discrepancies (Fig. 4). For example, flavonoids were more frequent in the acquired species than in the targeted species (16.5% vs. 12.9%), while species reported to have isoquinoline alkaloids and indole alkaloids were somewhat more frequent in the acquired species than in the targeted collections (12.4% of acquired species contained isoquinolines vs. 9% of targeted species; 9.9% of acquired species were reported to contain indole alkaloids, vs. 7.4% of targeted species).

Table 3
Frequency of folk use categories in targeted and acquired collections.

Folk use	Targeted (number)	Targeted (%)	Collected (number)	Collected (%)
Intoxicant	84	27.0	29	24.0
Hallucinogen	76	24.4	31	25.6
Analgesic	61	19.6	35	28.9
Stimulant	51	16.4	21	17.4
Geriatric	47	15.1	16	13.2
Nervousness	41	13.2	10	8.3
Magical	40	12.9	20	16.5
Antipyretic	38	12.2	22	18.2
Tranquilizer	35	11.3	9	7.4
Insanity	35	11.3	19	15.7
Anxiolytic	34	10.9	9	7.4
Sedative	34	10.9	9	7.4
Headache	33	10.6	16	13.2
Spasmolytic	28	9.0	8	6.6
Dementia	26	8.4	7	5.8
Narcotic	23	7.4	5	4.1
Tonic	23	7.4	8	6.6
Anticonvulsant	22	7.1	9	7.4
Insomnia	21	6.8	4	3.3
Susto	20	6.4	5	4.1
Memory	18	5.8	6	5.0
Depressant	16	5.1	4	3.3
Hysteria	16	5.1	3	2.5
Paralytic	15	4.8	3	2.5
Depression	15	4.8	9	7.4
Snuff	12	3.9	12	9.9
Aphrodisiac	12	3.9	6	5.0
Nerv. disorders	11	3.5	5	4.1
Tremors	10	3.2	5	4.1
Sudorific	9	2.9	5	4.1
Epilepsy	9	2.9	9	7.4
Smoked	8	2.6	8	6.6
Vertigo	6	1.9	1	0.8
Tremorogenic	5	1.6	2	1.7
Antitussive	4	1.3	2	1.7
Convulsant	2	0.6	1	0.8

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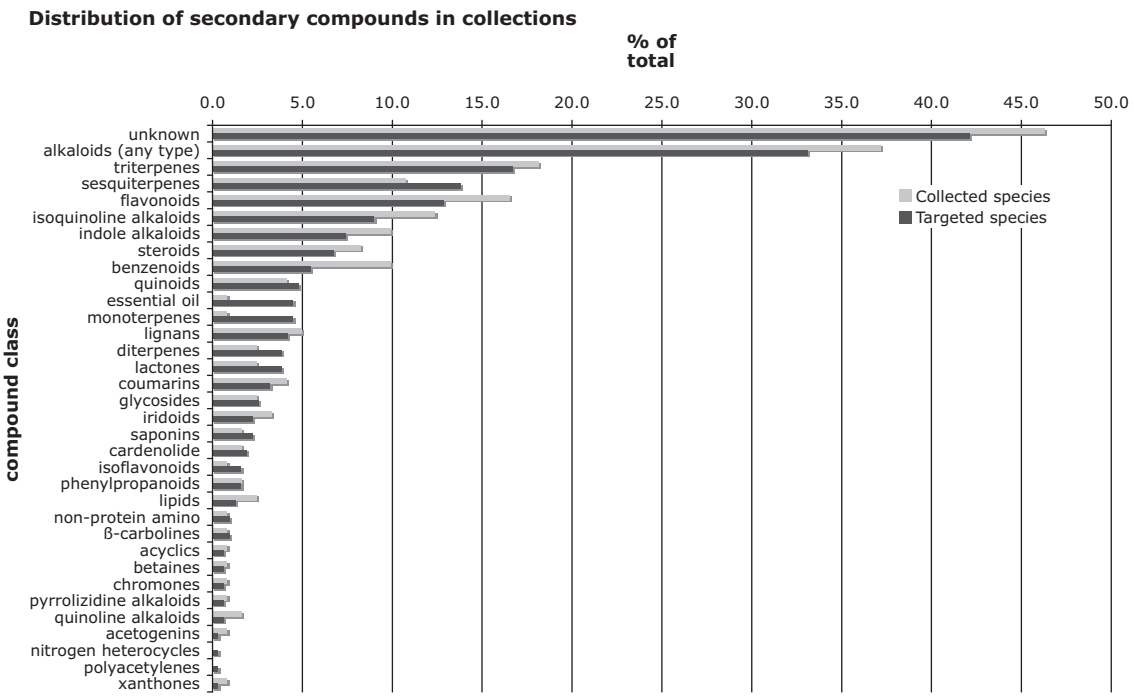

Fig. 4. Distribution of secondary compound classes in targeted and acquired species.

3.2. Receptor binding assays

A total of 228 crude extracts and fractions were screened in the radioligand receptor assays. Of these, 91 samples displayed 'hits' in one or more receptor assays, with a 'hit' being defined as ≥60% inhibition of the radioactive ligand (Table 4). Table 5 presents the data according to the receptor subtypes screened. A total of 39 genera displayed 'hits'; Table 6 displays the genera displaying 'hits' ranked by the number of active fractions for each genus.

Table 4
Distribution of samples showing 'hits' in receptor assays.

Receptors assayed[a]	Number of samples displaying 'hits' in binding assays	
	≥60% inhibition	≥75%inhibition
$5HT_{1A}$	11	7
$5HT_{1B}$	25	10
$5HT_{1D}$	13	12
$5HT_{1E}$	18	9
$5HT_{2A}$	7	1
$5HT_{2C}$	0	0
$5HT_3$	4	3
$5HT_{5A}$	5	4
$5HT_6$	6	2
$5HT_7$	18	10
D_1	6	4
D_2	3	2
D_3	4	1
D_4	2	1
D_5	5	2
α_{1A}	3	2
α_{1B}	3	2
α_{2A}	8	7
α_{2B}	16	4
α_{2C}	15	10
DOR	17	12
MOR	29	17
H_2	24	17
M_2	1	0
DAT	8	7
NET	6	0
SERT	2	1

[a] Abbreviations for binding sites assayed are listed in Table 2.

