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Further Evaluation of the Tropane Analogs of Haloperidol

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Abstract

Previous work from our labs has indicated that a tropane analog of haloperidol with potent D_2 binding but designed to avoid the formation of MPP+-like metabolites, such as 4-(4-chlorophenyl)-1-(4-(4-fluorophenyl)-4-oxobutyl)pyridin-1-ium (BCPP+) still produced catalepsy, suggesting a strong role for the D_2 receptor in the production of catalepsy in rats, and hence EPS in humans. This study tested the hypothesis that further modifications of the tropane analog to produce compounds with less potent binding to the D_2 receptor than haloperidol, would produce less catalepsy. These tests have now revealed that while haloperidol produced maximum catalepsy, these compounds produced moderate to low levels of catalepsy. Compound **9**, with the least binding affinity to the D_2R, produced the least catalepsy and highest Minimum Adverse Effective Dose (MAED) of the analogs tested regardless of their affinities at other receptors including the 5-HT_{1A}R. These observations support the hypothesis that moderation of the D_2 binding of the tropane analogs could reduce catalepsy potential in rats and consequently EPS in man.

Keywords

Haloperidol; Tropane Analogs; Antipsychotic agents; Catalepsy; Pyridinium metabolite

Haloperidol is an effective antipsychotic medication that is used to treat schizophrenia; however, it is associated with producing short and long term extrapyramidal side effects (EPS), i.e., Parkinsonism-like symptoms. These adverse effects may be due to its potent binding at the D_2 receptor and/or its metabolism into quaternary pyridinium species, such as $BCPP^+$ and $RHPP^+$ [1,2,3,4] (Fig 1).

Previous studies in our laboratories have shown that modifications in the butyrophenone and the piperidine moieties of haloperidol significantly altered its binding affinities at the D_2,

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D_4, and 5-HT_{1A} receptors.[1,5,4] Modifications of the piperidine ring resulting in D_2 receptor binding profiles that are similar or better than haloperidol and which could not form toxic pyridinium metabolites, were also identified.[4,6,7] These studies have indicated that adding an ethylene bridge to the piperidine moiety to form a tropane analog, maintained or increased affinity for the D_2 receptor subtype. It was also observed that replacement of the carbonyl functional group in haloperidol with an oxygen or a sulfur atom retains D_2 binding and increases affinity at the 5-HT_{1A} receptor, indicating that the carbonyl group is not essential for binding at the D_2R but can be replaced with other groups to produce compounds that bind with high affinity to the receptors associated with antipsychotic properties.[8]

While the tropane analog of haloperidol (**5**) appears to have a better binding profile than haloperidol, in vivo studies in our hands have confirmed that its similar potent antagonism at the D_2R subtype resulted in very significant catalepsy in rats.[5,9] Thus, it was of interest in this study, to further test the hypothesis that moderation of the binding affinity at the D_2 receptor could attenuate catalepsy in the tropane analogs of haloperidol. To test this hypothesis, we modified the fluorobutyrophenone moiety while replacing the piperidinol with 3-tropanol to obtain compounds **2-10** as shown in Fig 2 and then evaluated their binding affinities at receptors associated with antipsychotic activity, i.e., D_1 and D_2-like receptors, 5-HT_{1A}R, 5-HT_{2A}R and 5-HT_7R (Table 1). Subsequently, binding affinities at off-target receptors were also evaluated and reported in Table 2.

The syntheses of compounds **2-7** were previously reported.[8–10] The alkylating agent, (3-chloropropyl)(4-fluorophenyl)sulfane, was prepared using the procedure in our previously published paper,[8] with slight modification, by refluxing 4-fluorobenzenethiol and 1-bromo-3-chloropropane in the presence of K_2CO_3 in iPrOH. The other two alkylating agents were similarly prepared by using the corresponding n-fluorobenzenethiol. The target compounds, **8-10** were obtained by alkylating 3-(4-chlorophenyl)-8-azabicyclo[3.2.1]octan-3-ol, in the presence of K_2CO_3, KI and DME with the appropriate alkylating agent as shown in Scheme 1.

