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Evaluation of the eutomer of 4-{3-(4-chlorophenyl)-3-hydroxypyrrolidin-1-yl}-1-(4-fluorophenyl)butan-1-one, {(+)-SYA 09}, a pyrrolidine analog of haloperidol

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Abstract—Enantiomeric separation of the racemic 4-{3-(4-chlorophenyl)-3-hydroxypyrrolidin-1-yl}-1-(4-fluorophenyl)butan-1-one, a pyrrolidine analog of haloperidol, {(±)-SYA 09}, and subsequent binding studies revealed that most of the binding affinity at dopamine and serotonin receptors resides in the (+)-isomer {(+)-SYA 09} or the eutomer. Further pharmacological evaluation of the eutomer revealed that it has a higher affinity for the dopamine D4 (DAD4) receptor subtype (K_i = 3.6 nM) than for the DAD2 subtype (K_i = 51.1 nM) with a ratio of 14.2 (D2K_i/D4K_i ratio = 14.2). In an animal model of antipsychotic efficacy, the (+)-SYA 09 was efficacious with an ED_{50} value of 1.6 mg/kg, ip, and at twice this value, (+)-SYA 09 did not induce significant catalepsy in rats.

Previous studies in several laboratories including ours[1–3] have revealed that haloperidol is converted to quaternary pyridinium metabolites ($BCPP^+$ and $RHPP^+$) that, based on its structural similarity to MPP+, may possess the potential to induce irreversible Parkinsonism-like side effects. Consequently, we[4] have hypothesized that agents possessing binding profiles similar to that of haloperidol but lacking the structural features required to form quaternary pyridinium metabolites should produce little or no long-term Parkinsonism-like side effects.

In designing agents that could not undergo metabolic transformation to pyridinium species, several compounds were synthesized including the tropane analog[5] (**1**) and the pyrrolidine analog[4,6] (**2**) of haloperidol. Subsequent pharmacological evaluation of **1**, however, showed that its capacity to induce acute catalepsy in animal models is identical to that of haloperidol.[5] On the other hand, compound **2** which could not undergo similar metabolic transformation but binds with only moderate affinity to DAD2 receptors showed significantly reduced extrapyramidal reaction in an animal model.[4] Thus, the notion that haloperidol's acute catalepsy may be associated with its high DAD2 binding affinity is supported by the observation.

Others[7–9] have suggested that the low affinity binding of clozapine (**3**) at DAD2 receptors and the higher binding affinity at DAD4 receptors are an important element in its superior therapeutic action in treating schizophrenia. Hence, the identification of SYA 09 (**2**) with moderate binding affinity for the DAD2 receptor and a D2 to D4 binding profile {D2, K_i = 33 nM; D3, K_i = 200 nM; and D4, K_i = 11 nM; [K_i(D2)/K_i(D4)] = 3.0}[4] similar to that of clozapine[10] {D2, K_i = 130 nM; D3, K_i = 240 nM; and D4, K_i = 54 nM; [K_i(D2)/K_i(D4) = 2.4]} was considered significant. Unlike clozapine or haloperidol however, compound **2** has a chiral center, and thus is a mixture of two enantiomers. The purpose of this study, therefore, was to synthesize, separate the enantiomers of **2**,

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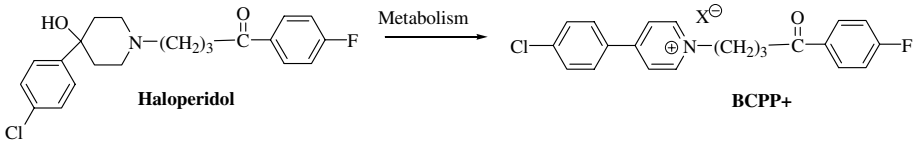
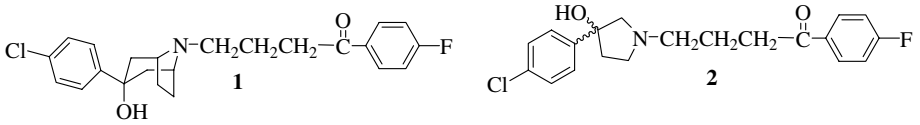
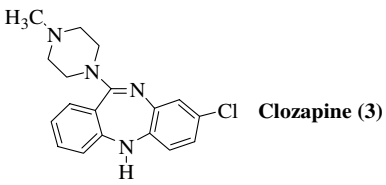




evaluate the eutomer's in vitro and in vivo pharmacological profile, and to compare the activities to that of clozapine, compound **1**, and haloperidol.

