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Evaluation of SYA16263 as a new potential antipsychotic agent without catalepsy

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ABSTRACT

SYA16263 exhibited moderate radioligand binding affinity at the D_2 receptor and produced inhibition of apomorphine-induced climbing behavior in mice with an ED_{50} value of 3.88 mg/kg IP, predicting potential antipsychotic effects in humans.

Analysis of plasma and brains from rats injected IP with SYA16263 over the course of 24 h revealed a log [brain]/[plasma] (log BB) at Cmax observed equal to 1.08, indicating that SYA16263 enters the brain and is predicted to cross the blood brain barrier (BBB) readily.

When tested in animal behavior tests for catalepsy, SYA16263 did not produce catalepsy at doses up to 19 times the apomorphine ED_{50} value predicting little or no extra-pyramidal (EPS) side effects in humans. This is similar to aripiprazole, which is associated with a low incidence of EPS in humans, but unlike haloperidol which is known to cause severe EPS in humans.

Functional activities for SYA16263 show that it acts as a D_2 agonist at both the Gi and β-arrestin pathways, similar to, but better than aripiprazole, which could account for the absence of the catalepsy observed.

Taken together, the receptor binding profile, the functional status, the animal behavioral tests and the log BB value, all provide evidence for further pre-clinical testing of SYA16263 as a potential antipsychotic agent with an interesting profile and a unique mechanism of action resulting in no EPS even up to 19 times the ED_{50} value.

1. Introduction

Schizophrenia affects about 1% of the world's population (Saha et al., 2005) and here in the US, the estimate is 1.1% (NIMH, 2017). The symptoms of schizophrenia are three-fold: a) positive b) negative and c) cognitive symptoms. The positive symptoms often involve hallucinations, delusions, thought disorders and movement disorders. The negative symptoms include disruptions to normal emotions and behaviors, poverty of speech and a lack of the ability to plan and sustain activities. Cognitive symptoms are subtle and may involve trouble focusing, poor executive functioning and problems with working memory. Schizophrenia is also associated with suicidal ideation and 9–13% of patients eventually take their own lives (Inskip et al., 1998; Meltzer, 1999). While estimates vary, up to 1/3 of patients are treatment resistant (TRS) (Essock et al., 1996; Lehman et al., 2004) and current drugs are either ineffective or they produce serious adverse side effects which lead to patient non-compliance. These findings underscore the need to seek novel treatment options for patients with schizophrenia.

First generation antipsychotic (FGA) drugs such as haloperidol presumably act primarily by blocking dopamine D_2 receptors (D_2Rs) (Besnard et al., 2012; Kapur and Mamo, 2003; Wang et al., 2018). The efficacy of the FGAs is however hampered by their capacity to induce Parkinsonism-like side effects that become very debilitating with long-term use (Correll et al., 2004; Girgis et al., 2011; Tapp et al., 2008). The second generation antipsychotic (SGA) drugs are efficacious against the positive symptoms and may also attenuate some of the negative symptoms (Boter et al., 2009; Tandon et al., 2008). In addition to their interaction at the D_2R, each interacts with additional receptors including $5HT_{1A}$, $5HT_{2A}$, $5HT_7$, alpha (α), and H_1 receptors which may be responsible for the subtle differences in their clinical profiles. While the EPS side effects of the SGAs are somehow muted, they produce weight gain and metabolic disorders which limit their widespread utility (Lieberman et al., 2005), (Boehm et al., 2004; Buckley et al., 2005;

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Wong et al., 2010).

Many CNS disorders are treated with therapeutic agents that directly or indirectly target G protein-coupled receptors (GPCR). In the canonical or conventional model of GPCR signaling, trimeric G protein signaling is initiated at the plasma membrane and is terminated by β-arrestins, which prevent coupling between receptors and G-proteins, and promote receptor internalization (Watari et al., 2014). The internalization process contributes further to the mechanism of G-protein signal desensitization by promoting receptor lysosomal degradation (Delom and Fessart, 2011; Mills, 2007). Studies using biochemical, pharmacological, biophysical, and mice studies have now revealed that several GPCRs, including the D_2 receptor subtype (D_2R), do not always follow this conventional signaling and desensitization paradigm. In fact, it is now widely accepted that GPCRs can signal not only through G protein activation but also through β-arrestins to scaffold intracellular signaling molecules with distinct pharmacological properties that could result in favorable therapies. Work by Caron and others (Urs et al., 2014; Urs et al., 2012) has now provided additional validation of the physiological role of this signaling pathway, using genetic approaches such as the deletion of GSK3β from D_2 receptor-expressing neurons, to study the effects of antipsychotics downstream of the D_2 receptor, confirming the role of the β-arrestin pathway. Thus, agonists and antagonists that selectively discriminate between these signaling mechanisms, through the stabilization of an associated GPCR conformation, might become novel therapeutic tools that can lead to obtaining compounds with reduced side effects.

