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In Vivo Phenotypic Drug Discovery: Applying a Behavioral Assay to the Discovery and Optimization of Novel Antipsychotic Agents†

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Abstract

Phenotypic drug discovery (PDD) is increasingly being recognized as a viable compliment to target-based drug discovery (TDD). By measuring functional changes, typically at a systems level, PDD can facilitate the identification of compounds having a desirable pharmacology. This capability is particularly important when studying CNS diseases where drug efficacy may require modulation of multiple targets in order to overcome a robust, adaptive biological system. Here, we report the application of a mouse-based high-dimensional behavioral assay to the discovery and optimization of a structurally and mechanistically novel antipsychotic. Lead optimization focused on optimizing complex behavioral features and no explicit effort was made to identify the target (or targets) involved.

Introduction

Target-based drug discovery (TDD) has been the dominant platform for discovering new drugs for over a quarter century. During this time, development of exquisitely potent and selective drug candidates has become the key metric of the drug discovery process. While medicinal chemists have become quite good at achieving these goals, the rate at which new drugs are approved has stayed largely flat.[1] At the same time, R&D expenditures have increased dramatically.[2] This can be attributed, in part, to clinical attrition rates that remain stubbornly high. Today, on average, only 13.3% of molecules that enter the clinic achieve NDA approval and success rates in some therapeutic areas such as CNS (4.7%) are even lower.[3] In terms of cost per drug approval, the era of TDD has been accompanied by a decline in Pharma productivity.[4]

Since biological systems tend to be complicated and difficult to study, TDD acts by deconvoluting a system into its constituent parts in order to provide detailed information about a single molecular target. However, an unintended consequence of this reductionist approach is that critical information may be lost. In particular, it can be difficult to understand the full functional role of a molecular target within a biological system or how

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system adaptation might compensate for the disruption of a single target. The role that reductionism may have played in the decline in Pharma productivity has been noted[5] and has spurred efforts to identify alternate approaches to drug discovery. One strategy that has (re)emerged is phenotypic drug discovery (PDD) which focuses on functional rather than target activity. Since a phenotypic assay probes a compound's effect on a typically complex biological response, it may afford a read-out that is more physiologically relevant than that of a target-based assay. Importantly, no knowledge of the molecular target (or targets) is required. The process can be driven entirely by measuring changes in functional endpoints and so might more effectively identify molecules that engage multiple targets.

Despite the central influence of TDD, phenotypic screening continues to have a surprisingly significant impact on new drug approvals. Recent reports[6, 7] have confirmed that phenotypic screening remains a relevant approach for drug discovery. Today, PDD, aided by such critical technological advances as high-content screening and big data analysis, is experiencing a renaissance. A number of reasons for this have been postulated[8-10] but PDD has emerged as an alternative, and complimentary, strategy to TDD. Phenotypic screens are being used to drive the discovery process in a variety of therapeutic areas (e.g., infectious diseases,[11-13] oncology,[14] CNS[15, 16]). To date, cell-based assays, which tend to be more reproducible and amenable to high-throughput screening than *ex vivo* or *in vivo* assays, have predominated. However, there have also been reports of whole animal assays being used in phenotypic screens. These largely focus on organisms (e.g., parasites,[17, 18] nematodes,[19, 20] xenopus embryos,[21, 22] zebrafish[15]) that can be adapted to automated, high-throughput screens. While *in vivo* assays can be challenging (e.g., lower reproducibility, lower throughput, higher cost), there are some clear advantages associated with *in vivo* PDD. For example, animal-based screening can select for compounds having superior ADME (absorption, distribution, metabolism and excretion) properties and brain exposure.

The complex biology associated with most CNS diseases can explain, at least in part, the poor clinical success rate in this therapeutic area. Drug efficacy may require modulation of multiple targets in order to overcome a robust, adaptive biological system. Notably, most currently marketed CNS drugs act on multiple molecular targets. This is especially true for drugs that treat psychiatric diseases such as schizophrenia. While all known drugs that treat schizophrenia block the dopamine D_2 receptor,[23] activity at other receptors (e.g., serotonergic, adrenergic, muscarinic, histaminergic) also contributes to their efficacy.[24] Unfortunately, because of the rudimentary understanding of CNS network topologies, it is exceptionally difficult to select an appropriate target combination and design a polypharmacological molecule that is both safe and efficacious. As a consequence, most antipsychotic drugs were discovered serendipitously. This realization led us to develop a phenotypic strategy for the discovery and optimization of novel antipsychotics. Here we report the application of a mouse-based *in vivo* phenotypic assay for both hit identification and lead optimization resulting in the discovery of a compound that is structurally and mechanistically novel as well as active in known animal models relevant to schizophrenia.

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Results and Discussion

At the outset of our program, we selected the SmartCube® system, a mouse-based, high-throughput automated behavioral platform developed by PsychoGenics Inc as our screening assay. The system presents a sequence of challenges to a mouse through its customized hardware, extracts more than 2000 features per session, and, using proprietary bioinformatics tools, predicts potential therapeutic efficacy of compounds from the changes that the drug induces in those behavioral features. A thorough description of the platform and its validation has been described elsewhere.[25] Additionally, the system had previously been validated using a collection of marketed antipsychotic drugs[26] as well as in other drug discovery programs.[27] While analysis of these data can be complex, two basic analytical measures were used. The first was signal strength (shown as a bar with color bands and normalized to a scale of 0 to 100%), which is a measure of the probability of behavioral activity differing from vehicle (i.e., a cross-validated accuracy of a binary classifier of discriminating between the vehicle and drug mice based on their behavioral features is a measure of activity, where 0 corresponds to a chance level in which drug and vehicle are indistinguishable and 100% accuracy representing perfect discrimination or clear differentiation from vehicle). Signal strength is a measure of efficacy with a minimum efficacious dose (MED) defined for this program as the lowest dose administered (i.p.) in which a compound's non-vehicle signal remains above 50%. The second measurement was class signature (represented as one or more of 15 colors; Figure 1A) which is a probabilistic outcome of a multi-class support vector machines (SVM) based classifier mapping behavioral features to the class labels defined by know reference drugs (CNS indications). Both SVM[28] and probabilistic neural network based methods[29] performed very similarly on all cross-validation tests. A SVM based method was selected for speed considerations. The size of a given color band in the signal bar indicates the probability that the observed behavior matches that of a set of reference drugs for each therapeutic indication (e.g., Figure 1B). For example, an antipsychotic signature is represented by a purple color. Since, in our hands, we encountered no instance where a single molecule was found to have two different class colors that are both greater than 30% at a single dose, we considered a class signature of 30% or more at a specific dose as representing a high probability that the test compound will be behaviorally similar to known drugs from that class.

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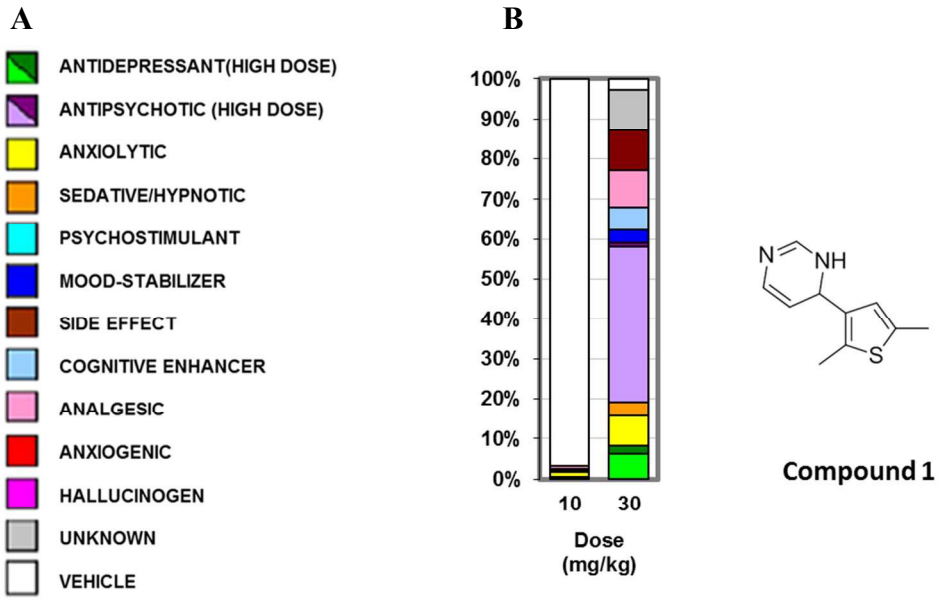

Figure 1.