3.3. Functional assays

A subset of extracts and fractions were screened in functional assays against a limited selection of receptors using the standard functional assay protocol described in the PDSP Protocol Handbook, to determine if any showed evidence of agonist or antagonist activity. The receptors screened were 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}, adrenergic α_{1A}, and muscarinic M_1-, M_3- and M_5, based on prior evidence implicating these targets in the treatment of schizophrenia and related disorders (Roth et al., 2004a; Gray and Roth, 2007a,b). Of a total of 169 fractions screened, 135 showed evidence of agonist or antagonist activity, or both. "Activity" in the assays was defined as activation of the response at 5X baseline activity (baseline was defined at 100%) for agonists, and for antagonists, activity was defined as 50% or less of baseline. The overall results of the functional assays are summarized in Figs. 5 and 6, and Table 7 lists the full results of the samples screened in the functional assays for 5HT receptors.

4. Discussion

A major finding of this study is that the largest number of receptor 'hits' was concentrated in those genera that characteristically contain indole alkaloids (Table 6); secondarily, that the greatest number of 'hits' overall were with one or more 5HT receptor (Fig. 5). The taxonomic distribution of the collected species corresponded in most respects to that of the species on the expanded list of targeted species. From an expanded list of 311 species and 172 genera, we were able to acquire 121 species in 90 genera. Eighty species in 62 genera were on the original list of targeted collections, while 38 species and 34 genera collected were on the expanded target list, but not on the original list. One hundred and sixteen genera, representing 178 species, were on the original list but were not collected. Forty genera and 70 species were collected that were related, but not identical, to species on the original or expanded target list (Fig. 2).

Examination of the family distribution of collected and targeted species and genera indicates a fairly good correspondence in both

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Table 5
Collections and fractions showing ≥60% inhibition in receptor assays. Percent inhibition is shown in the right hand column under each receptor subtype.[a]

PDSP sample number	Collection #, fraction #	Genus/species	$5HT_{1A}$
4949	069Bk-ACN	*Aspidosperma excelsum* Benth. (Apocynaceae)	91.4
2425	069A	*Aspidosperma excelsum*	63.6
2402	073	*Hamelia patens* Jacq. (Rubiaceae)	77.4
2401	72	*Indigofera suffruticosa* Mill. (Fabaceae)	64.3
4884	087Bk2-ACN	*Tabernaemontana heterophylla* Vahl (Apocynaceae)	81.7
2451	087B	*Tabernaemontana heterophylla*	60.6
4901	097Lv-ACN	*Tabernaemontana sananho* Ruiz & Pav. (Apocynaceae)	83.2
4902	097Rt-ACN	*Tabernaemontana sananho*	80.1
4928	122Lv-ACN	*Zanthoxylum kellermanii* P. Wilson (Rutaceae)	90.9

PDSP sample number	Collection #, fraction #	Genus/species	$5HT_{1B}$
4914	106Bk-ACN	*Abuta rufescens* Aubl. (Menispermaceae)	92.1
4917	109Wp-ACN	*Alternanthera halimifolia* (Lam.) Stand. ex Pittier (Amaranthaceae)	80.7
2425	069A	*Aspidosperma excelsum*	90.0
2432	071 A/B	*Erythrina ulei* Harms (Fabaceae)	60.5
4955	048Lv-ACN-SH11	*Gloeospermum sphaerocarpum* Triana & Planch (Violaceae)	82.0
4956	048Lv-ACN-SH12	*Gloeospermum sphaerocarpum*	78.3
4957	048Lv-ACN-SH13	*Gloeospermum sphaerocarpum*	64.9
2402	073	*Hamelia patens*	69.3
4892	090Lv-ACN	*Lippia alba* (Mill.) N.E. Br. Ex Britton & P. Wilson (Verbenaceae)	68.2
4836	042Lv-ACN	*Mansoa alliaceae* (Lam.) A.H. Gentry (Bignoniaceae)	82.3
4911	104Rt-ACN	*Memora cladotricha* Sandwith. (Bignoniaceae)	74.5
4952	074Bk-ACN	*Potalia resinifera* Mart. (Loganiaceae)	60.3
4912	105Br-ACN	*Schlegelia macrophylla* Ducke (Bignoniaceae)	78.3
2414	085	*Sida setosa* Mart. ex Colla (Malvaceae)	61.3
4915	107Bk-ACN	*Siparuna mollicoma* (Mart. ex Tul.) A. DC. (Monimiaceae)	93.0
4916	107Lv-ACN	*Siparuna mollicoma*	84.7
4908	102Bk-ACN	*Sloanea brachytepala* Ducke (Eleaocarpaceae)	68.9
2451	087B	*Tabernaemontana heterophylla*	61.3
4902	097Rt-ACN	*Tabernaemontana sananho*	66.8
4901	097Lv-ACN	*Tabernaemontana sananho*	63.8
4891	089Bk-ACN	*Triplaris peruviana* Fisch. & Meyer ex C.A. Meyer (Polygonaceae)	62.3

PDSP sample number	Collection #, fraction #	Genus/species	$5HT_{1D}$
2425	069A	*Aspidosperma excelsum*	82.7
4949	069Bk-ACN	*Aspidosperma excelsum*	90.3
2431	070B	*Duroia duckei* Huber (Rubiaceae)	100.8
4881	084Wp-ACN	*Lantana trifolia* L. (Verbenaceae)	102.5
4915	107Bk-ACN	*Siparuna mollicoma*	78.5
4885	087Bk-ACN	*Tabernaemontana heterophylla*	90.5
4889	087Rt-ACN	*Tabernaemontana heterophylla*	76.4
4901	097Lv-ACN	*Tabernaemontana sananho*	92.6
4928	122Lv-ACN	*Zanthoxylum kellermanii*	78.6

PDSP sample number	Collection #, fraction #	Genus/species	$5HT_{1E}$
4914	106Bk-ACN	*Abuta rufescens*	92.1
4917	109Wp-ACN	*Alternanthera halimifolia*	80.7
2424	068B	*Ambelania occcidentalis* Zarucchi (Apocynaceae)	86.0
4955	048Lv-ACN-SH11	*Gloeospermum sphaerocarpum*	95.9
4956	048Lv-ACN-SH12	*Gloeospermum sphaerocarpum*	86.2
4957	048Lv-ACN-SH13	*Gloeospermum sphaerocarpum*	71.4
4892	090Lv-ACN	*Lippia alba*	63.6
4911	104Rt-ACN	*Memora cladotricha*	74.5
4952	074Bk-ACN	*Potalia resinifera*	74.8
4912	105Br-ACN	*Schlegelia macrophylla*	73.2
4915	107Bk-ACN	*Siparuna mollicoma*	93.0
4916	107Lv-ACN	*Siparuna mollicoma*	80.1
4908	102Bk-ACN	*Sloanea brachytepala*	64.2
4901	097Lv-ACN	*Tabernaemontana sananho*	63.8
4902	097Rt-ACN	*Tabernaemontana sananho*	66.8
4958	060Rz-4	*Teliostachya lanceolata* Nees (Acanthaceae)	82.9
4960	060Rz-13	*Teliostachya lanceolata*	82.9
4891	089Bk-ACN	*Triplaris peruviana*	62.3