It has previously been shown that the D_2R is the main target of clinically effective antipsychotics and D_2R engages an Akt/GSK3 signaling pathway via the scaffolding of a β-arrestin2/Akt/PP2A/GSK3 complex (Beaulieu et al., 2005). Therefore, a strategy to activate β-arrestin2 selectively and spare the G-protein dependent signaling has been proposed and validated as a novel approach to the development of new antipsychotic agents. There is also now a preponderance of evidence in rodent models indicating that activation of $5HT_{1A}R$ produces several effects including prevention of extrapyramidal symptoms (EPS) induced by dopamine D_2R blockade, enhancement of dopaminergic neurotransmission in the frontal cortex and inhibition of NMDA receptor antagonist-induced cognitive and social interaction deficits (Newman-Tancredi, 2010). Furthermore, blocking the $5HT_7R$ is suggested to improve cognitive impairments in animals (Bonaventure et al., 2011). Lurasidone, a more recently introduced antipsychotic drug with favorable cognitive outcomes, activates $5\text{-}HT_{1A}R$ and blocks $5\text{-}HT_7R$ (Huang et al., 2012). Therefore, we have hypothesized that an antipsychotic agent which activates β-arrestinergic signaling through the D_2R, activates $5HT_{1A}R$ [Ki < 10 nM] and blocks $5HT_7R$ [Ki < 10 nM] should constitute an effective antipsychotic agent. It is equally important that the compounds avoid H_1R, $5HT_{2C}R$, $5HT_{2B}R$ and not activate $5HT_{2A}R$. Weight gain and the metabolic syndrome are associated with binding to H_1R and $5HT_{2C}R$ (Kroeze et al., 2003; Matsui-Sakata et al., 2005; Opgen-Rhein et al., 2010) while induction of valvular heart disease is associated with activating the $5HT_{2B}R$ (Rothman et al., 2000; Setola et al., 2003; Setola and Roth, 2005). While often targeted in antipsychotic drug development, activating the $5HT_{2A}R$ could produce hallucinogenic effects (Glennon et al., 1982; Glennon et al., 1980; Rasmussen et al., 1986; Smith et al., 1998).

The present study investigates the potential of SYA16263 to serve as an antipsychotic agent using the apomorphine-induced climbing behavior test in mice. The results are then compared to another potential antipsychotic, SYA013, which does not activate the β-arrestin pathway at the D_2R, the typical antipsychotic haloperidol, and the β-arrestin pathway activator aripiprazole. In addition, the potential of these compounds to induce catalepsy in rats, an animal behavioral test with predictive ability for possible extra-pyramidal side effects in humans, was investigated, and the results compared and discussed in light of their CNS receptor binding profiles.

2. Materials and methods

2.1. Animals

Reversal of apomorphine-induced climbing behavior experiments were performed using male, albino, Swiss-Webster mice (21–29 g), (5–7 weeks old). Catalepsy experiments were carried out on male Sprague-Dawley rats (107–168 g), (5–6 weeks old). All animals were from Harlan Laboratories, Inc. Animals were housed in the Florida A & M University Animal Care facility which is fully AAALAC accredited, and operates with a 12 h light/dark cycle and controlled temperature (24 ± 2 °C). Animals were given free access to food and water and at least 5 days to adjust before experiments were begun. Animals were fasted the night before each experiment. All experimental procedures were performed in accordance with protocols approved by the Florida A & M University Institutional Animal Care and Use Committee.

2.2. Drugs and chemicals

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated. SYA16263 (CLog *P* = 4.36) and SYA013 (CLog *P* = 5.15) were synthesized and characterized by us (Ablordeppey et al., 2008; Peprah et al., 2012) at Florida A & M University with CHN values within 0.4% of theoretical values. The compounds were dissolved in filtered (0.22 μ) 1% lactic acid vehicle for all of the animal studies. Haloperidol and apomorphine hydrochloride hemihydrate (APO), were obtained from Sigma-Aldrich. Haloperidol and aripiprazole were dissolved in filtered (0.22 μ) 1% lactic acid. APO was dissolved in HPLC grade water, followed by ascorbic acid (0.1% w/w) dissolution followed by sodium chloride (0.9% w/w) on the morning of the experiments in an amber vial. Lactic acid (ACROS), ascorbic acid, phosphate buffered saline (PBS) (Fisher) and sodium chloride (Fisher) were ACS reagent grade). The d-amphetamine hemisulfate salt was from Sigma-Aldrich, and the water used to make solutions was HPLC grade. Diethyl ether was from Fluka, residue analysis grade. The acetic acid, water, methanol, and acetonitrile were HPLC grade. The sodium sulfate was analytical reagent grade. Doses are expressed as the free base for all compounds except SYA013 which is the HCl salt, and were given in a volume of 10 mL/kg by intraperitoneal (ip) injection, except for apomorphine, which was given by subcutaneous (sc) injection. The HPLC internal standard DS-49 (CLog *P* = 5.17) used for calibration was also synthesized at Florida A & M University (Sikazwe et al., 2004) with CHN values within 0.4% of theoretical values as determined by CHN analysis.