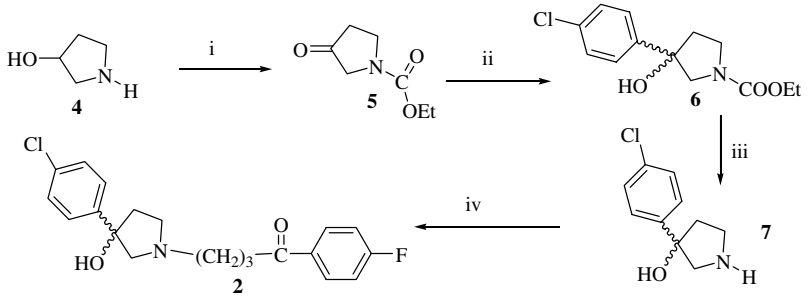


Clozapine (3)

The synthetic procedures for obtaining **1** and **2** are similar and were previously reported.[4–6] Briefly, the procedure for obtaining compound **2** requires a carbamate-protected ketone (**5**) as the key intermediate. This intermediate was obtained by carbamylation of commercially available pyrrolidin-3-ol (**4**) and oxidation to the carbamate-protected pyrrolidinone (**5**). Reaction of **5** with freshly generated 4-chlorophenyl magnesium bromide produced a carbamate-protected aminoalcohol (**6**). After decarbamylation in alcoholic KOH, the resulting intermediate (**7**) was alkylated with the commercially available fluorobutyrophenone chloride to obtain the desired product (Scheme 1). Compound **1** was similarly obtained starting with carbamate-protected tropanone as previously reported.[5]

Separation of the enantiomers of **2** was achieved on a Chiralpak AD-H column from Chiral Technologies, Inc. Column dimensions were 250×4.6 mm. Mobile phase was a volume ratio of 70:25:5 hexane/EtOH/*i*-PrOH at a flow rate of 1 ml/min. The separation was monitored at 254 nm and 10–20 μl of 30–65 mg/ml racemate solution was injected. Two fractions were collected and their purities were verified by re-injecting isolated fractions into the HPLC system and examining the chromatograms as shown in Fig. 1.

Chiral chromatographic separation of racemic compound **2** was accomplished in high enantiomeric purity (**2a** = 100% and **2b** > 95%) as shown in Figure 1. The specific rotation, [α] at 25 °C, of the (−)-enantiomer (t_R = 13.5 min) is −8.1 and the (+)-enantiomer (t_R = 18.3 min) is +8.9. The binding profiles of these enantiomers along with haloperidol, clozapine, and compound **1** are presented in Table 1. These results indicate that the (+)-SYA 09 binds with higher affinity at both DA and 5HT receptors investigated, while (−)-SYA 09 binds with a higher affinity at α and H-1 receptors. Thus, the (+)-isomer is the active enantiomer or eutomer at dopamine and serotonin receptors. (+)-SYA 09 also binds with a higher affinity at the D4 subtype than that at the D2 subtype with a ratio of 14.2 ($D2K_i$ = 51.1 nM and $D4K_i$ = 3.6 nM; $D2K_i/D4K_i$ = 14.2). This ratio is different from that of the racemic mixture ($D2K_i/D4K_i$ = 3.0) but is consistent with our proposed hypothesis[4] that a ratio greater than



Scheme 1. Synthesis of the racemic mixture of the pyrrolidine analog (**2**). Reagents and conditions. (i) a—ClCOOEt; b—$K_2Cr_2O_4$; (ii) 4-Cl-C_6H_4MgBr, Et_2O (anhydrous), N_2, reflux, 12 h, 42%; (iii) KOH, EtOH, reflux, 73%; (iv) 4-chloro-4′-fluorobutyrophenone, KI, K_2CO_3, DME (anhydrous), N_2, reflux, 12 h, 23%.