PDSP sample number	Collection #, fraction #	Genus/species	$5HT_{2A}$
2423	068A-2-2-1	*Ambelania occcidentalis*	66.7
2425	069A	*Aspidosperma excelsum*	64.3
2447	076 A/B	*Eucharis ulei* Kraenzl. (Amyrillidaceae)	62.3
2440	073-2-2	*Hamelia patens*	60.1
2403	74	*Potalia resinifera*	96.5
2443	074-1	*Potalia resinifera*	67.6
2451	087B	*Tabernaemontana heterophylla*	61.7

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Table 5 (*Continued*)

PDSP sample number	Collection #, fraction #	Genus/species	$5HT_{2C}$
No inhibition greater than 60% detected			

PDSP sample number	Collection #, fraction #	Genus/species	$5HT_3$
2436	073 A/B	*Hamelia patens*	122.0
2442	074 A/B	*Potalia resinifera*	64.0
4931	126Bk-ACN	*Sloanea terniflora* (Sessé & Moc. ex DC.) Standl. (Eleaocarpaceae)	99.1
4932	126Lv-ACN	*Sloanea terniflora*	99.5

PDSP sample number	Collection #, fraction #	Genus/species	$5HT_{5A}$
2423	068A-2-2-1	*Ambelania occcidentales*	68.0
2426	069A A/B	*Aspidosperma excelsum*	92.6
2438	073-1-2	*Hamelia patens*	99.2
2444	074A	*Potalia resinifera*	75.0

PDSP sample number	Collection #, fraction #	Genus/species	$5HT_6$
2432	071 A/B	*Erythrina ulei*	60.4
4836	042Lv-ACN	*Mansoa alliaceae*	87.1
2451	087B	*Tabernaemontana heterophylla*	61.2
4889	087Rt-ACN	*Tabernaemontana heterophylla*	60.2

PDSP sample number	Collection #, fraction #	Genus/species	$5HT_7$
4914	106Bk-ACN	*Abuta rufescens*	78.4
4949	069Bk-ACN	*Aspidosperma excelsum*	64.7
4874	075Bk-ACN	*Byrsonima stipulina* J.F. Macbr. (Malpighiaceae)	67.8
2447	076 A/B	*Eucharis ulei*	78.0
2418	067B A/B	*Gnetum leyboldii* Tul. (Gentaceae)	63.9
2402	073	*Hamelia patens*	85.4
2436	073 A/B	*Hamelia patens*	63.3
4803	004Bk-ACN	*Remijia peruviana* Standl. (Rubiaceae)	87.6
4915	107Bk-ACN	*Siparuna mollicoma*	65.9
4916	107Lv-ACN	*Siparuna mollicoma*	67.3
2449	087A	*Tabernaemontana heterophylla*	61.6
2451	087B	*Tabernaemontana heterophylla*	75.2
4884	087Bk2-ACN	*Tabernaemontana heterophylla*	79.6
4902	097Rt-ACN	*Tabernaemontana sananho*	82.6
4928	122Lv-ACN	*Zanthoxylum kellermanii*	91.0

PDSP sample number	Collection #, fraction #	Genus/species	α_{1A}
2425	069A	*Aspidosperma excelsum*	79.4
2401	72	*Indigofera suffruticosa*	68.6

PDSP sample number	Collection #, fraction #	Genus/species	α_{1B}
2402	073	*Hamelia patens*	85.1
2401	72	*Indigofera suffruticosa*	75.4

PDSP sample number	Collection #, fraction #	Genus/species	α_{2A}
2421	068A-1-2	*Ambelania occcidentales*	89.2
2424	068B	*Ambelania occcidentales*	60.6
2400	71	*Erythrina ulei*	96.1
2402	073	*Hamelia patens*	90.2
2437	073-1-1	*Hamelia patens*	82.5
2401	72	*Indigofera suffruticosa*	95.6
2403	74	*Potalia resinifera*	91.4
2450	087A A/B	*Tabernaemontana heterophylla*	85.2

PDSP sample number	Collection #, fraction #	Genus/species	α_{2B}
2425	069A	*Aspidosperma excelsum*	81.9
2406	77	*Cybianthus spichigeri* Pipoly (Myrsinaceae)	70.9
2434	071-2/3-1-3	*Erythrina ulei*	68.0
2438	073-1-2	*Hamelia patens*	62.2
2439	073-2-1	*Hamelia patens*	60.5
2415	86	*Justicia comata* (L.) Lam. (Acanthaceae)	67.7
2413	84	*Lantana trifolia*	70.1
2409	80	*Mayna grandiflora* (Spruce ex Benth.) R.E. Schult. (Flaucortiaceae)	61.1
2410	81	*Philodendron cuneatum* Engl. (Araceae)	63.8
2443	074-1	*Potalia resinifera*	71.8
2449	087A	*Tabernaemontana heterophylla*	62.0
2450	087A A/B	*Tabernaemontana heterophylla*	68.3

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Table 5 (*Continued*)

PDSP sample number	Collection #, fraction #	Genus/species	α_{2C}
2402	073	*Hamelia patens*	71.1
2416	067A	*Gnetum leyboldi*	100.1
2419	068A	*Ambelania occcidentales*	75.9
2436	073 A/B	*Hamelia patens*	99.9
2440	073-2-2	*Hamelia patens*	71.0
2442	074 A/B	*Potalia resinifera*	101.6
2443	074-1	*Potalia resinifera*	100.0
2444	074A	*Potalia resinifera*	64.5
2445	074A A/B	*Potalia resinifera*	93.8
2447	076 A/B	*Eucharis ulei*	100.0
2451	087B	*Tabernaemontana heterophylla*	97.1

PDSP sample number	Collection #, fraction #	Genus/species	D_1
4914	106Bk-ACN	*Abuta rufescens*	100.3
4865	064Lv-ACN	*Annona muricata* L. (Annonaceae)	65.6
2415	86	*Justicia comata*	95.1
2414	085	*Sida setosa*	102.7
4827	033BkRt-ACN	*Xylopia frutescens* Aubl. (Annonaceae)	60.4
4828	036Bk-ACN	*Xylopia micans* R.E. Fr. (Annonaceae)	82.1