The CLog *P* values in this report were calculated using ChemDraw Ultra, version 11.0.1 obtained from CambridgeSoft.

2.3. Animal behavioral experiments

All behavioral experiments were conducted under standard temperatures (68–79 °F) and humidity (30–70%) in the animal care facility.

2.3.1. Reversal of apomorphine-induced climbing

A modified climbing test (Needham et al., 1996; Protais et al., 1976) was used with Swiss-Webster mice to predict potential antipsychotic activity. Inhibition of the apomorphine-induced climbing indicates antipsychotic properties. Five mice per dose were injected first with haloperidol, SYA16263, or vehicle and returned to their home cage, then 30 min later injected with 1.5 mg/kg (as free base) apomorphine, and placed in cylindrical wire cages (12 cm diameter, 14 cm height) and observed for climbing behavior at 10 and 20 min after the apomorphine injection. Climbing behavior was assessed as follows: For all 4 paws up on the cage wall, not on the floor, a score of 2 was assigned (0% inhibition). For 3 or 2 paws on the cage wall, a score of 1 was assigned. For 1 or 0 paws on the cage wall, a score of 0 was assigned (100% inhibition). Scores were expressed as mean percent climbing inhibition, and plotted in Fig. 4. Prism 5.03, GraphPad software, Inc., non-linear regression software was used to calculate ED_{50}s.

2.3.2. Catalepsy bar test

To assess the potential for extrapyramidal side effects (EPS) in humans, the catalepsy bar test was used with rats (Kleven et al., 2005) utilizing a semi-automated instrument from Med Associates, Inc., St. Albans, VT (Bricker et al., 2014). We have used a second test of catalepsy that is reported to be sensitive to the anticataleptic actions of $5HT_{1A}$ receptor agonists, the crossed-legs position (CLP) test (Depoortere et al., 2007; Kleven et al., 2005). A righting test (McCreary et al., 2007; Reeve et al., 1992) was performed following the two catalepsy tests to check for sedation or other effects.

Rats were injected ip, then 60 min later, tested in the CLP test for 30 s, followed by the bar test for 30 s, followed by the righting test, then returned to their home cage. This was repeated at 3 and 6 min from the start of the first trial. Five rats were used for each dose. The mean of the 3 trials was used as the catalepsy response for each test for each rat (Depoortere et al., 2007; Kleven et al., 2005).

2.3.3. Catalepsy crossed-legs position (CLP) test

Rats were place on the stainless steel floor of the catalepsy test chamber, the abdomen toward the floor, and the hind paws brought forward and the front paws backward so that the ipsilateral hind paws could hold onto the top of the front paws and the time (measured with a stopwatch) the rat stayed in this position with either one or both sets of paws was recorded up to 30 s (Depoortere et al., 2007; Kleven et al., 2005).

2.3.4. Righting test

Rats were placed gently on their back and observed immediately following the bar and CLP tests. If the rat did not stay in this position, and flipped over without assistance it was scored as “righted” (McCreary et al., 2007; Reeve et al., 1992).

2.4. Statistical analyses

All statistical analyses were performed using Prism 5.03, GraphPad Software Inc. All error bars are standard error of the mean (SEM). One-way ANOVA with Dunnett's post tests were used, with *, **, and *** indicating $p < 0.05$, 0.01, and 0.001 respectively compared with the vehicle group.

2.5. Receptor binding and GloSensor cAMP functional assays

Protocols for D_2, and other receptor radioligand binding assays and GloSensor cAMP functional assays for G_i- or G_s-coupled GPCRs are available at the National Institute of Mental Health Psychoactive Drug Screening Program Web site, verified 12-7-18: https://pdspdb.unc.edu/pdspWeb/content/PDSP%20Protocols%20II%202013-03-28.pdf.