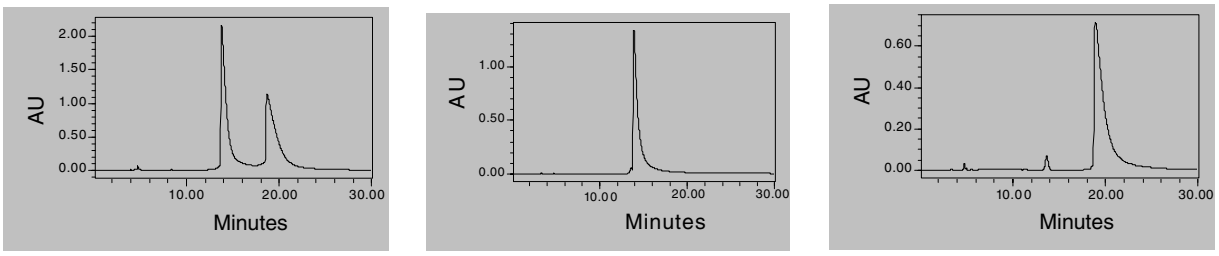


Figure 1. Chromatograms of the chiral separation of (±)-SYA 09, (−)-SYA 09, and (+)-SYA 09, respectively. Mobile phase: 70:25 hexane/EtOH/*i*-PrOH.

Table 1. Binding affinity data for (+) and (−)-SYA 09, compound **1**, clozapine, and haloperidol

Receptor	(+)-SYA 09 K_i (nM) mean	(−)-SYA 09 K_i (nM) mean	Compound **1** K_i (nM) mean	Clozapine K_i[a] (nM) mean	Haldol K_i[a] (nM) mean
hDA D2	51.1 ± 6.0	489 ± 119	1.6 ± 0.1	130	2.0
hDA D3	1069.0 ± 292	1177 ± 149	5.1 ± 1.4	240	12.0
hDA D4.4	3.6 ± 0.5	245 ± 29	5.3 ± 0.1	54	15.0
h5-HT1A	722.3 ± 90	832 ± 126	27.7 ± 6.0	140	1202
h5-HT2A	75.8 ± 12	241.5 ± 41.8	30.9 ± 3.0	8.9[a]	435
h5-HT2C	3598.0 ± 162	1252 ± 461	872.1 ± 178	17.0[a]	>5.454
hα-1A	114.8 ± 7.0	34 ± 4	39.3 ± 6.6	1.6	12.0
hα-1B	102.4 ± 8.0	41.7 ± 1.3	42.7 ± 1.4	7.0	8.0
hα-2A	2239.0 ± 403	5817 ± 419	2835.0 ± 220	142	473.0
hα-2B	4559.0 ± 1075	1543 ± 85	515.5 ± 100.5	27.0	480.0
hα-2C	94.3 ± 14.4	16.4 ± 4.2	142.8 ± 12.7	34.0	550.0
hHis H1	1467.0 ± 473	259.6 ± 34.0	8780.0 ± 1625	1.8	3002.0

[a] Data obtained from Ref. 10 and/or kidb.case.edu.

one (D2K_i/D4K_i = >1) and a D2 receptor binding within a K_i range of 30–150 nM are therapeutically desirable.

Further evaluation of the binding profile of (+)-SYA 09 at 5HT and α-adrenergic receptors was carried out in order to compare its profile with that of clozapine. The D2K_i/5HT2A K_i ratio (D2K_i = 51.1 nM and 5HT2A K_i = 75.8 nM) was found to be 0.67 compared to that of clozapine's ratio of 24.1 and indicates a significant difference in binding at these receptors. In addition, (+)-SYA 09 binds poorly to 5HT1A (K_i = 722 nM), 5HT2C (K_i = 3598 nM), and H-1 (K_i = 1462 nM) receptors, while clozapine binds with moderate affinities at 5HT1A and 5HT2C receptors (K_i = 105 nM and K_i = 29 nM) (Table 1), and with high affinity at H-1 receptors (1.8 nM).[10] Interestingly, compound **1**, which was previously shown to induce catalepsy in rats,[4] binds with a much higher affinity at 5HT1A (K_i = 27.7 nM) and 5HT2A (K_i = 30.9 nM) receptors. These receptors have been shown to play significant roles in the superior therapeutic profile of atypical antipsychotics such as aripiprazole.[11] The low affinity of (+)-SYA 09 for the H-1 receptor is therapeutically advantageous since the H-1 receptor is implicated in weight gain associated with atypical antipsychotic medication.[2]