A. SmartCube® class signature color key (15 classes represented including high-dose antidepressant, high-dose antipsychotic and vehicle); *includes antidepressant (green) and high-dose antidepressant (dark green); **includes antipsychotic (purple) and high-dose antipsychotic (dark purple)

B. Structure and SmartCube® class signature of compound **1** (administered i.p. 15 min prior to testing). The predominant purple color is characteristic of an antipsychotic signature.

The initial screening campaign had two primary goals. The first goal was to identify hits having a predominant antipsychotic signature. In order to ensure that the process did not simply identify another compound acting via a D_2 mechanism, a second goal was to avoid any D_2 receptor activity. Screening of a 1000-compound library led to the identification of multiple hits, including compound **1** (Figure 1B).[30] This hit was commercially acquired as the HCl salt and was screened as the racemate. As shown in Figure 1B, racemic **1** displayed a strong signal (>95%) at a dose of 30 mg/kg (i.p.) as well as a predominant antipsychotic signature (~40% purple). Given the unusual dihydropyrimidyl (DHP) moiety, initial hit follow-up focused on confirming compound stability. When stored and used as the HCl salt, **1** was found to be sufficiently stable for *in vivo* studies, although it proved to be less stable as the freebase, decomposing over the course of days. Additionally, **1** was found to have a favorable *in vitro* ADME profile[31] (see Table 1). Further profiling of this hit in a receptor panel screen[32] confirmed that it had no D_2 binding at 10 μM. Encouraged by these data, we decided to bypass the hit-to-lead phase and directly initiate lead optimization using the SmartCube® system as the primary assay. Several goals were identified, most notably maximization of the antipsychotic signature and improvement of the MED, while avoiding D_2 receptor activity.

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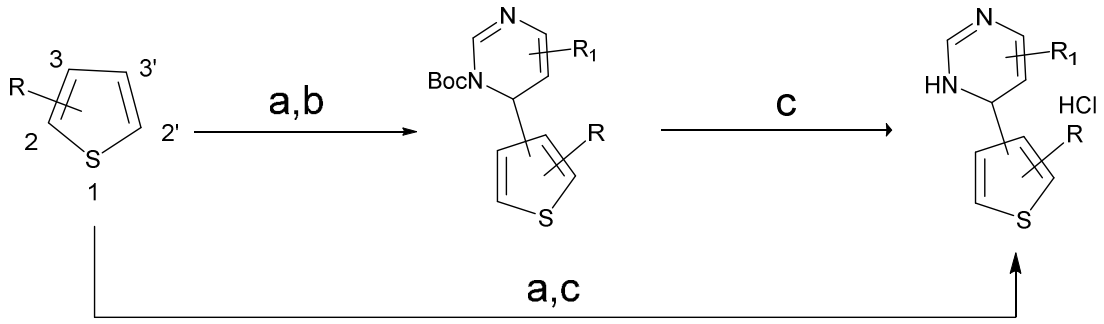
hERG Inhibition IC_{50} (μM)	CYP450 Inhibition IC_{50} (μM)					Plasma Protein Binding Fraction bound (%)			Microsomal Stability $t_{1/2}$ min		
	CYP1A	CYP2C19	CYP2C9	CYP2D6	CYP3A4	*Human*	*Mouse*	*Rat*	*Human*	*Mouse*	*Rat*
>25	>25	12.7	>25	6.3	19.8	100	76	70	48	155	39

Table 1. *In vitro* ADME profile of compound **1**. All assays are commercially available from Cyprotex.[31]

Scheme 1 depicts the general route for the synthesis of a variety of 2- and 3-substituted dihydropyrimidinothiophenes. Addition of a pyrimidine moiety to the thiophene substrates was generally straightforward and afforded the desired compounds in one step in fair to good yields. To facilitate product isolation, in some instances, the unpurified reaction mixture was treated directly with Boc anhydride to give a Boc protected precursor, which after flash chromatography, was de-protected with HCl to afford the final product as the salt in 10 to 83% overall yields (see supplemental material). We also discovered that, if the thiophene was substituted with an electron-withdrawing group, the reaction could be assisted with microwave irradiation or ultrasonication. Addition occurred preferentially at the 2 or 2' position of the thiophene. When both of these positions were blocked, then addition occurred at the 3 or 3' positions to give 3-substituted dihydropyrimidinothiophenes as racemates.[33, 34]



Scheme 1. General synthetic route to DHP-substituted thiophenes. Reagents and conditions: (a) pyrimidines, TFA, MW or ultrasonication; (b) Boc_2O, Na_2CO_3; (c) HCl.

In the interest of expediency, initial exploration of the thiophene scaffold was limited to racemates. With a DHP group at the 3-position, we found that structural modifications that included large functional groups or functional group changes at multiple positions frequently resulted in substantial transformations in the class signature (see Figure 2, compounds **5-9**). Conservative structural changes were better tolerated, but, even then, sometimes dramatic class signature changes could be observed. For example, compounds **2, 3** and **4** differ from **1** by only a single atom or methyl group and yet the predominant class signature for each of these compounds is different (i.e., anxiolytic, antidepressant and analgesic classes respectively). While the reason for this is not known, these results may be consistent with a polypharmacological mechanism. If the assay is measuring how modulation of multiple molecular targets affects an intact biological system, then it is conceivable that, as the structure changes, the targets modulated may also change in ways that lead to different behavioral outcomes. Based on these findings, we decided to mostly limit SAR studies to conservative changes.

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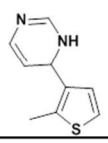
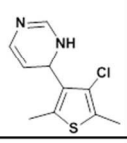
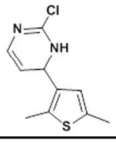
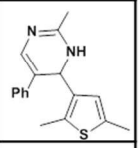
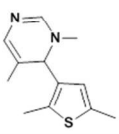
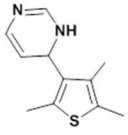
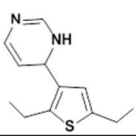
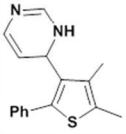
Compound	2	3	4	5
Structure				
Compound	6	7	8	9
Structure				

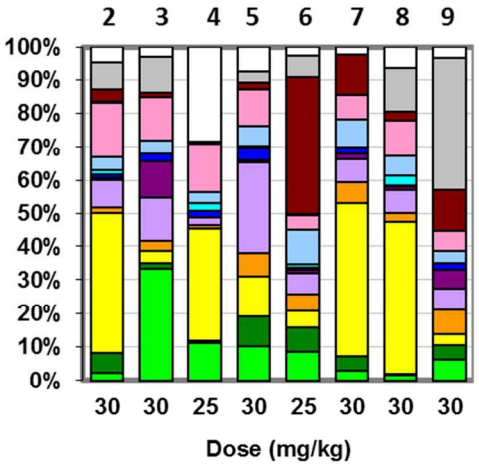
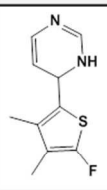
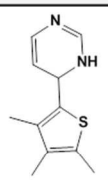
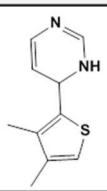
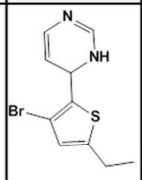
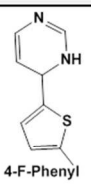
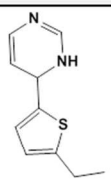


Figure 2. Structure and SmartCube® class signatures of representative substituted 3-dihydropyrimidinothiophenes (administered i.p. 15 min prior to testing).