The first modifications to moderate the D_2R binding involve replacement of the carbonyl group in haloperidol and the results are reported in Table 1. Deoxygenation (**2**), replacement with oxygen (**3**) or sulfur (**4**) resulted in significant improvements in binding to the 5-HT_{1A}, D_1 and D_4 receptors. Affinity however, was decreased at the 5-HT_7R while binding to the D_2 and 5-HT_{2A} receptors remained about the same except for the deoxygenated compound (**2**) which had a 27-fold decrease at the D_2 receptor (Ki = 24 nM, haloperidol Ki = 0.89 nM). The next compounds (**5-8**), the tropane analogs of haloperidol and compounds **2-4,** were also screened at the same receptors and the results are also presented in Table 1. The methylene (**6**) and the oxygen (**7**) analogs had similar binding across all 7 receptors of interest (Table 1); however, their affinities were significantly lower than those of the previously reported carbonyl analog (**5**). The binding affinities of compound **8**, the sulfur analog of **6** and **7**, turned out to be generally more potent at the primary receptors of interest, i.e., D_2, 5-HT_{1A}, 5-HT_{2A} and 5-HT_7 receptors. Thus, compound **8** was selected for further investigation.

Having been aware that replacement of the fluorine atom on the butyrophenone moiety with other substituents resulted in significant changes in binding affinities,[8] we synthesized the other two fluorinated analogs with the fluorine atom located at the *meta* (**9**) and the *ortho* (**10**) positions on the butyrophenone moiety. The analogs were subsequently screened and the results are recorded in Table 1. Compounds **8**, **9** and **10** bind to the 5-HT_{1A}R with similar affinities (Ki = 20.0, 13.0 & 8.2 nM respectively) as expected. At the 5-HT_{2A}R, the binding Ki of the *para*- (**8**) and *ortho*- (**10**) substituted analogs were similar, and 4-6 times more potent that of the *meta*-substituted analog (**9**). Apart from compound **8** which binds with a Ki of 97.0 nM at the 5-HT_7R, both **9** and **10** have about 9-fold weaker affinity. All three analogs, **8-10**, have potent binding at the D_2-like receptors although the *meta*-substituted analog, **9**, displayed an 8-fold lower affinity (Ki = 27.0 nM) at the D_2R subtype. This D_2 binding affinity turned out to be similar to that of the literature reported[12] value for olanzapine (Ki = 20.0 nM). Spurred on by this observation, (albeit superficial since the binding studies were reported from different laboratories) and the potent binding to the 5-HT_{1A}R, purportedly associated with attenuating catalepsy in animal models,[11] we selected compounds **9** and **8** (for comparison**)** for evaluation in animal models predictive of antipsychotic efficacy and EPS side effects.

The compounds were also evaluated for off-target interactions at receptors that may produce side effects such as 5-HT_{2B}, (valvular heart disease) 5-HT_{2C}, (induction of weight gain), H_1, (induction of weight gain and/sedation) M_1-M_3, (cognitive symptoms) DAT, NET and SERT which might interfere with *in vivo* observations. The results reported in Table 2, indicate that all the compounds have relatively low affinities at these receptors and hence are expected to have low potentials to induce weight gain, sedation and other potential side effects associated with these receptors.[13]

To test for a compound's ability to display antipsychotic properties in humans, the inhibition of apomorphine (APO)-induced climbing behavior in mice is evaluated.[14,15] Thus, in this study, the reversal of APO-induced climbing was assessed in Swiss Webster mice given 1.5 mg/kg of APO in a manner consistent with previous reports.[1,5] Doses were expressed as the free base for haloperidol and apomorphine, and as the HCl salt for compounds **8** and **9** and were given in a volume of 10 mL/kg for the highest dose given by intraperitoneal (ip) injection, except for APO, which was given by subcutaneous (sc) injection. Five mice were each dosed, at various concentrations with compounds **8** or **9**, then 30 min later with APO (1.5 mg/kg). Each mouse was subsequently placed in a cylindrical wire cage to assess the climbing behavior. Compound **8,** the *para*-fluoro substituted analog had significant inhibition of the APO-climbing behavior, with an ED_{50} value of 0.165 mg/kg (R^2 = 0.9511) compared to its *meta*-substituted counterpart, **9** (ED_{50} = 3.137 mg/kg; R^2 = 0.9132). These results are consistent with the fact that the *para* analog is more potent than the *meta* analog and possesses APO-induced climbing reversal properties similar to haloperidol (ED_{50} = 0.122 mg/kg) as expected, while **9** is less potent at the D_2 receptor and displayed an equally lower potency in the in vivo model. The results are reported in Fig. 3 and Table 3.