2.6. β-Arrestin Tango assay

Recruitment of β-arrestin to agonist stimulated D_2R or $5\text{-}HT_{1A}$ were performed using the “Tango”-type assay described in Barnea (Barnea et al., 2008), with modifications, to evaluate SYA16263 and SYA16264. Briefly, HTLA cells stably expressing β-arrestin-TEV protease and a tetracycline transactivator-driven luciferase were plated in 15-cm dishes in DMEM containing 10% FBS and transfected with D2 or $5\text{-}HT_{1A}$ Tango construct (PMID25895059). The next day the cells were plated and challenged with the reference agonist or test ligand (dose-responses with 16 concentrations). After 18 h, the medium was removed and replaced with BriteGlo reagent (Promega), and luminescence per well was read using a TriLux plate reader. Data were normalized to vehicle (0%) and quinpirole (100%) controls and regressed using the sigmoidal dose-response function built into GraphPad Prism 5.0. The results are reported in Table 2.

2.7. Pharmacokinetic analyses

2.7.1. Sample preparation for HPLC-PDA

Plasma and brain tissues were stored at −20 °C until ready for extraction, and then prepared and extracted as described in (Sampson et al., 2014; Shimokawa et al., 2005). Briefly, 0.5 mL rat plasma plus internal standard was made basic with 100 µL 2 M NaOH and extracted with diethyl ether. Supernatant was dried under nitrogen and then reconstituted in 200 µL of mobile phase. Centrifugation at 10,000*g* afforded a supernatant for HPLC injection. Frozen rat brains were homogenized with 2 parts phosphate buffered saline (PBS), sonified, and internal standard added to 1 mL (~1 g) of homogenate which was then extracted with acetonitrile, centrifuged, and the supernatant evaporated under nitrogen. Residue was mixed with water and sodium hydroxide, extracted with diethyl ether, dried under nitrogen, and reconstituted with 200 µL of mobile phase. After centrifugation at 10,000*g*, the extract was injected into the HPLC.

2.7.2. HPLC conditions

As reported in (Sampson et al., 2014; Shimokawa et al., 2005), reversed phase HPLC with PDA detection at 254 nm was used to analyze the extracts. The mobile phase was 55% methanol 45% (0.01 M sodium sulfate in 4% *v*/v acetic acid) at a flowrate of 1 mL/min, and run time of 15 min. Retention time and PDA spectra were used to identify analytes.

2.7.3. Determination of SYA16263 levels in plasma and brain samples

A single bolus dose of 50 mg/kg SYA16263 (12.9 × ED_{50}) was given by IP injection to an average of 4 rats per time point group, and blood and brains collected at 5, 10, 15 and 30 min, 1 h, 2 h, 3 h, 6 h, 9 h, and 24 h. For control purposes, vehicle was injected into 4 rats as well. The internal standard method was used to correct for % recoveries. Calibration lines were calculated using linear regression from 7 to 8 points, and had coefficients of determination of 0.9988 or better.

3. Results and discussion

3.1. Biochemistry studies

3.1.1. Evaluation of chemical stability in plasma

The chemical stability of SYA16263 in rat plasma incubated at 37 °C was evaluated prior to the in vivo studies in order to ascertain that the drug remained intact during the experiments.

HPLC analysis indicated no change in concentration over a 24 h period for a 0.71 mg/mL preparation. One-way ANOVA indicated no significant difference [$F(2,6) = 0.4361$; $p > 0.05$] between concentration means at 0, 2 and 24 h time points (Fig. 1).

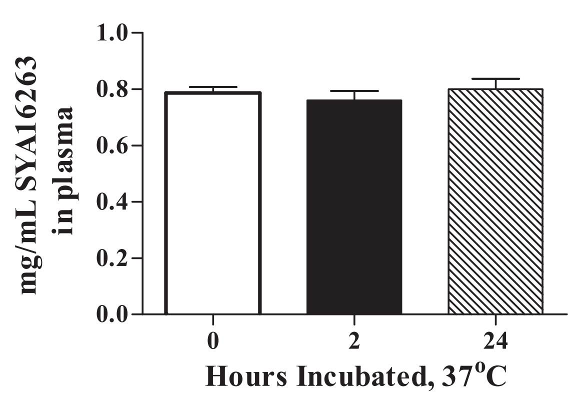

Fig. 1. Chemical stability of SYA16263 in plasma. No significant difference in means (one-way ANOVA), $n = 3$ was observed. Error bars are SEM.

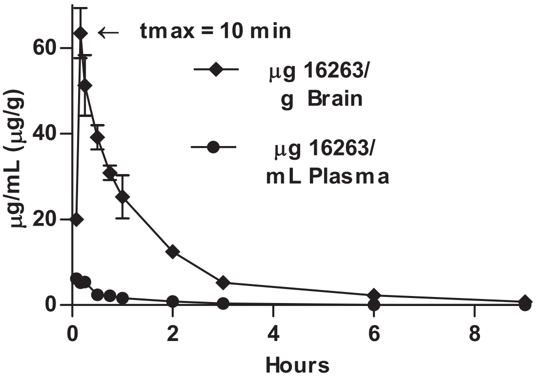

Fig. 2. Time profile of plasma and brain concentrations of SYA16263 in a rat, n = 3–4 rats/group. Error bars are SEM.