Moving the DHP moiety to the 2-position of the thiophene maintained or enhanced the antipsychotic signature at a dose of 30 mg/kg (Figure 3). Additionally, the size of the thiophene substituents was found to be important for signal strength. Small substituents tended to maintain a strong antipsychotic signature and often improved the MED (e.g., **10**, **12**). As previously noted, larger substituents (e.g., **14**, **15**) usually altered the predominant class signature. In this series, compound **12** was found to have the strongest antipsychotic signature, the best MED and little (if any) binding to the D_2 receptor (18% at 30 μM) (see supplemental material).

Compound	10	11	12
Structure			
MED	10	30	3
Compound	13	14	15
Structure			
MED	30	3	10

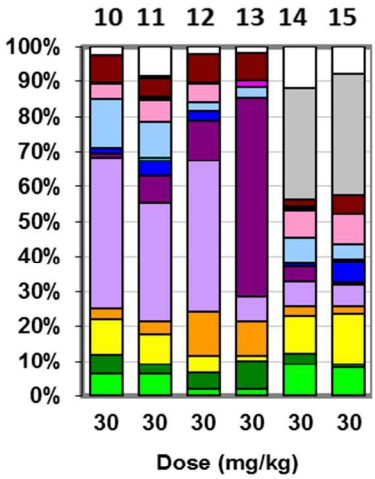


Figure 3. Structure and SmartCube® class signatures of representative substituted 2-dihydropyrimidinothiophenes (administered i.p. 15 min prior to testing).

12 was submitted to chiral chromatography to obtain the pure enantiomers (**12a, 12b**). For the purposes of this study, no effort was made to determine the absolute

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stereochemistry and all stereochemical assignments were arbitrary. The full dose-response signatures of these compounds are shown in Figure 4A. Most of the activity was found to reside with one enantiomer, **12a,** having a MED of 3 mg/kg, the same as the racemate. Additionally, **12a** was found to have a strong antipsychotic signature at three doses (3, 10, 30 mg/kg). While the other enantiomer (**12b)** showed a similar signature, it had a much weaker activity (MED=30 mg/kg).

During the isolation of the enantiomers, we noticed that concentration of the free bases at elevated temperatures resulted in modest decomposition but no changes in enantiomeric purities. To explore the extent of this decomposition, a stability study of **12a** was conducted. At pH 2 the compound was found to be stable in aqueous solution at room temperature. However, when the pH was adjusted to 7.1, a slow decomposition was observed (see supplemental material), affording a complex mixture. We anticipated that re-aromatization of the DHP ring by oxidation could be a route of decomposition so an authentic standard of the presumed aromatized decomposition product **16** was prepared by H_2O_2 oxidation of **12** (Figure 4B). While **16** did display some SmartCube activity, we were unable to detect the formation of **16** during either chemical stability studies or the mouse and human liver microsomal stability assays of **12a**.

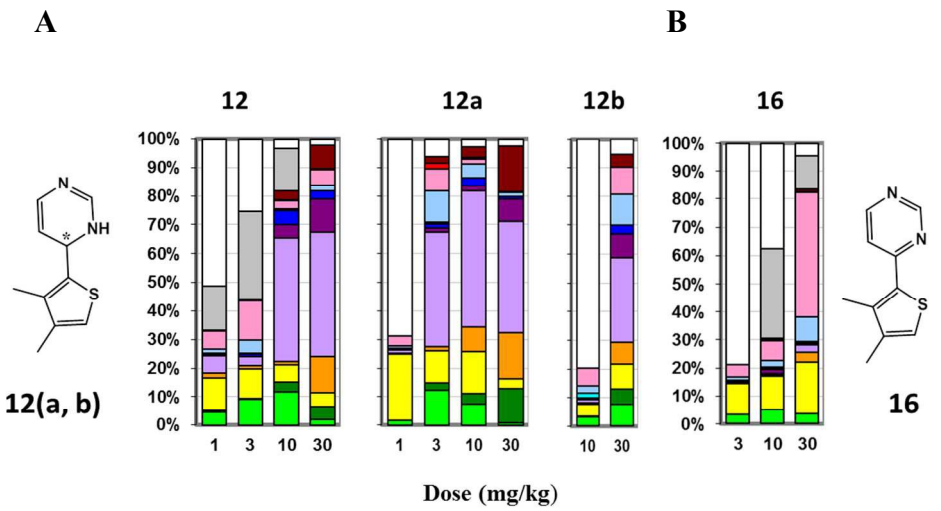

Figure 4.

A. Structure and SmartCube® class signatures of **12** (racemate)**, 12a** (enatiomer 1) and **12b** (enatiomer 2) at multiple doses (administered i.p. 15 min prior to testing). Absolute stereochemistry was not determined.

B. Structure and SmartCube® class signatures of **16** at multiple doses (administered i.p. 15 min prior to testing).

Since the observed degradation appeared to be a consequence of the thiophene ring instead of the DHP moiety, we expanded the scope of our SAR studies to include dihydropyrimidinobenzothiophenes, which could be prepared by the same general synthetic route shown in Scheme 1 (see supplemental material). In order to probe whether the SAR developed for the thiophene series translated to the benzothiophenes, we prepared a series of 2-substituted benzothiophenes as shown in Figure 5. To our surprise, these compounds (**17-22**) exhibited little of the desired antipsychotic signature,

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although most maintained a strong signal at 30 mg/kg. Because of the generally undesired class signatures, the MED for these compounds was not determined.

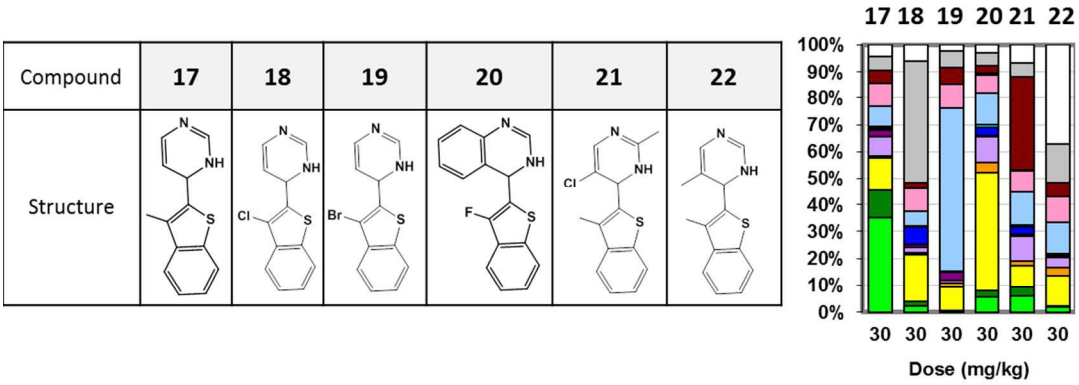

Figure 5. Structure and SmartCube® class signatures of representative substituted 2-dihydropyrimidinobenzothiophenes (administered i.p. 15 min prior to testing).

In contrast, when the DHP group was moved to the 3-position, the antipsychotic signature tended to be maintained when dosed at 30 mg/kg. Additionally, improved MEDs were more frequently found (Figure 6). While these results were unanticipated by the earlier thiophene SAR, they did offer an alternative to the 2-DHP substituted thiophenes. The SAR around the phenyl ring was quickly established. Small functional groups at the 4 or 5 positon of the benzothiophene ring were preferred (e.g., **23, 24, 25**) for both antipsychotic signature and improved MEDs. Substitutions at positions 6 or 7 (e.g., **26, 27, 28**) were less tolerated, exhibiting weaker antipsychotic signatures and/or weaker MEDs. Finally, replacement of the phenyl ring with saturated carbocyclic rings (e.g., **29, 30**) was also acceptable.