PDSP sample number	Collection #, fraction #	Genus/species	D_2
2422	068A-1-3	*Ambelania occcidentales*	62.9
2425	069A	*Aspidosperma excelsum*	80.6
2447	076 A/B	*Eucharis ulei*	101.1

PDSP sample number	Collection #, fraction #	Genus/species	D_3
4914	106Bk-ACN	*Abuta rufescens*	88.1
2425	069A	*Aspidosperma excelsum*	61.1
4949	069Bk-ACN	*Aspidosperma excelsum*	74.3
2402	073	*Hamelia patens*	74.4

PDSP sample number	Collection #, fraction #	Genus/species	D_4
4892	090Lv-ACN	*Lippia alba*	60.2
4914	106Bk-ACN	*Abuta rufescens*	90.1

PDSP sample number	Collection #, fraction #	Genus/species	D_5
4914	106Bk-ACN	*Abuta rufescens*	102.1
4865	064Lv-ACN	*Annona muricata*	62.0
4858	058Lv-ACN	*Bixa orellana* L. Bixaceae	72.2
2432	071 A/B	*Erythrina ulei*	61.2
2414	085	*Sida setosa*	83.1

PDSP sample number	Collection #, fraction #	Genus/species	DOR
2425	069A	*Aspidosperma excelsum*	87.1
2429	069B	*Aspidosperma excelsum*	104.6
2417	067B	*Gnetum leyboldii*	90.8
2411	82	*Guarea cristata* T.D. Penn (Meliaceae)	104.6
2436	073 A/B	*Hamelia patens*	64.8
2415	86	*Justicia comata*	164.5
2413	84	*Lantana trifolia*	81.2
2409	80	*Mayna grandifolia*	61.3
2446	074B	*Potalia resinifera*	122.0
2414	085	*Sida setosa*	75.8
2449	087A	*Tabernaemontana heterophylla*	60.9
2407	78	*Tovomita laurina* Planch & Triana (Clusiaceae)	97.3
2412	83	*Witheringia solanacea* L'Hér. (Solanaceae)	69.7

PDSP sample number	Collection #, fraction #	Genus/species	MOR
2425	069A	*Aspidosperma excelsum*	79.2
2404	75	*Byrsonima stipulina*	70.6
2431	070B	*Duroia duckei*	81.8
2400	71	*Erythrina ulei*	82.7
2432	071 A/B	*Erythrina ulei*	61.8
2405	76	*Eucharis ulei*	80.7
2447	076 A/B	*Eucharis ulei*	74.4
2416	067A	*Gnetum leyboldii*	92.7
2417	067B	*Gnetum leyboldii*	62.3
2418	067B A/B	*Gnetum leyboldii*	62.9
2411	82	*Guarea cristata*	78.1
2436	073 A/B	*Hamelia patens*	60.2

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Table 5 (*Continued*)

PDSP sample number	Collection #, fraction #	Genus/species	MOR
2401	72	*Indigofera suffruticosa*	67.4
2415	86	*Justicia comata*	86.4
2413	84	*Lantana trifolia*	70.5
2409	80	*Mayna grandifolia*	97.5
2410	81	*Philodendron cuneatum*	81.0
2444	074A	*Potalia resinifera*	75.6
2446	074B	*Potalia resinifera*	80.3
2408	79	*Psychotria remota* Benth. (Rubia eae)	77.2
2414	085	*Sida setosa*	86.6
2449	087A	*Tabernaemontana heterophylla*	68.7
2451	087B	*Tabernaemontana heterophylla*	61.6
2407	78	*Tovomita laurina*	68.5
2412	83	*Witheringia solanaceae*	87.0

PDSP sample number	Collection #, fraction #	Genus/species	H_2
2419	068A	*Ambelania occcidentales*	68.8
2420	068A A/B	*Ambelania occcidentales*	70.2
2421	068A-1-2	*Ambelania occcidentales*	62.8
2422	068A-1-3	*Ambelania occcidentales*	120.2
2426	069A A/B	*Aspidosperma excelsum*	67.3
2427	069A-5	*Aspidosperma excelsum*	94.1
2448	077 A/B	*Cybianthus spichigeri*	87.1
2431	070B	*Duroia duckei*	79.7
2432	071 A/B	*Erythrina ulei*	97.4
2433	071-1-4	*Erythrina ulei*	81.1
2405	76	*Eucharis ulei*	63.9
2447	076 A/B	*Eucharis ulei*	110.4
2417	067B	*Gnetum leyboldii*	91.1
2418	067B A/B	*Gnetum leyboldii*	100.7
2436	073 A/B	*Hamelia patens*	102.1
2437	073-1-1	*Hamelia patens*	150.6
2439	073-2-1	*Hamelia patens*	150.4
2413	84	*Lantana trifolia*	62.5
2443	074-1	*Potalia resinifera*	107.5
2445	074A A/B	*Potalia resinifera*	78.5
2450	087A A/B	*Tabernaemontana heterophylla*	68.7

PDSP sample number	Collection #, fraction #	Genus/species	M_1
No inhibition greater than 60% detected			

PDSP sample number	Collection #, fraction #	Genus/species	M_2
2425	069A	*Aspidosperma excelsum*	61.9

PDSP sample number	Collection #, fraction #	Genus/species	M_3
No inhibition greater than 60% detected			

PDSP sample number	Collection #, fraction #	Genus/species	M_4
No inhibition greater than 60% detected			

PDSP sample number	Collection #, fraction #	Genus/species	M_5
No inhibition greater than 60% detected			

PDSP sample number	Collection #, fraction #	Genus/species	NET
2425	069A	*Aspidosperma excelsum*	71.5
2401	72	*Indigofera suffruticosa*	61.3
2410	81	*Philodendron cuneatum*	74.2
2451	087B	*Tabernaemontana heterophylla*	63.7

PDSP sample number	Collection #, fraction #	Genus/species	SERT
4848	052Lv-ACN	*Norantea guianensis* Aubl. (Marcgraviaceae)	67.4
4827	033BkRt-ACN	*Xylopia frutescens*	95.8

PDSP sample number	Collection #, fraction #	Genus/species	DAT
2415	86	*Justicia comata*	77.3
2413	84	*Lantana trifolia*	82.7
2409	80	*Mayna grandifolia*	115.0
2443	074-1	*Potalia resinifera*	82.3

[a] Column 1: PDSP sample number. PDSP = Psychoactive Drug Screening Program; Column 2: specimen collection # and fraction number: Rt = roots; Lv = leaves; Bk = bark; Rz = rhizome; Br = branch; Wp = whole plant; ACN = acetonitrile extract; collection # A = bark; collection # B = leaves; A/B = base (alkaloid) fraction prepared by acid/base partitioning of crude extracts; hyphenated fractions = fractions of indicated collection number obtained from column.