3.1.2. Brain uptake of SYA16263: Plasma and brain concentrations of SYA16263

Log [brain]/[plasma] (Log BB) at C_{max} is a predictor of the degree of drug incorporation into the brain (Palenicek et al., 2011) and a value > 0.3 predicts compounds will readily cross the blood brain barrier (BBB) (Olsen et al., 2008; Vilar et al., 2010). To evaluate the brain permeability of SYA16263, a time profile of the plasma and brain concentrations was conducted after IV injections of 50.0 mg/kg using previous methods (Bricker et al., 2012). As shown in Fig. 2, SYA16263 enters the brain quickly with t_{max} = 10 min. In addition, the brain levels significantly exceeded those in the plasma with log BB = 1.08 (log BB of haloperidol is 1.34) indicating a high degree of brain permeability.

3.2. Neurochemistry studies I of the CNS: Radioligand receptor binding studies

3.2.1. Prediction and comparison of the potential of compounds to exhibit antipsychotic properties in animal behavioral studies: Binding of analogs at CNS receptors

We have previously synthesized SYA16263 and several haloperidol analogs and screened them at CNS receptors including D_2R, $5HT_{1A}$, $5HT_{2A}$, $5HT_{2C}$, $5\text{-}HT_7$ and H_1 receptors (Peprah et al., 2012) (Table 1) due to their relevance in antipsychotic drug discovery. The structures of key compounds discussed in this article are presented in Fig. 3. The compounds were found to bind to the D_2R with moderate affinity and with varying affinities at the other receptors. Of particular note is the high affinity of SYA16263 at the $5HT_{1A}R$ which may suggest a potential to overcome EPS and to contribute to the treatment of the negative and cognitive symptoms of schizophrenia (Bardin et al., 2006; Broekkamp et al., 1988; Christoffersen and Meltzer, 1998; Diaz-Mataix et al., 2005; Erhart et al., 2006; Goff et al., 1991; Ichikawa and Meltzer, 2000; Invernizzi et al., 1988; Kapur and Remington, 1996; Kleven et al., 2005; Leucht et al., 1999; Liebman et al., 1989; Moss et al., 1993; Rollema et al., 2000; Rollema et al., 1997; Sprouse et al., 1999). In addition to its affinity to D_2R and $5HT_{1A}R$, SYA16263 also binds with high affinity to the D_4R, and moderately to D_3R and $5HT_7R$ and does not bind significantly to $5HT_{2C}R$ or M_1R-M_5R. These observations are noteworthy since these latter two receptors are associated with weight gain (Kroeze et al., 2003; Matsui-Sakata et al., 2005; Opgen-Rhein et al., 2010) and cognitive deficits (Anagnostaras et al., 2003; Seeger et al., 2004) respectively. SYA16263 also binds about 100-fold more poorly to H_1R compared to clozapine, suggesting a lower propensity to induce sedation and/or weight gain.

3.3. Behavioral studies

3.3.1. Evaluation of haloperidol, SYA16263 and SYA013 in an in vivo mouse model of schizophrenia, reversal of apomorphine-induced climbing

Based on its binding profile in comparison to clozapine (Table 1), SYA16263 was selected for evaluation in a mouse model of the positive symptoms of schizophrenia (Needham et al., 1996; Protais et al., 1976) and the results are reported in Fig. 4. SYA16263 exhibited dose dependent antagonism to apomorphine climbing behavior in mice, with higher doses producing greater inhibition [$F(6, 28) = 24.53$; $p < 0.0001$]. The results were also compared to those of haloperidol [$F(6, 28) = 13.09$; $p < 0.0001$] and SYA013 [$F(7, 42) = 6.495$; $p < 0.0001$] which were previously reported by our lab (Ablordeppey et al., 2008; Sampson et al., 2014). The results suggest that SYA16263 is predicted to produce antipsychotic-like effect as expected, by its inhibition of APO-induced climbing behavior (ED_{50} = 3.88 mg/kg, 95% CI 3.03–4.97, n = 5 mice per dose).