(+)-SYA 09 was also subjected to evaluation in animal models for antipsychotic efficacy. When given by ip injection, (+)-SYA 09 was found to be effective in inhibiting apomorphine-induced climbing with an ED_{50} of 1.6 mg/kg compared to that of clozapine and haloperidol of 5.5 and 0.007 mg/kg, respectively. These results are consistent with the binding affinities of these agents at DAD2 receptor subtype supporting the critical role played by D2 receptors in these animal models for testing antipsychotic efficacy. It was also of interest to evaluate the potential of (+)-SYA 09 to induce catalepsy in rats. Lack of catalepsy combined with inhibition of apomorphine-induced climbing would indicate that the compound is efficacious and is unlikely to induce extrapyramidal side effects (EPS) in man. The results are shown in Figure 2. These results confirm the previous observation that racemic **2** does not induce catalepsy to the same extent as haloperidol or compound **1**[4] at equivalent doses. Indeed, the catalepsy profile of (+)-SYA 09 is similar to those of clozapine at the doses investigated. Since the ED_{50} value (1.6 mg/kg) of (+)-SYA 09 falls within the doses tested for catalepsy (0.1–4.0 mg/kg), this suggests that the compound may not induce EPS at the therapeutic dose in man. Since the common element in receptor binding between (+)-SYA 09 and clozapine is related primarily to their DAD2 binding affinity, it can be deduced that the major pharmacological actions observed are probably due to their D2-like receptor binding profile rather than to their serotonin or alpha receptor profiles.

In this paper, we have synthesized and separated enantiomers of a pyrrolidine analog of haloperidol incapable of undergoing metabolism to pyridinium-type metabolites but possess moderate binding affinity at D2-like receptors. Since haloperidol produces pyridinium-like metabolites with the potential to contribute to some of