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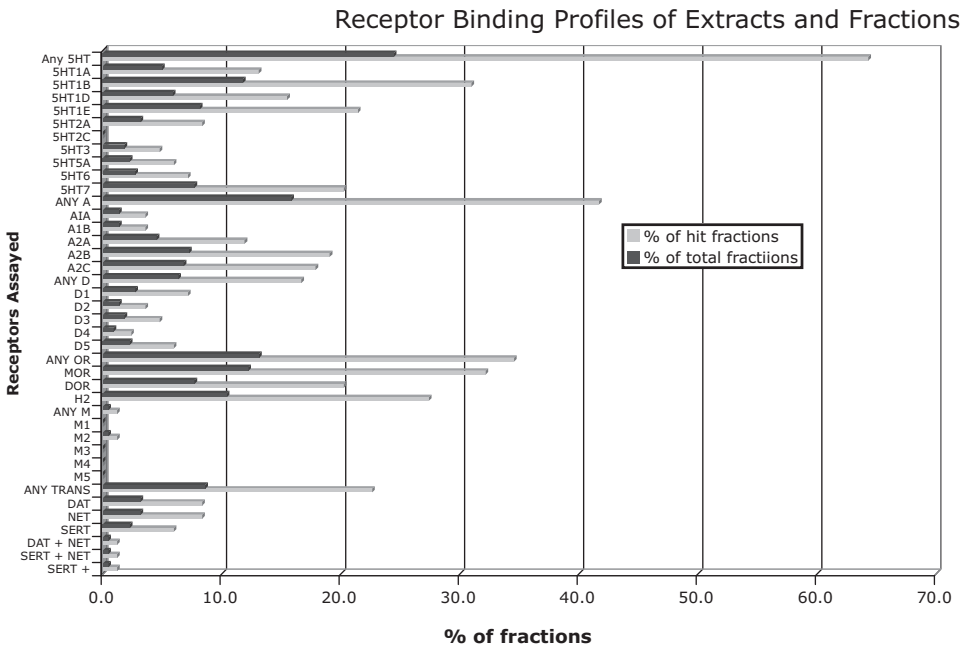

Fig. 5. Receptor binding profiles of extracts and fractions.

categories, i.e. those families that were most represented in the target list were also most frequent in the acquired collections (Fig. 2). The Apocynaceae, Araceae, Bignoniaceae, Fabaceae, Rubiaceae, and Solanaceae were among the most frequently represented families in both the target list and the acquired list. The distribution of the genera and species on the lists appears to be determined by the criteria for folk use (CNS activity) more than the natural distribution of genera and species in the Amazonian biome. Although the families cited above contain some of the largest numbers of species and genera in Amazonian flora, other families are underrepresented in the sample, compared to their abundance in the Amazonian flora (Ayala, 2003). Underrepresented families in the target and collection lists include the Asteraceae, Cucurbitaceae, Cyperaceae, Euphorbiaceae, Melastomataceae, Orchidaceae, and Poaceae.

The most frequently encountered categories of folk use based on published reports were similar in both the targeted collections and the acquired collections (Table 3). The most frequent categories in both were intoxicants or hallucinogens, followed by analgesics, stimulants, and those used in geriatrics. The acquired collections contained a greater percentage of analgesics than the targeted collections (28.9% vs. 19.6% in the targeted collections). The acquired collections also contained relatively greater proportions of 'magical' plants, antipyretics, plants used for insanity, and headache remedies than the targeted species, and a

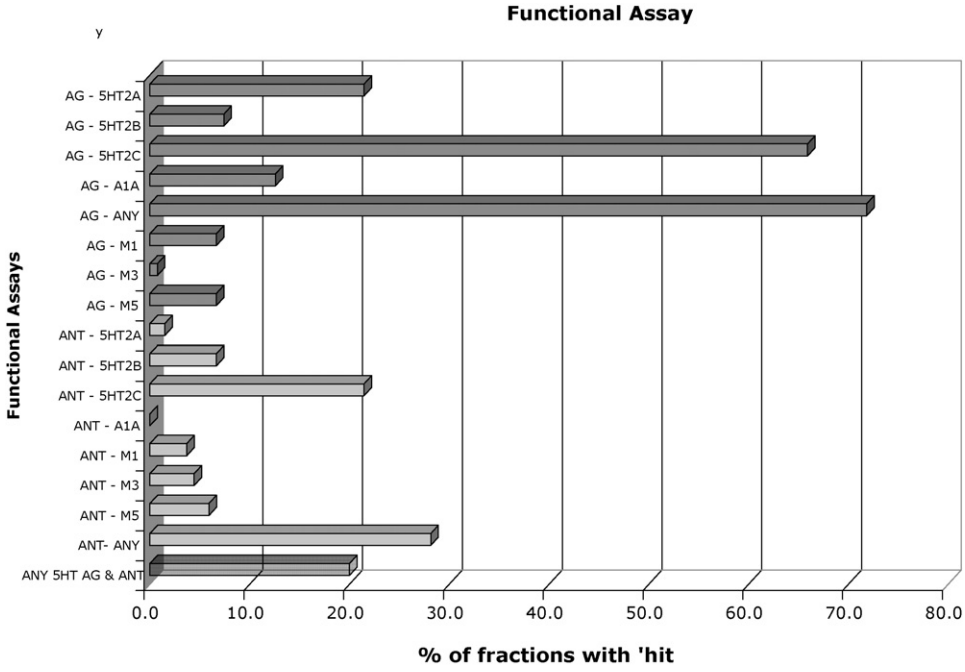

Fig. 6. Profile of agonist/antagonist activity.

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Table 6
Genera displaying 'hits' in receptor assays, ranked by the number of active fractions.