3.3.2. Evaluation of SYA16263, haloperidol, SYA013, and aripiprazole for induction of catalepsy in rats

Most FGAs produce undesirable EPS in humans which manifests as catalepsy in animals. This occurs in addition to their antipsychotic actions, presumably as a result of blockade of D_2R. However, compounds such as aripiprazole do not produce significant catalepsy, presumably as a result of their activation rather than blockade of the D_2R (Bardin et al., 2006; Broekkamp et al., 1988; Christoffersen and Meltzer, 1998; Goff et al., 1991; Invernizzi et al., 1988; Kleven et al., 2005; Liebman et al., 1989; Moss et al., 1993; Rollema et al., 2000; Rollema et al., 1997).

Thus, an evaluation of the potential of an agent to induce catalepsy in animals is a required step in the development of new antipsychotic agents. When SYA16263 was evaluated in rats for catalepsy induction, there was an absence of catalepsy (< 20 s on bar) even up to 19 times its ED_{50} value (75 mg/kg), suggesting it may have a unique mechanism of action. The results are shown in Fig. 5 for SYA16263 [$F(6, 32) = 2.736$; $p < 0.05$] along with the standards, haloperidol [$F(6, 45) = 66.37$; $p < 0.0001$], SYA013 [$F(3, 49) = 0.9125$; $p > 0.05$], and aripiprazole [$F(2, 29) = 21.09$; $p < 0.0001$]. All animals righted themselves following each test.

We have also evaluated the potential of SYA 16263 to induce catalepsy in the CLP catalepsy test to confirm the absence of catalepsy. As shown in Fig. 6, while catalepsy produced by haloperidol [$F(4, 20) = 32.25$; $p < 0.0001$] and aripiprazole [$F(2, 12) = 11.30$; $p < 0.01$] showed a dose dependent trend of catalepsy in this test, SYA

Table 1
*Binding affinities [Mean K_i (nM) n = 3/4 experiments] of SYA16263, SYA013, and several standard antipsychotics at relevant CNS receptors.

Compound	$5HT_{1A}$	$5HT_{2A}$	$5HT_7$	D_1	D_2	D_3	D_4	D_5	$5HT_{2C}$	H_1	M_1
[a]Aripiprazole	50.6	80.7	10.3	1960	30.3	90.7	510	2590	76	25.1	6780
[b]Haloperidol	3600	120	1100	120	10.4	20.5	30.3	147[c]	4700	440	1600
[b]Clozapine	140	80.9	66	290	130	240	54.0	454[d]	17.0	10.8	1.8
SYA013	117	23.6	78.0	163	43.3	159	60.6	NA	1425	189	872
SYA16263	10.1	50.0	90.0	238	124	86	30.5	1451	> 10 K	167	MPA

* All standard errors are within 20% of the mean values indicated. Each result represents the mean of at least two determinations, each in triplicate. Data for aripiprazole, haloperidol and clozapine were obtained from [a]ref (Shapiro et al., 2003), [b]ref (Schmidt et al., 2001), [c]ref (Tyler et al., 2017), and [d]ref (Web ref. 3). MPA = Missed primary assay threshold of 50% inhibition at 10 µM. NA = not available.

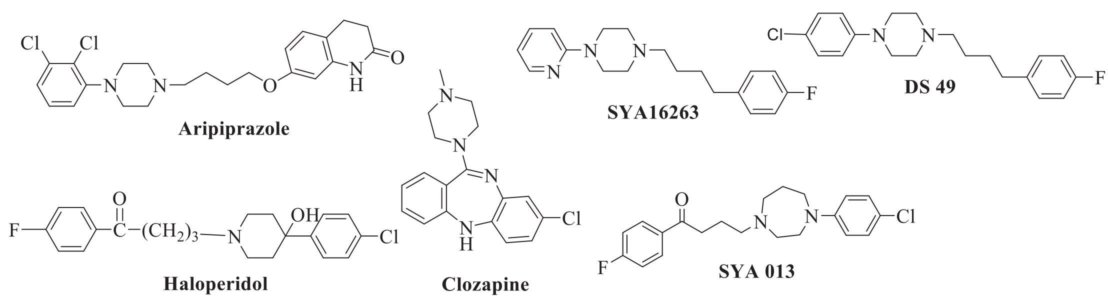

Fig. 3. Structures of key agents discussed in this article.

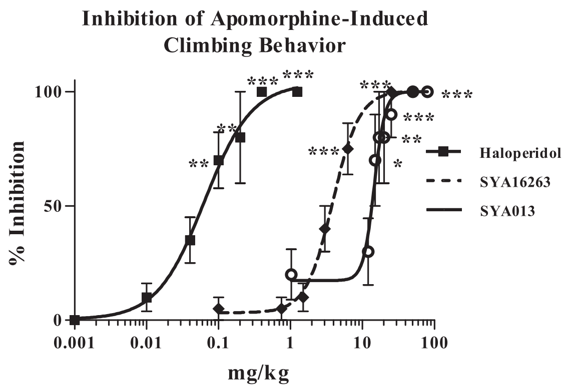

Fig. 4. SYA16263, haloperidol, and SYA013 inhibited APO-induced climbing behavior in mice. $N = 5$ mice per dose. Error bars are SEM. One-way ANOVA with Dunnett's post-tests. *, **, and *** indicate $p < 0.05$, 0.01, and 0.001 respectively compared with the vehicle group.