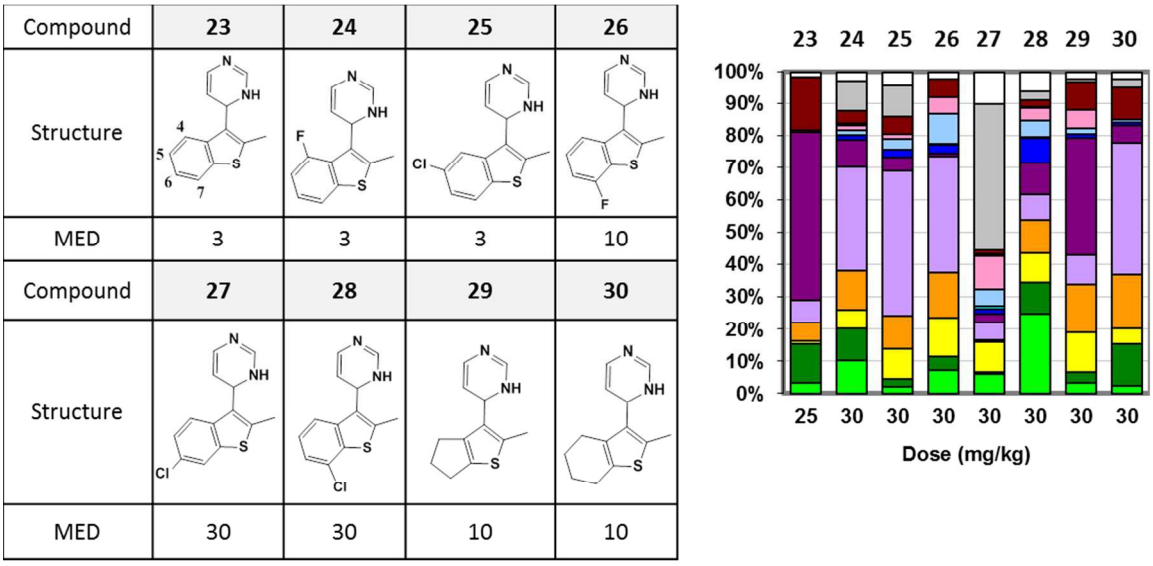

Figure 6. Structure and SmartCube® class signatures of representative substituted 3-dihydropyrimidinobenzothiophenes (administered i.p. 15 min prior to testing).

We also studied the effects of DHP group substitutions on activity. As illustrated in Figure 7, while some modifications of the DHP moiety maintained the antipsychotic

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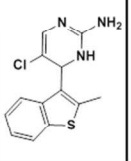
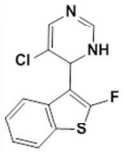
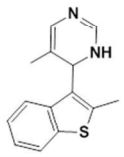
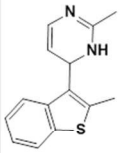
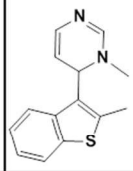
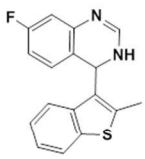
signature (e.g., **31**), the trend was for less active compounds or compounds having undesired class signatures such as sedative (orange bar) or adverse effects (brown bar) (e.g., **32-36**). In this series all measured MEDs were greater than 10 mg/kg.

Compound	**31**	**32**	**33**
Structure			
MED	30	30	30
Compound	**34**	**35**	**36**
Structure			
MED	30	30	30

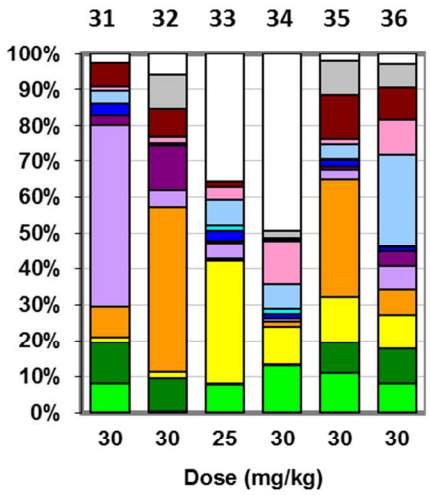


Figure 7. Structure and SmartCube® class signatures of representative DHP-substitutions of 2-dihydropyrimidinobenzothiophenes (administered i.p. 15 min prior to testing). All signatures shown are at a 30 mg/kg dose.

Based on its *in vitro* profile, compound **23** was selected from the benzothiophene series for further evaluation. After chiral separation, it was determined that the antipsychotic signature largely resided with enantiomer **23a** which exhibited a high-dose antipsychotic (dark purple) signature at 30 mg/kg and the more commonly encountered antipsychotic (purple) signature at 10 mg/kg (Figure 8). The class signature changes that were observed for **23a** in going from a 10 mg/kg dose to a 3 mg/kg dose was not uncommon in our experience. The reasons for this are not known but may be attributable to the nature of *in vivo* assays. Compound **23a** is an example of how a signature can become unstable near the MED. As the signal becomes weaker, the test animals' behavior becomes more vehicle-like as indicated by the white bar. We believe that this could lead to signatures becoming less reproducible.

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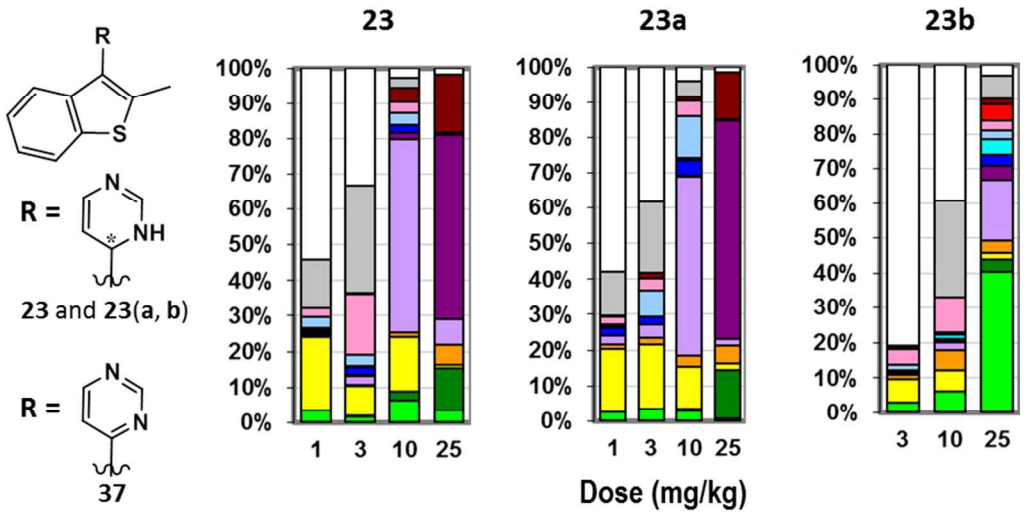

Figure 8. Structure and SmartCube® class signatures of **23** (racemate)**, 23a** (enatiomer 1) and **23b** (enatiomer 2) at multiple doses (administered i.p. 15 min prior to testing). Absolute stereochemistry was not determined.

We were pleased to find that, in contrast to **12a**, **23a** (free base or HCl salt) was determined to be stable at room temperature (see supplemental material) with no detectable amount of the aromatized pyrimidine **37** observed in the chemical stability studies. Formation of **37** was also not observed in mouse, rat or human liver microsomal assays. This improved chemical stability led to the selection of **23a** for additional profiling and the results are summarized in Table 2. This is a very small molecule (MW=228) with excellent physicochemical properties and an acceptable *in vitro* ADME profile. Receptor panel profiling confirmed that there was no detectable D_2 receptor binding at 10 μM, although binding to other receptors was observed. Several of these have been associated with molecular mechanisms-of-action (MMOA) of known antipsychotics.[24] Most significantly, it was determined that **23a** was a 5-HT_{2A} antagonist (IC_{50}=356 nM).