Genera	Family	Fractions displaying 'hits'	No. of species collected
Tabernaemontana	Apocynaceae	8	2
Hamelia	Rubiaceae	7	1
Potalia	Gentianaceae	7	1
Ambelania	Apocynaceae	6	1
Aspidosperma	Apocynaceae	5	1
Erythrina	Fabaceae	4	1
Gloeospermum	Violaceae	3	1
Gnetum	Gnetaceae	3	1
Sloanea	Elaeocarpaceae	3	2
Byrsonima	Malpighiaceae	2	1
Cybianthus	Myrsinaceae	2	1
Eucharis	Amaryllidaceae	2	1
Lantana	Verbenaceae	2	1
Mimosa	Fabaceae	2	1
Siparuna	Monimiaceae	2	2
Teliostachya	Acanthaceae	2	1
Xylopia	Annonaceae	2	2
Abuta	Menispermaceae	1	1
Alternanthera	Amaranthaceae	1	1
Annona	Annonaceae	1	1
Bixa	Bixaceae	1	1
Duroia	Rubiaceae	1	1
Guarea	Meliaceae	1	1
Indigofera	Fabaceae	1	1
Justicia	Acanthaceae	1	1
Lippia	Verbenaceae	1	1
Mansoa	Bignoniaceae	1	1
Mayna	Flaucortiacea	1	1
Memora	Bignoniaceae	1	1
Norantea	Marcgraviaceae	1	1
Philodendron	Araceae	1	1
Psychotria	Rubiaceae	1	1
Remijia	Rubiaceae	1	1
Schlegelia	Bignoniaceae	1	1
Sida	Malvaceae	1	1
Tovomita	Clusiaceae	1	1
Triplaris	Polygonaceae	1	1
Witheringia	Solanaceae	1	1
Zanthoxylum	Rutaceae	1	1

smaller proportion of plants used for nervousness, tranquilizers, anxiolytics, or dementia than the proportions represented in the targeted collections. Some folk categories do not have any correspondence to Western diagnostic criteria, for example, "susto" which resembles chronic depression but is not classified as such (Logan, 1993).

The phytochemical distribution of secondary compounds, based on published literature, in the targeted and acquired collections show approximately the same frequencies, with the most frequent phytochemical categories being (1) unknown constituents; (2) alkaloids of any type; (3) triterpenes; (4) sesquiterpenes; (5) flavonoids; (6) isoquinoline alkaloids; (7) indole alkaloids (Fig. 4). These similarities are probably a reflection of our limited knowledge of the overall abundance of secondary compounds in the Amazonian flora, and are an indication of what has been reported in published literature rather than the actual distribution. There are some discrepancies; for example, flavonoids were more frequent in the acquired species than in the targeted species (16.5% vs. 12.9%), while species reported to have isoquinoline alkaloids and indole alkaloids were somewhat more frequent in the acquired species than in the targeted collections (12.4% of acquired species contained isoquinolines vs. 9% of targeted species; 9.9% of acquired species were reported to contain indole alkaloids, vs. 7.4% of targeted species). It is noteworthy that the largest category of secondary compounds in both the acquired and targeted collections is "unknown" which is a reflection of the paucity of phytochemical investigations in this subset of Amazonian flora, and of the Amazonian flora as a whole. There are some indications of clustering with respect to the phytochemical profiles, in that the largest numbers of overall receptor interactions were samples from characteristically alkaloidal families (Table 6). These results should not be over-interpreted, however, since alkaloids were the second most abundant category of secondary constituents in this sample, after 'unknown' constituents (Fig. 4). Since the sample contains such a large proportion for which the phytochemical profiles are 'unknown', it is difficult to gain an accurate picture of the correlations that may exist between phytochemical profiles and receptor interactions.

A total of 228 crude extracts and fractions were generated from the acquired collections, and of these, 91 generated 'hits' in one or more receptor binding assay, with a 'hit' being defined as ≥60% inhibition of radioligand binding. This data is summarized and presented in various ways in Tables 4–6. Perhaps unsurprisingly, the greatest number of active fractions ('hits') was clustered in genera in families that are known to be rich in alkaloids, since these secondary products frequently affect the central nervous system. The six top-ranked genera in Table 6 are from alkaloid-rich families, and of these, three of the six belong to the Apocynaceae, a family that is well known for its abundance of indole alkaloids.

The overall distribution of 'hits' at all receptors screened is graphed in Fig. 5. Ninety one active fractions displayed inhibition in receptor binding assays of ≥60%; over 60% of 'hit' fractions (vs. approximately 25% of total fractions) were at one or more 5HT receptor subtypes. Of the 5HT subtypes screened, none of the samples displayed ≥60% inhibition at $5HT_{2C}$ receptors. The most frequent 5HT receptors displaying 'hits' were $5HT_{1B}$, $5HT_{1D}$, $5HT_{1E}$, $5HT_7$, and $5HT_{1A}$, respectively, with all other 5HT receptors screened showing less than 10 'hits' out of the 91 samples screened (Table 4 and Fig. 5). More than 10% of samples displayed 'hits' at α_{2A}, α_{2B} and α_{2C} receptors, while less than 5% showed activity at α_{1A} and α_{1B}-adrenergic receptors. Only about 5% of the sample showed activity at any dopamine (D) receptors; the most frequent dopaminergic receptor showing activity was D_1, at which 6 out of 91 samples displayed more than 60% inhibition. Over 30% of samples displayed 'hits' at μ opiate receptors and about 20% inhibited binding at δ opioid receptors; altogether, 46 samples gave 'hits' at one or both opiate receptors. Histamine H_2 receptors also gave relatively large percentages of 'hits' (28%). Surprisingly, only one sample yielded a 'hit' on any muscarinic cholinergic receptor (M_2) (Table 5).

Table 8 shows the distribution of receptor activities with respect to folk classification of the CNS activities of the collections. Analgesia was the most frequent folk use of the collections indicative of probable CNS activity, followed by antipyretics and headache remedies. There does not appear to be any clear correlation between receptor binding and folk uses. The receptor 'hits' are distributed in about the same proportions in all folk categories, with 5HT, α-adrenergic, and opiate receptor inhibition the most common, with lower levels of activity at the remaining receptors assayed.

A subset of fractions and extracts were screened in functional assays at three 5HT receptors, one adrenergic receptor, and three muscarinic receptors. The results are summarized in Fig. 6, and Table 7 presents the full results for the 5HT functional assays. These data must be considered incomplete, because none of the samples that showed activity in the functional assays were subsequently screened in receptor binding assays at the same receptors. Therefore it is unclear whether a correlation exists between the receptor binding data and the functional activity observed for these samples. These gaps in the data set should be considered a high priority for follow-up investigations.

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Table 7
Distribution of 5HT agonist and antagonist activity in extracts and fractions screened in functional assays.