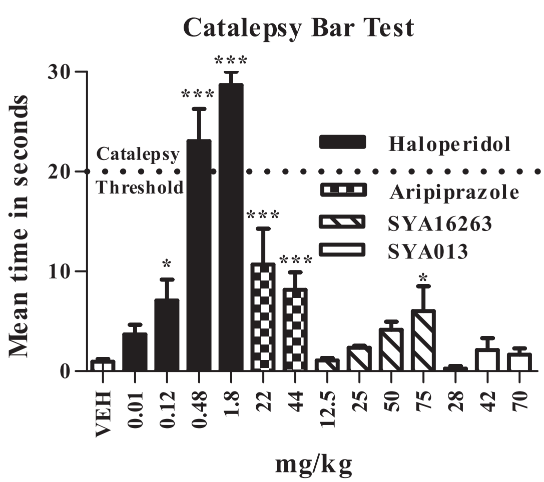

Fig. 5. Haloperidol produced catalepsy in rats at clinical doses while SYA16263, SYA013, and aripiprazole did not (< 20s on bar). In fact, no catalepsy was observed up to 75 mg/kg SYA16263, or at 19× the ED_{50} value of SYA16263. $N = 5$ mice per dose. Error bars are SEM. One-way ANOVA with Dunnett's post-tests. *, **, and *** indicate $p < 0.05$, 0.01, and 0.001 respectively compared with the vehicle group.

16263 failed to elicit any signs of catalepsy up to 19 times its ED_{50} value. All animals righted themselves following each test. It is worthy to note that the aripiprazole catalepsy result is consistent with a meta-analysis (Bernagie et al., 2016) indicating a mean EPS incidence of 17.1 % (95 % CI 0.128–0.223) in pediatric and adolescent patients.

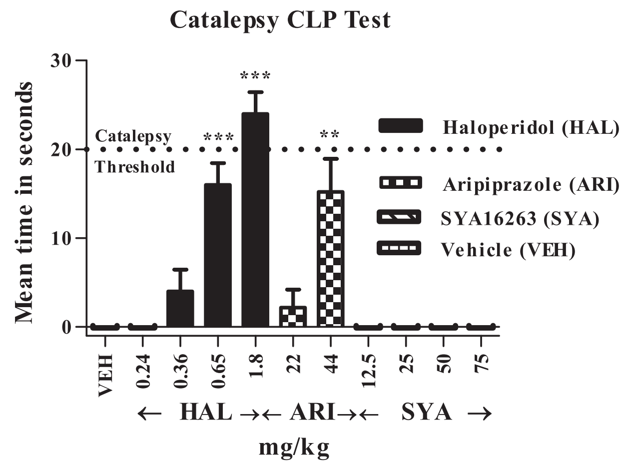

Fig. 6. Haloperidol produced catalepsy in the CLP test (> 20 s position maintained) in rats while aripiprazole and SYA16263 did not. A mean of zero seconds was observed for vehicle, 0.24 mg/kg haloperidol, and all doses of SYA16263 up to 19 times the ED_{50} value. $n = 5$–9 rats/dose. Error bars are SEM. One-way ANOVA with Dunnett's post-tests. *, **, and *** indicate $p < 0.05$, 0.01, and 0.001 respectively compared with the vehicle group.

3.4. Neurochemistry studies II of the CNS

3.4.1. Evaluation of functional selectivity of SYA16263 and SYA013 compared to aripiprazole

To ascertain whether SYA16263 acts as an agonist or antagonist at the D_2R, a functional assay was carried out on SYA16263 and SYA013 with aripiprazole as the positive control given that functionally selective ligands promoting β-arrestin recruitment to D_2R may produce antipsychotic-like properties without catalepsy (Allen et al., 2011; Chen et al., 2012). Using the D_2R β-arrestin Tango Assay (Barnea et al., 2008; Kroeze et al., 2015) the effects of the compounds on signaling were obtained and are reported in Tables 2 and 3.