***in vitro* Pharmacology**		**Found for Compound 23a**
Receptor Panel Profile	Binding (% at 10 μM))	D2 (0); α_1 (88); α_2 (97); D_1 (77); 5-HT_{2A} (99); 5-HT_{2B} (81); 5-HT_3 (64); 5-HT_6 (61); 5-HT_7 (94); H1 (86); 5-HT trans.(94); DA trans. (78); NE trans. (51)
	Functional Activity	5HT_{2A}: IC_{50} = 356 nM
***in vitro* ADMET**		
CYP profile	5 isozymes (μM)	2C19 (25); 2D6 (3.1); 2C9 (6.9);1A (6.9); 3A4 (11)
LM profile	$t_{1/2}$ (min)	137 (h), 29(r), 242(m)
hERG	(μM)	>25
Physicochemical		
Solubility	pH 7.4	>100 μg/mL
ALogP		1.9
Protein Binding	Fraction Unbound (%)	30 (h), 18 (m)

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Table 2. *In vitro* profile of **23a**. Assays are commercially available from CEREP S.A.[32] (*in vitro* pharmacology), or Cyprotex[31] (*in vitro* ADMET and physicochemical); (h) = human, (r) = rat, (m) = mouse.

Compound **23a** was also determined to be orally active in two rodent tests associated with antipsychotic behavior. The reversal of stimulant-induced hyperlocomotor activity is commonly used to assess antipsychotic activity in preclinical models.[35] Similar to the clozapine positive control, **23a** (3, 10 and 30 mg/kg, p.o.) reversed phencyclidine (PCP)-induced hyperlocomotor activity in mice (Figure 9A). Although, baseline locomotor activity was also decreased at the two highest doses of **23a** (10 and 30 mg/kg), there was a separation of dose for efficacy (3 mg/kg) that did not alter baseline activity, an effect also reported for some other antipsychotics.[35] Prepulse inhibition of acoustic startle (PPI) can be used to assess sensory gating mechanisms[36] and PPI deficits are widely seen in schizophrenia.[37] Treatment of mice with **23a** (3 and 10 mg/kg, p.o.) significantly increased PPI compared to vehicle (Figure 9B).

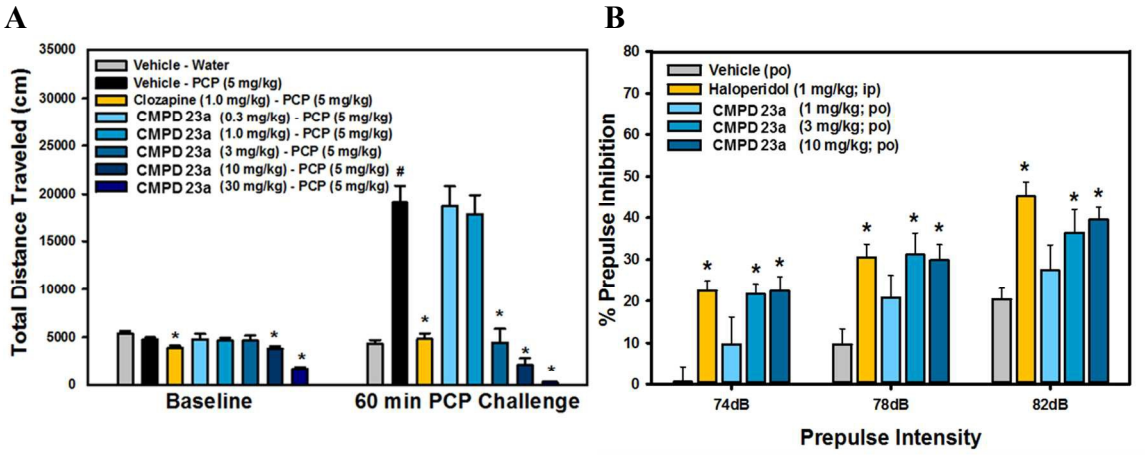

Figure 9.

A. Effects of **23a** (p.o. administration) in mice on baseline locomotor activity and after a PCP challenge (5 mg/kg). Clozapine was used as a positive control.

B. Effects of compound **23a** (p.o. administration) on pre-pulse inhibition in mice. Haloperidol was used as a positive control.

Conclusions

A mouse-based behavioral platform, the SmartCube® system, was successfully used in a phenotypic screening campaign leading to the identification of a hit (compound **1).** Lead optimization of **1** was then efficiently conducted using the same phenotypic assay to afford compound **23** having a strong antipsychotic signature. The more active enantiomer, **23a,** was found to have good chemical stability, good *in vitro* ADME and DMPK profiles and excellent CNS drug-like properties. Furthermore, this compound exhibited potent oral activity in two rodent models relevant to schizophrenia despite having no D_2 receptor activity. The absence of any D_2 activity is important considering that the MMOA of all approved drugs for schizophrenia includes inhibition of the D_2 receptor, making compound **23a** a potentially useful research tool. Throughout the course of this program, no effort was made to understand the MMOA associated with **23a**

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but we believe that it may likely be polypharmacological in nature and possibly includes 5-HT_{2A} receptor antagonism. These results support the utility of the SmartCube® system as a tool for PDD and demonstrate that a molecular target need not be a required part of the drug discovery process.

Experimental Section: *In vivo* Pharmacology

All housing and testing was in accordance with the Principles of Laboratory Animal Care and the approval of the PsychoGenics, Inc., Institutional Animal Care and Use Committee in AAALAC-accredited facilities. Experimental details are described in the supplementary.

Supplementary Data

Supplementary data associated with this article can be found in the online version.

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30. For all SmartCube® studies, compounds were formulated in a cocktail consisting of 5% 1-methyl-2-pyrrolidone, 30% 1:1:1 solution of PEG200, PEG400, propylene glycol, and 65% saline. Additionally, PsychoGenics' standard operating procedures include the routine testing of reference compounds to confirm signature. For example, haloperidol has been run multiple times. The antipsychotic signature was consistent and no drift was seen.
31. *In vitro* ADME, toxicology and physicochemical profiling assays were all selected from the Cyprotex (Watertown, MA, USA) services catalog (http://www.cyprotex.com/services) and used without modification.
32. A customized Cerep S. A. (Poitiers, France) panel was used that included the "ExpresSProfile" of 55 targets (http://www.cerep.fr/cerep/users/pages/catalog/profiles/catalog.asp). Screening concentrations of 10 μM or 30 μM were used. See the supplementary material for the full 52 assay list.
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Figure 1