The potential for compounds **8** and **9** to induce catalepsy in rats and hence extrapyramidal side effects in human beings[16,11] was also assessed using the catalepsy bar test as previously reported.[17,18] A secondary evaluation was also made using the crossed-legs position (CLP)

test, which is reported to be more sensitive to the anticataleptic actions of 5-HT_{1A} receptor agonists.[19,11] Five rats at each dose point, for **8** (ED_{50}-9 ED_{50}) or **9** (ED_{50}-5 ED_{50}), were injected i.p. and tested in the CLP test for 30 sec followed by the bar test for 30 sec. A righting test[20,21] was performed immediately after, to assess the potential of the compounds to induce sedation. This was repeated at 3 and 6 minutes after the start of the first trial. The mean of the three trials was used. The results are reported in Fig. 3 and Table 3.

Compound **8** induced catalepsy in both the bar and CLP tests. One-way ANOVA with Bonferroni post tests with $p<0.05$ indicated that there was a significant difference between vehicle (1% lactic acid) and compound 8. The Minimum Adverse Effective Dose (MAED) for the bar test was observed at 0.578 mg/kg or 3.5 times its ED_{50} value for reversal of APO-induced climbing behavior. Since all animals "righted" themselves after the catalepsy tests, catalepsy displayed by compound **8** was not associated with a sedative effect by the compound, which is consistent with its poor binding at the 5-HT_{2C} and H_1 receptors (Ki = 1498 & 977 nM respectively; Table 2). Compound **9** also demonstrated catalepsy with a MAED of 12.7 mg/kg or 4.0 times the ED_{50} of its reversal of APO-induced climbing behavior. Given that the MAED of haloperidol was only 2.0 times its ED_{50} value of reversal of the APO-induced climbing behavior, the observed trend supports the hypothesis that potent binding at the D_2 receptor is associated with the potential of a compound to induce catalepsy and that its moderation would result in attenuation of catalepsy induction.

In conclusion, initial modifications of haloperidol have produced analogs with antipsychotic potential as demonstrated by inhibition of apomorphine-induced climbing behavior. Catalepsy testing revealed that a tropane analog with potent binding to the D_2 receptor, still had potent catalepsy, suggesting that binding to the D_2R plays a key role in EPS in humans. Further modifications of the tropane analog produced compounds with relatively lower binding affinities to the D_2 receptor compared to haloperidol. While haloperidol produced maximum catalepsy, these compounds produced moderate to low levels of catalepsy. Compound **9**, with the least binding to the D_2R, produced the least catalepsy and the highest MAED of the analogs tested even though the Ki values for the 5-HT_{1A}R were similar and moderately potent. These observations are consistent with the hypothesis that further moderation of the binding affinity of the tropane analogs at the D_2R, would attenuate their catalepsy potential in rats.

Acknowledgments

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22. Binding affinities reported in Tables 1–2 were conducted by the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP). Details of the methods and radioligands used for the binding assays were previously reported in Shapiro DA, Renock S, Arrington E, Chiodo LA, Liu LX, Sibley DR, Roth BL, Mailman R. Neuropsychopharmacology. 2003; 28:1400. [PubMed: 12784105] . In the initial screening assays, test compounds were tested at a concentration of 10 mM in quadruplicate at selected GPCRs. For molecular targets at which >50% inhibition was measured, Ki determinations were obtained using at least six concentrations of the test compound; Ki values were calculated in quadruplicate using GraphPad Prism