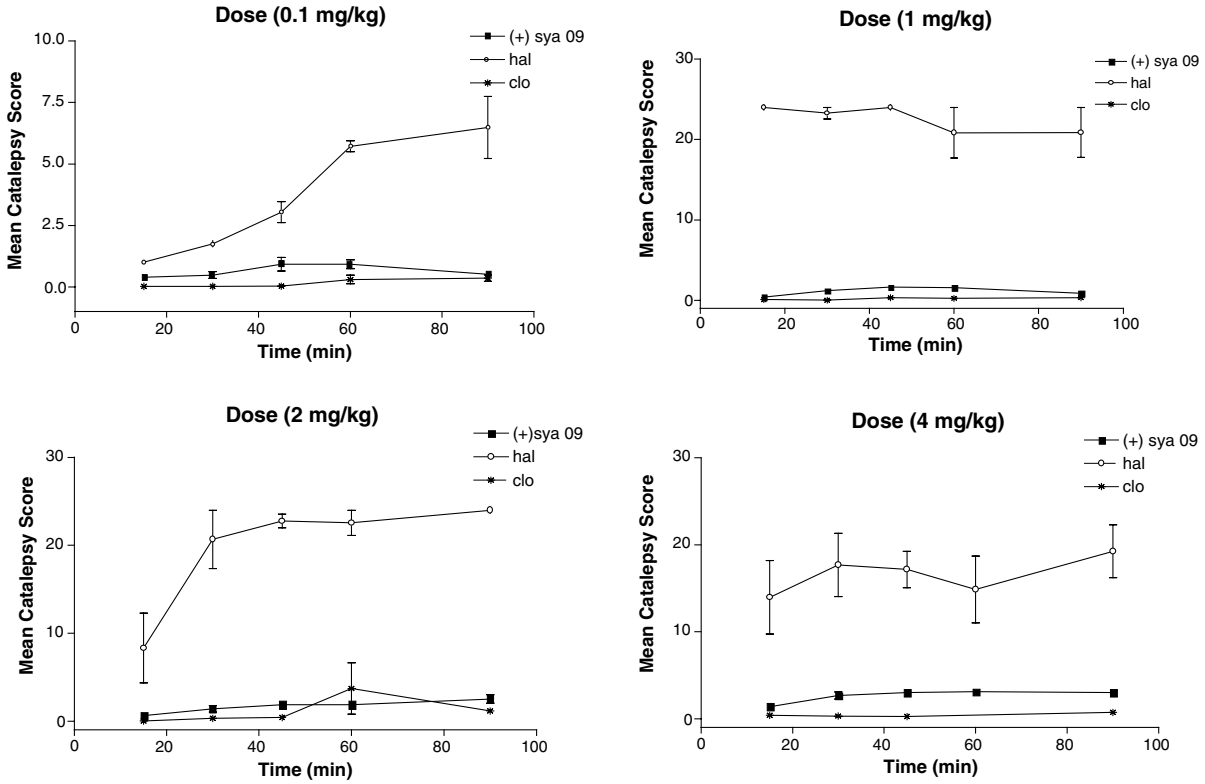


Figure 2. Mean catalepsy scores at various doses and times for (+)-SYA 09, haloperidol, and clozapine.

the long-term side effects through destruction of dopaminergic neurons in the nigrostriatum, (+)-SYA 09 has a potential therapeutic advantage over haloperidol. The fact that (+)-SYA 09 possesses D2/D4 binding affinity ratio greater than 1 and has a behavioral profile with similarities to clozapine is interesting and requires further evaluation in animal models. The hypothesis that low affinity for D2 receptor and $\{K_i(D2)/K_i(D4) > 1\}$ may lead to agents with efficacy against positive schizophrenia and a low propensity to induce movement disorders is thus supported by the observations on (+)-SYA 09.

Radioligand binding studies were performed by the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP) as previously described in Shapiro et al.[11] Briefly, a number of transiently and stably transfected cloned human cDNAs were used for radioligand binding assays as previously detailed in Rothman et al.[14] and Tsai et al.[13] In initial screening assays, compounds were tested at a concentration of 10 μM in quadruplicate at all receptors evaluated.

Inhibition of apomorphine induced climbing-stereotypy was determined using a modified climbing test by Needham et al.[12] Swiss male mice (20–25 gm, $N = 125$) in groups of five per time point (30 min, 1, 2, 4, and 6 h) were injected ip with 1.0 ml/100 g of vehicle (0.1% lactic acid and 0.9% saline) or increasing milligrams per kilogram equivalent doses of dopamine antagonists haloperidol, (+)-SYA 09, and clozapine. Animals were then challenged with 2.8×10^{-6} mol/kg of the agonist apomorphine, placed in cylindrical wire cages (12 cm in diameter, 14 cm in height), and observed for climbing behavior at 10 and 20 min post-dose. Climbing behavior was assessed as follows: 4 paws on the cage floor = 0 score; 2 or 3 paws on the cage = 1 score; 4 paws on the cage = 2 scores. Scores were expressed as mean percentage of climbing inhibition and the ED_{50} calculated (Table 2).

Catalepsy was determined using a modified bar test by Needham et al.[4,12] Male SD rats (60–100 gm, $N = 100$) were injected ip with 1 ml/kg of vehicle (<0.5% acetic acid in H_2O) or increasing doses of haloperidol, (+)-SYA 09, and clozapine (i.e., 0.1–4.0 mg/kg). Catalepsy severity was assessed immediately at various time points (15, 30, 45, 60, and 90 min) post-injection, by scoring how long the rat maintained both forepaws motionless on a horizontal metal bar (1.1 cm in diameter, 10 cm above the bench top in a box). A score of 1 was given for every 5 s (2 min maximum) the animal remained on the bar. Mean scores from five animals per time point were recorded for catalepsy (Fig. 2).

Table 2. Inhibition of apomorphine-induced climbing in Swiss–Webster mice by (+)-SYA 09, the eutomer of SYA 09

Drug	ED_{50} (mg/kg ip)
Haloperidol	0.007 ± 0.001
(+)-SYA-09	1.60 ± 0.70
Clozapine	5.54 ± 1.25

Each ED_{50} value was derived from a separate dose–response curve, using four doses and a vehicle-treated apomorphine control group; $n = 5$ mice/dose. ED_{50} values are expressed as free base equivalents.

The Student t test was used to compare the compounds used in the animal behavioral tests. Results were considered significant at $p < 0.05$.

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