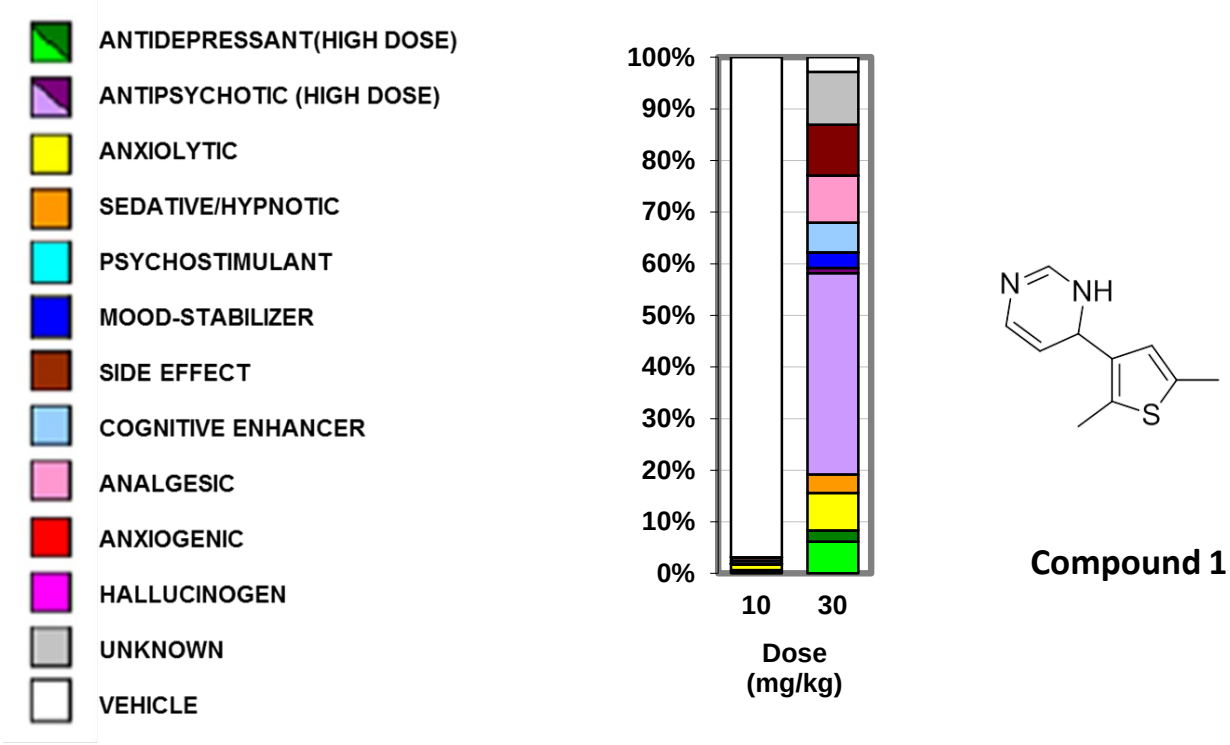


Table 1

hERG Inhibition IC_{50} (μM)	CYP450 Inhibition IC_{50} (μM)					Plasma Protein Binding Fraction bound (%)			Microsomal Stability $t_{1/2}$ min		
	CYP1A	CYP2C19	CYP2C9	CYP2D6	CYP3A4	*Human*	*Mouse*	*Rat*	*Human*	*Mouse*	*Rat*
>25	>25	12.7	>25	6.3	19.8	100	76	70	48	155	39

Scheme 1

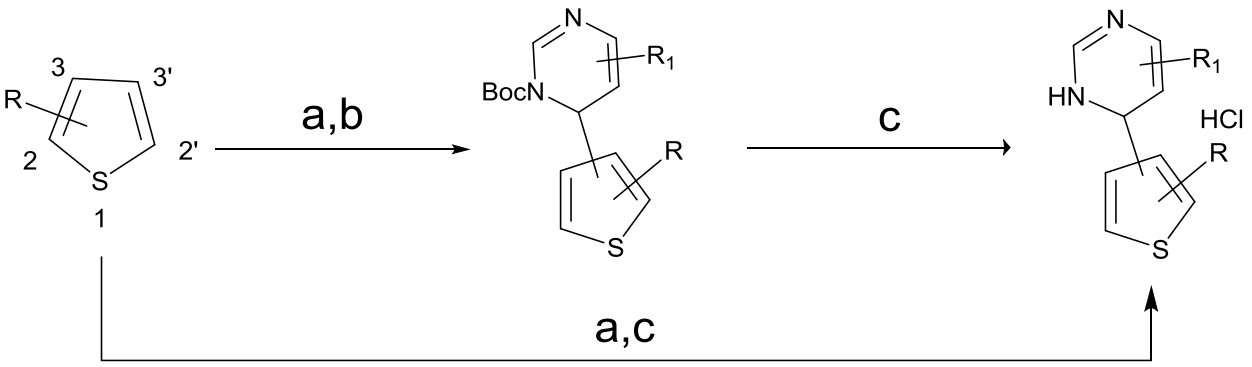
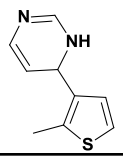
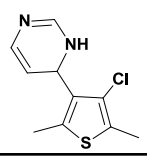
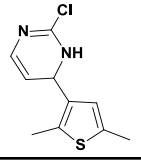
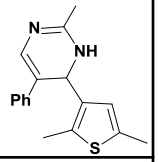
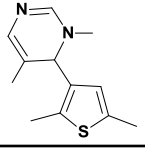
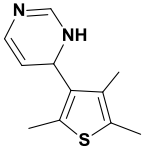
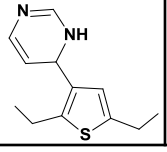
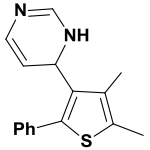


Fig 2

Compound	2	3	4	5
Structure				
Compound	6	7	8	9
Structure				

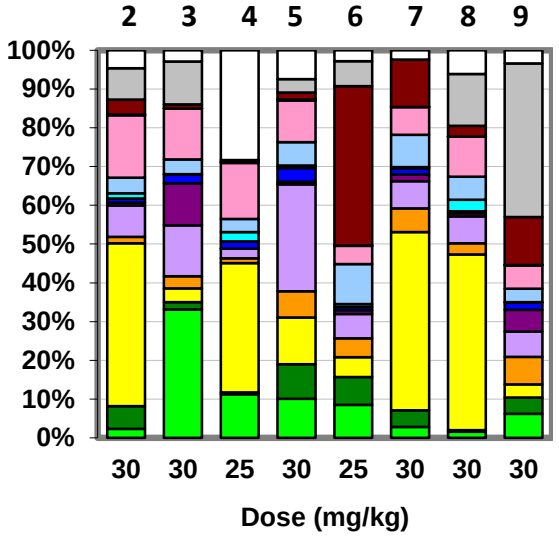
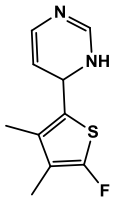
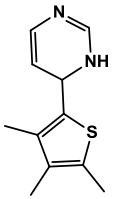
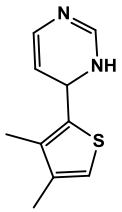
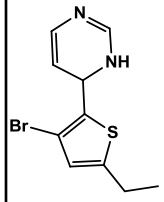
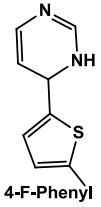
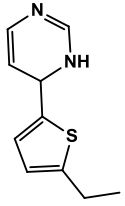


Figure 3

Compound	**10**	**11**	**12**
Structure			
MED	10	30	3
Compound	**13**	**14**	**15**
Structure			
MED	30	3	10