3.4.2. β-Arrestin Tango assay at D_2R and absence of catalepsy

Recruitment of β-arrestin-2 to agonist stimulated D_2R was performed using a "Tango" assay (Barnea et al., 2008; Kroeze et al., 2015) to evaluate SYA16263 and SYA013. The results show that SYA16263 acts as an agonist at both the G protein dependent and independent β-arrestin signaling pathways. Interestingly, its functional profile at the D_2R mimics that of aripiprazole albeit superior in its efficacy. For example, while it has only a moderate binding affinity to D_2R [$K_i = 124$ nM], SYA16263 is less potent but more efficacious as an agonist [$EC_{50} = 5.7$ nM; $E_{max} = 64.4\%$] than aripiprazole [$EC_{50} = 1.0$ nM; $E_{max} = 51.0\%$] in activating G_i pathway at the D_2R. Also, while less potent, it is more efficacious in promoting β-arrestin-2 recruitment to D_2R, [$EC_{50} = 10.5$ nM; $E_{max} = 92\%$] than aripiprazole [$EC_{50} = 4.0$ nM; $E_{max} = 62\%$] (Table 2). These results are consistent with the absence of catalepsy observed with SYA16263. On the other hand, SYA013 neither showed significant agonist or antagonist

Table 2
Radioligand binding affinity (K_i) and functional activities at D_2 receptor.
(EC_{50}/IC_{50}, nM; E_{max} %).[a]

CMPD	D_2 binding (K_i nM)	D_2 agonist G_i-cAMP	D_2 antagonist	D_2 Tango agonist	D_2 Tango antagonist
[b]Aripiprazole	(3.3,3.9)	1.0; 51.0	0.0	4.0; 62	0.0
SYA16263	124	5.7; 64.4	0.0	10.5; 92	0.0; −3.8
SYA013	43.3	0.0;16.4	> 10 K; 49.6	0.0	ND

ND = Not determined. Quinpirole is the positive controls for D_2R. Tango assays are for β-arrestin-2 recruitment.
[a] Results from NIMH PDSP.
[b] Ref (Chen et al., 2012; Shapiro et al., 2003).

Table 3
Radioligand binding (K_i, nM) and functional activities at 5-$HT_{1A}R$.
(EC_{50}/IC_{50}, nM; E_{max} %)[a].

CMPD[a]	5-$HT_{1A}R$ binding (K_i nM)	Agonist G_i-cAMP	Antagonist	Tango agonist	Tango antagonist
Methysergide	25.0	ND	ND	ND	ND
SYA16263	1.1	0.0; −23.7	0.0	149; 54.4	0.0
SYA013	117.4	0.0;4.4	0.0; 6.5	ND	ND

ND = Not determined. Methysergide is the positive control for 5-$HT_{1A}R$. Tango assays are for β-arrestin-2 recruitment.
[a] Results from NIMH PDSP.

properties at the D_2R and thus, the results have not provided an explanation as to why SYA013 produces antipsychotic-like activity and has limited catalepsy. Further experiments to evaluate the functional behavior of these compounds at other D_2-like and 5-HT receptors are planned to elucidate the basis of these observations.

3.4.3. *Functional selectivity at the 5-HT_{1A} receptor*

The results of functional evaluation of SYA16263 at 5-$HT_{1A}R$ showed that it does not activate G protein coupled $5HT_{1A}R$ signaling [EC_{50} = 0; E_{max} = −23.7] and instead, shows extreme bias in β-arrestin recruitment to the $5HT_{1A}R$ [IC_{50} = 149 nM; E_{max} = 54.4%, Table 3]. The implication of this unique signaling is under further investigation.

4. Conclusion

In summary, we have identified 1-(4-(4-fluorophenyl)butyl)-4-(pyridin-2-yl)piperazine or SYA16263 as a D_2R ligand with an ED_{50} value of 3.88 mg/kg, in reversing APO-induced climbing behavior in mice. Evaluation of catalepsy induction indicated that SYA16263 does not produce catalepsy even at greater than 19 times its ED_{50} value. Functional analysis revealed SYA16263 activates both G-protein and β-arrestin signaling at the D_2R, thus suggesting that SYA16263 has a signaling profile similar to that of aripiprazole at the D_2R. However, SYA16263 binds with a higher affinity at the $5HT_{1A}R$ than at the D_2R and functional evaluation at the 5-$HT_{1A}R$ demonstrated SYA16263 selectively activates β-arrestin recruitment to the $5HT_{1A}R$ and spares any significant interaction at the G-Protein dependent signaling pathway. The studies herein support this unique mechanism of action, making SYA16263 a potential antipsychotic to be further studied.

Author information

BAB contributed to experimental design, collected, analyzed and interpreted the in vivo studies data and the writing of the manuscript. K.P. synthesized the compounds tested in this manuscript. H.D.K. contributed to the functional assays under the supervision of the Director of the NIMH PDSP, Prof Bryan L Roth. SYA originated the ideas expressed in the manuscript, contributed to the experimental design, analyzed and interpreted the data, wrote and edited the manuscript.

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