PDSP sample #	Coll. #/fraction	Genus and species	$5HT_{2A}$ agonist	$5HT_{2A}$ antagonist	$5HT_{2B}$ agonist	$5HT_{2B}$ antagonist	$5HT_{2C}$ agonist	$5HT_{2C}$ antagonist
4802	003Lv-ACN	*Marcgravia longifolia* J.F. Macbr. (Marcgraviaceae)	1768	144	806	112	572	97
4803	004Bk-ACN	*Remijia peruviana*	2125	89	498	23	1885	8
4825	032Bk-ACN	*Rinorea racemosa* (Mart.) Kuntze (Violaceae)	2004	150	1116	111	1123	104
4827	033BkRt-ACN	*Xylopia frutescens*	1853	138	1163	112	1161	122
4828	036Bk-ACN	*Xylopia micans*	1519	137	751	101	1341	97
4829	038ST-ACN	*Philodendron solimoesense*	1278	120	308	42	1685	15
4831	039Ft-ACN	*Byrsonima poeppigiana* A. Juss. (Malpighiaceae)	1566	150	718	122	1613	48
4843	048Bk-ACN	*Gloeospermum sphaerocarpum*	341	134	280	58	1888	29
4845	049Bk-ACN	*Unonopsis floribunda* Diels (Annonaceae)	783	135	354	79	1744	58
4849	053Wp-ACN	*Sauvagesia erecta* L. (Ochnaceae)	1520	122	614	70	1761	49
4851	054Lv-ACN	*Duroia hirsuta* (Poepp.) K. Schum. (Rubiaceae)	1600	152	350	121	1634	93
4864	063Lv-ACN	*Vismia macrophylla* Kunth (Hypericaceae)	277	120	179	22	241	116
4874	075Bk-ACN	*Byrsonima stipulina*	2003	107	447	60	2082	6
4877	078Bk-ACN	*Tovomita laurina*	1475	123	453	89	2169	6
4882	085Wp-ACN	*Sida setosa*	204	127	65	76	1408	6
4884	087Bk2-ACN	*Tabernaemontana heterophylla*	1562	84	559	45	1863	3
4889	087Rt-ACN	*Tabernaemontana heterophylla*	768	132	286	62	1471	10
4901	097Lv-ACN	*Tabernaemontana sananho*	695	127	90	24	1483	33
4902	097Rt-ACN	*Tabernaemontana sananho*	1245	107	296	36	1610	3
4903	098Bk-ACN	*Stylogyne longifolia* (Mart. ex Miq.) Mez (Primulaceae)	1589	168	546	103	1675	79
4908	102Bk-ACN	*Sloanea brachytepala*	3939	156	1343	131	2150	65
4914	106Bk-ACN	*Abuta rufescens*	574	138	159	11	1565	46
4916	107Lv-ACN	*Siparuna mollicoma*	781	154	395	110	1884	56
4923	117Lv-ACN	*Sloanea guianensis*	2438	138	925	117	1753	83
4928	122Lv-ACN	*Zanthoxylum kellermanii*	431	104	152	43	1195	4
4942	MHRt_ACN	*Mimosa hostilis* (Mart.) Benth (Fabaceae)	349	95	103	53	1216	4
4943	MHRt_Hex	*Mimosa hostilis*	944	79	165	51	1427	5
4949	069Bk-ACN	*Aspidosperma excelsum*	435	145	193	26	1278	72
4959	060Rz-8	*Teliostachya lanceolata*	104	96	98	101	90	114
4960	060Rz-13	*Teliostachya lanceolata*	79	95	123	100	74	106
4963	071Bk-4	*Erythrina ulei*	80	90	83	134	122	110
4968	204LV-1-1	*Potalia resinifera*	84	95	108	108	92	99

Agonist activity was defined as >5X baseline of 100%; antagonist activity was defined as <50% of baseline. Numbers are rounded to the nearest whole number.

Table 8
Summary of receptor binding profiles vs. categories of folk use.

Folk uses	5HT	ALPHA	DA	DOR/MOR	H_2	M_2	TRANS	Total
Analgesic	25	17	5	13	9	1	4	74
Antipyretic	15	13	5	6	10	1	4	54
Headache	11	10	5	6	6	0	1	39
Stimulant	13	5	3	7	4	0	3	35
Hallucinogen	11	5	2	9	4	0	1	32
Intoxicant	11	5	1	9	4	0	1	31
Geriatric	9	5	0	6	4	0	2	26
Magical	11	4	1	5	4	0	0	25
Sedative	6	6	2	3	1	0	3	21
Anxiolytic	6	5	2	3	1	0	1	18
Nervousness	7	5	1	3	1	0	1	18
Insanity	7	2	2	2	2	0	0	15
Tonic	5	2	1	4	3	0	0	15
Tremors	2	3	1	4	2	0	2	14
Narcotic	2	3	1	4	2	0	1	13
Memory	5	3	0	2	1	0	1	12
Depression	3	1	1	3	2	0	0	10
Spasmolytic	6	1	1	1	0	0	1	10
Aphrodisiac	3	1	2	1	0	0	2	9
Anticonvulsant	2	1	2	2	0	0	1	8
Sudorific	3	1	0	1	1	0	1	7
Epilepsy	1	1	0	1	0	0	1	4
Tranquilizer	2	2	0	0	0	0	0	4
Dementia	2	0	1	0	0	0	0	3
Insomnia	1	0	1	1	0	0	0	3
Nervous disorders	0	1	0	0	0	0	2	3
Antitussive	1	0	0	1	0	0	0	2
Paralytic	1	1	0	0	0	0	0	2
Susto	1	0	1	0	0	0	0	2
Convulsant	0	0	0	1	0	0	0	1
Hysteria	1	0	0	0	0	0	0	1
Depressant	0	0	0	0	0	0	0	0

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Table 9
Receptor profiles of anti-dementia plants reported by Schultes (1993).

Collection #	Genus and species	Receptor 'hits'
106	*Abuta rufescens*	$5HT_{1B}$, $5HT_{1E}$, $5HT_7$, D_3, D_4, D_5
067	*Gnetum leyboldii*	$5HT_7$, MOR, DOR, H_2, α_{2C}
105	*Schlegelia macrophylla*	$5HT_{1B}$, $5HT_{1E}$
087	*Tabernaemontana heterophylla*	$5HT_{1A}$, $5HT_{1B}$, $5HT_{1D}$, $5HT_{2A}$, $5HT_6$, $5HT_7$, α_{2A}, α_{2B}, α_{2C}, DOR, MOR, H_2, NET
097	*Tabernaemontana sananho*	$5HT_{1A}$, $5HT_{1B}$, $5HT_{1D}$, $5HT_{1E}$, $5HT_7$