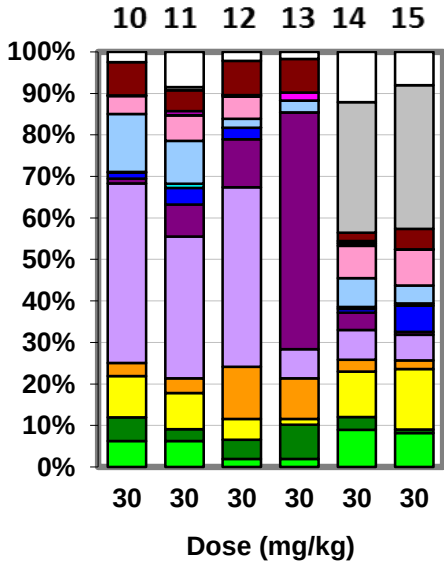


Figure 4

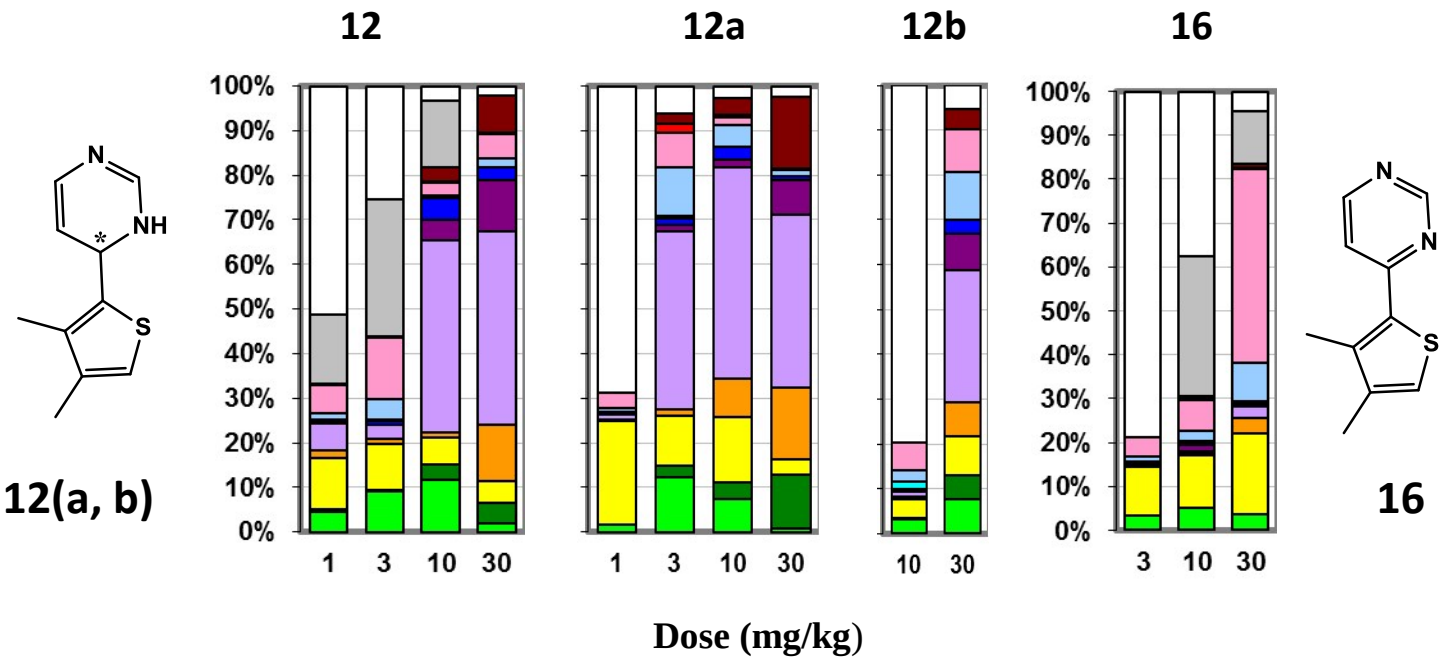
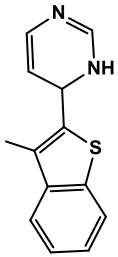
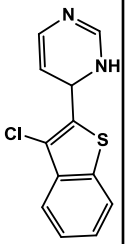
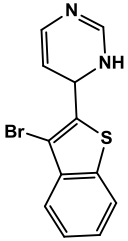
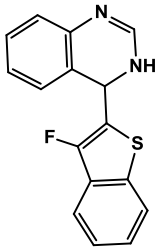
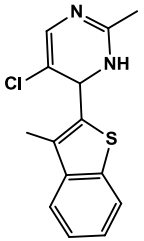
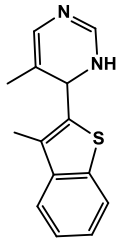


fig 5

Compound	17	18	19	20	21	22
Structure						

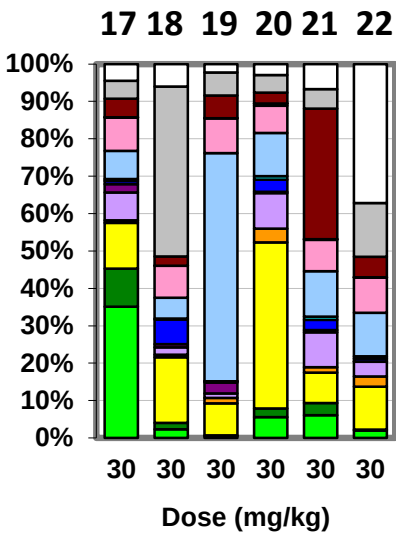
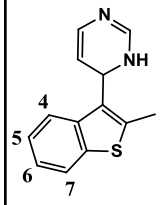
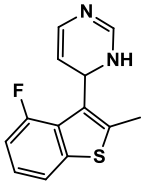
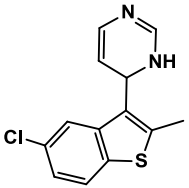
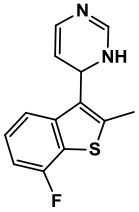
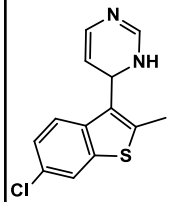
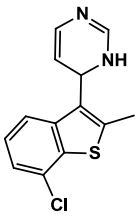
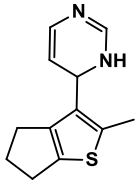
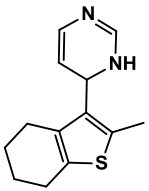


Fig. 6

Compound	**23**	**24**	**25**	**26**
Structure				
MED	3	3	3	10
Compound	**27**	**28**	**29**	**30**
Structure				
MED	30	30	10	10

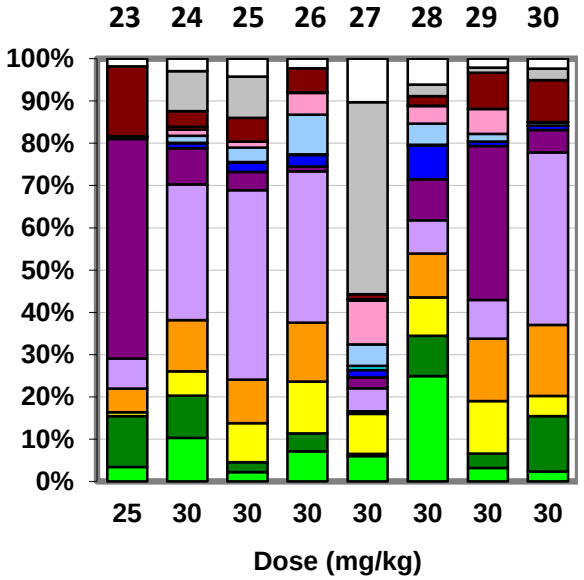
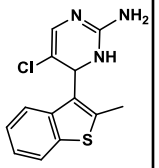
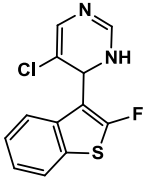
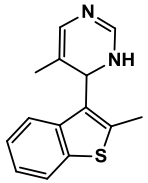
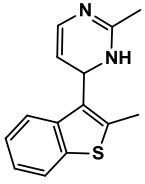
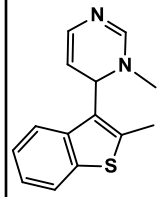
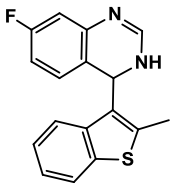


Fig 7

Compound	**31**	**32**	**33**
Structure			
MED	30	30	30
Compound	**34**	**35**	**36**
Structure			
MED	30	30	30

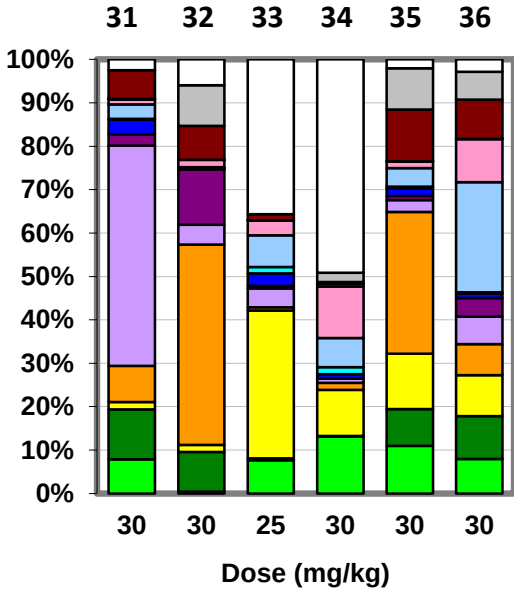


Fig 8

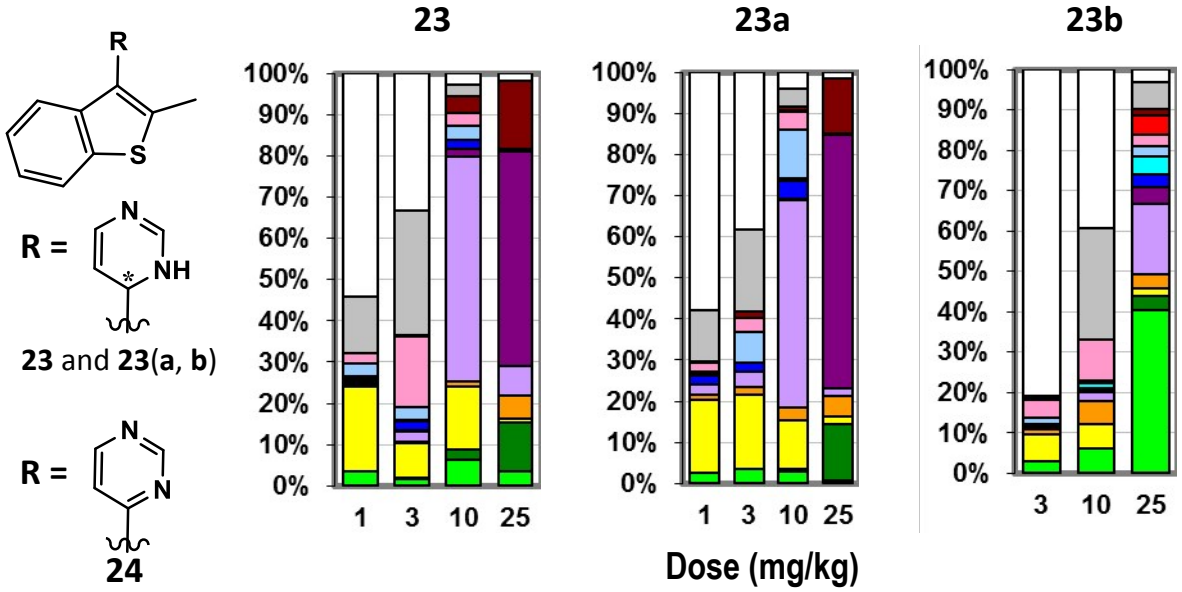
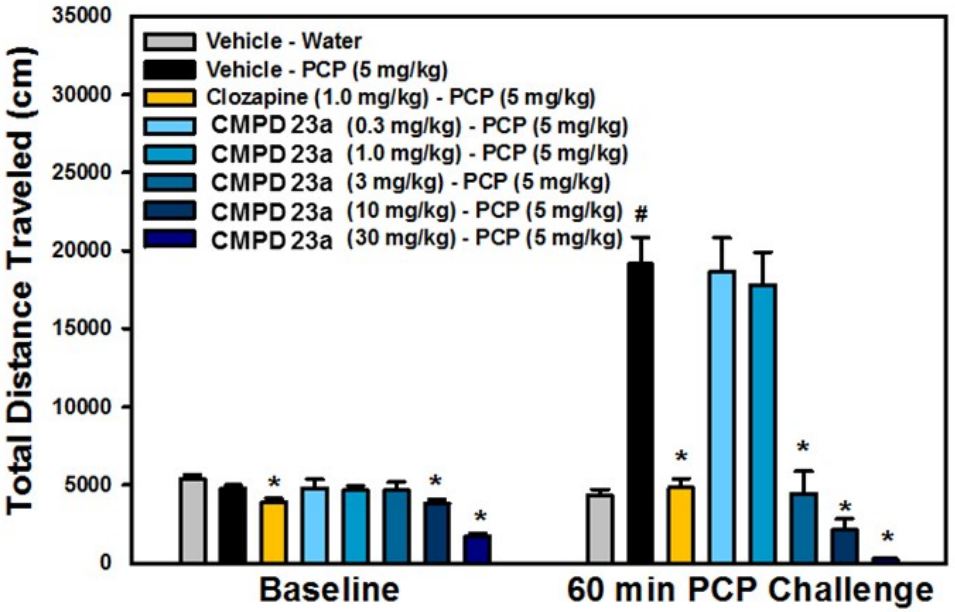
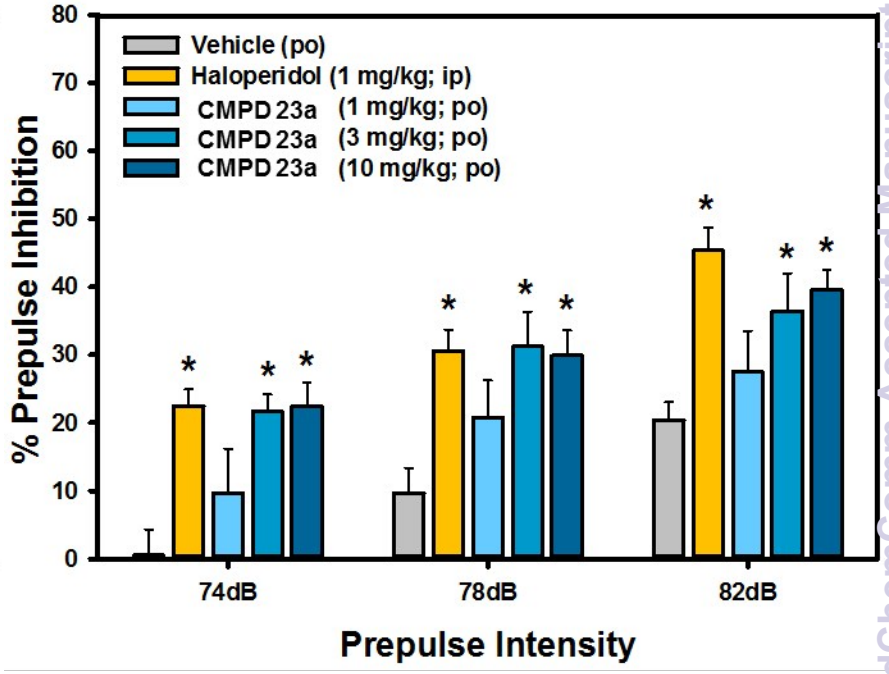


Table 2

***IN vitro* Pharmacology**		**Found for Compound 23a**
Anti-Target Profile	Binding (%)	D2 (0), α_1 (88); α_2 (97); D_1 (77); 5-HT_3 (64); 5-HT_6 (61); 5-HT_7 (94); H1 (86); 5-HT trans.(94); DA trans. (78); NE trans. (51); 5-HT_{2B} (81); 5-HT_{2A} (99),
	Functional Activity (%)	$5HT_{2A}$: IC_{50} = 356 nM
ADMET/PK		
CYP profile	5 isozymes (μM)	**2C19** (25); **2D6** (3.1); **2C9** (6.9);**1A** (6.9); **3A4** (11)
LM profile	$t_{1/2}$ (min)	137 **(h),** 29**(r),** 242**(m)**
hERG	(μM)	>25
Physicochemical		
Solubility	pH 7.4	>100 μg/mL
cLogP		1.9
Protein Binding	% Fu	30 **(h),** 18 **(m)**

Fig 9





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A mouse-based high-dimensional assay, the SmartCube, was used for both screening and lead optimization of a novel antipsychotic.