5. Conclusions

The study has shown that interactions with serotonin receptor subtypes were the most common activity detected, followed by α-adrenergic receptors, opiate receptors, and histamine H_2 receptors. In contrast, fewer than 10% of samples showed any interaction with dopamine receptors or monoamine transporters, and only one sample displayed any inhibition of muscarinic receptors (Fig. 5 and Table 5). Although inhibition of 5HT receptors was the most common, there were anomalies in this data as well; at the criteria level defined as a 'hit' (≥60% inhibition) none of the samples yielded 'hits' at $5HT_{2C}$ receptors, while 7 samples yielded 'hits' at the homologous $5HT_{2A}$ receptor, although only one of these (*Potalia resinifera* Mart. (syn. *Potalia amara* var. *resinifera* (Mart.) Progel)) (Loganiaceae) had an inhibitory value greater than 70%. The factors contributing to this distribution of receptor interactions in this sample may be both co-evolutionary (in the sense that plants evolve biologically active constituents to mediate their interactions with other organisms) and ethnobotanical (in the sense that indigenous populations will introduce an unconscious bias into their selection of plants with CNS activity). Since all of the plants screened have one or more folk uses related to CNS activity, it is unsurprising that there is a spectrum of receptor interactions; what is perhaps more surprising, however, is that there appears to be little correlation between folk uses and receptor interactions; the relative proportions of receptor inhibitions is about the same regardless of the folk use. Analgesics, for example, are not over-represented by opiate receptor interactions; this category contains nearly twice as many 5HT receptor interactions, although this may be misleading because serotonin is also involved in analgesia. There are also indications of correlations in some cases; nearly 50% of the plants used for 'insanity' show inhibition at 5HT receptors; similarly, 41% of plants used for 'memory' and 35% used for 'geriatric' show 5HT interactions. Plants used as 'hallucinogens' or 'intoxicants' have a high proportion of 5HT interactions (~35%) as might be expected since the actions of hallucinogens are known to be mediated through 5HT receptors (Nichols, 2004).

Roth (Roth et al., 2000, 2004a; Gray and Roth, 2007a,b) reviewed serotonin receptor subtypes that show promise for the development of medications to treat cognitive deficits in schizophrenia. These investigators highlight $5HT_{1A}$ partial agonists, $5HT_{2A}$ antagonists, $5HT_4$ partial agonists, and $5HT_6$ antagonists as likely targets for improving cognition in schizophrenia. On these criteria, 6 species in our collections displayed $5HT_{1A}$ receptor interactions, 5 species displayed $5HT_{2A}$ interactions, and 3 species displayed interactions at $5HT_6$ receptors. $5HT_4$ receptors were not included among the receptors assayed in this study. The species that displayed inhibition of binding at the receptors mentioned may represent candidates for further investigation. Functional data is missing or incomplete for all of these receptors except $5HT_{2A}$ receptors. In functional assays, 19 species displayed activity as $5HT_{2A}$ agonists, but none showed antagonist activity (Table 7). None of the species that were screened in functional assays were subsequently screened in the corresponding receptor assays, and this represents a deficiency in the data that should be resolved by additional receptor screens.

A review by Gray and Roth (2007b) discusses other receptors that bear investigation as potential targets for cognition enhancers, in addition to the 5HT receptors mentioned above. These include D_1 agonists, D_4 agonists and antagonists, nicotinic α_7 and nicotinic $\alpha_4\beta_2$ agonists, M_1 and M_4 agonists, M_5 antagonists, NMDA enhancers, glycine transport inhibitors, AMPA/kainite receptors, mGluR2/3 and mGluR5 agonists, α-adrenergic agonists, sigma agonists, and GABA-A agonists and antagonists. Of the receptors mentioned, our collections included 6 fractions that showed inhibition at D_1 sites, and 2 at D_4 sites. Thirty-five samples showed inhibition at one or more A_2 subtypes in binding assays, but no functional screens were carried out for these receptors. Only one sample was active at any muscarinic site (M_2) in binding assays; no other samples inhibited binding at other muscarinic sites. In functional assays, 13 samples displayed agonist activity at M_1 and M_5 sites (9 samples), M_3 (1 sample), with some showing activity at more than one site. With respect to antagonists at muscarinic sites, 3 samples were active at M_1, M_3, and M_5 sites, 1 at M_1 only, and 3 were active at M_3 and M_5 but not M_1. The other targets cited in this review were not screened in the present study, but those showing interactions with dopamine D_1 and D_4 sites, and activity as agonists or antagonists at muscarinic receptors represent an additional subset of the samples that should be further investigated.

Schultes (1993) reported on 28 species that are used in the Northwest Amazon to treat dementia-like disorders in the elderly. Of the species cited by Schultes, 18 were represented in our collections and 4 displayed inhibition at various receptors, including 5HT, dopamine subtypes, opiate subtypes, and adrenergic subtypes (Table 9). Of the samples screened, various fractions from *Tabernaemontana heterophylla* Vahl (Apocynaceae) (collection # 87) displayed activity at 6 5HT subtypes, 3 adrenergic subtypes, both opiate subtypes, the histamine H_2 receptor, and the norepinephrine transporter (NET). *Tabernaemontana* is a chemically well-studied genus, known to contain indole alkaloids and with numerous folk uses in Amazonian ethnomedicine (Van Beek et al., 1984). Six of 8 fractions derived from this species were active in the receptor assays and 2 were active in 5HT functional assays. Another species, *Tabernaemontana sananho* Ruiz & Pavon (Apocynaceae) is not mentioned by Schultes but fractions showed an inhibition profile similar to *Tabernaemontana heterophylla* at several 5HT receptor subtypes, and both species were active as 5HT agonists in functional assays at 5HT2A receptors. These results suggest that future investigations should focus on a more complete characterization of *Tabernaemontana* alkaloids at receptor subtypes relevant to cognitive functions.

Other investigators have applied radioligand receptor binding assays as tools to detect potentially therapeutic activity in medicinal plant extracts (Zhu et al., 1996; Misra, 1998; Phillipson, 1999; Sampson et al., 2000; Jäger et al., 2004; Nielsen et al., 2004) but none have been applied to the investigation of Amazonian ethnomedicines. Moreover, most previous studies have utilized a restricted battery of receptor screens applied to a relatively few number of extracts. The present study is the first to apply an extended battery of receptor assays to a large number (>228) of extracts and fractions derived from 121 species in 90 genera. The data reported here must be considered incomplete or at least as a

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work in progress. The results reported highlight both the promise and the limitations of such an approach. It has provided, for example, a picture of the distribution of CNS activity (to the extent that this is reflected in receptor interactions) in a subset of Amazonian flora, sampled according to both ethnobotanical and phytochemical criteria. It has not succeeded in definitively identifying one or more Amazonian species that are certain to lead to the development of medications that will find clinical use for the treatment of cognitive deficits in schizophrenia or dementias. It has, however, identified a subset of species that are promising candidates for further investigation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.jep.2010.12.037.

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