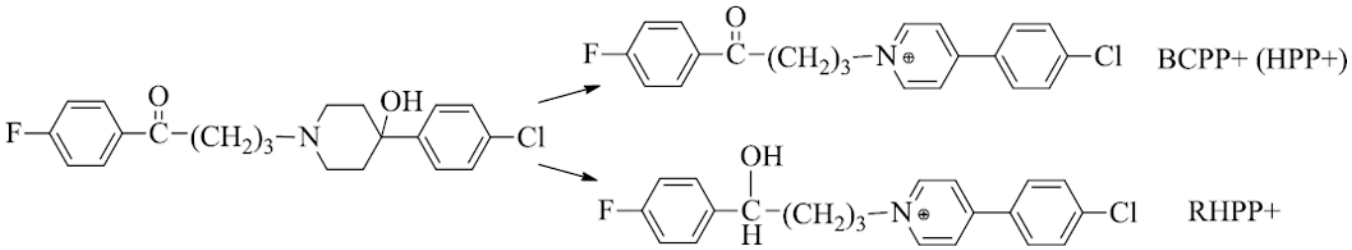


Figure 1.
Quaternary Pyridinium Metabolites of Haloperidol

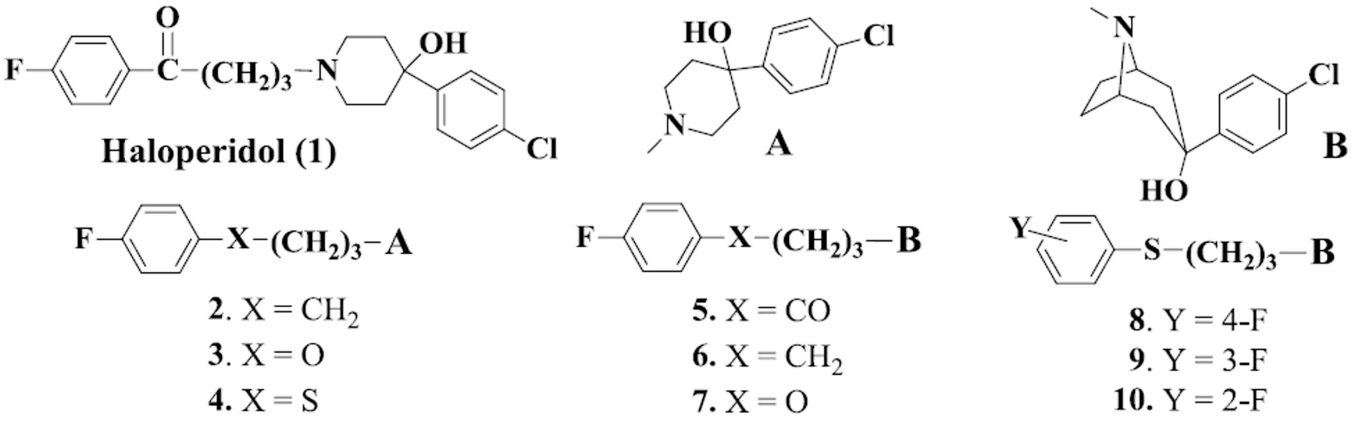


Figure 2.
Structures of Compounds Evaluated

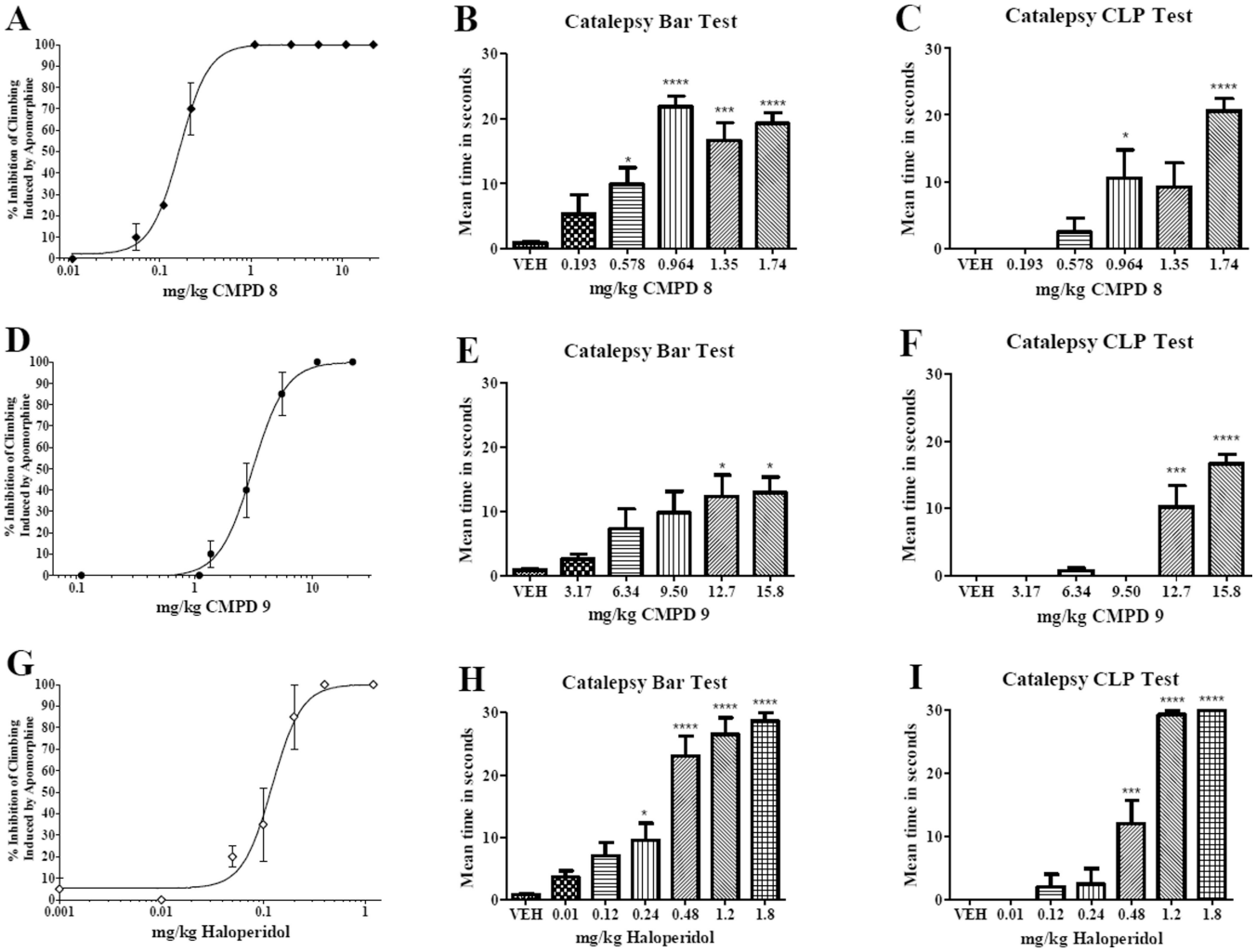
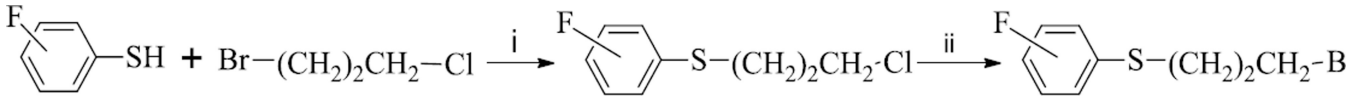


Figure 3.

Inhibition of APO-induced climbing behavior in Swiss-Webster mice, n=5/dose, by Compound 8, Compound 9, and haloperidol in graph 1A, 1D, and 1G respectively. Mean time in seconds on the bar or in the CLP stance for Sprague-Dawley rats, n=5. Graphs 1B and 1C are compound 8. Graphs 2E and 2F are compound 9. Graphs 2H and 2I are haloperidol. Significant difference between vehicle and dose of compound indicated by * ($p<0.05$), *** ($p<0.001$), and **** ($p<0.0001$).



Scheme 1.

Reagents: (i) iPrOH, K_2CO_3, reflux 12 h; (ii) 3-(4-chlorophenyl)-8-azabicyclo[3.2.1]octan-3-ol (B), DME, KI, K_2CO_3, 100 °C, reflux, 12 h.

Table 1

Binding Affinities expressed as Mean Ki ± SEM (nM) of the Analogs at CNS receptors[22]

	Binding Affinities (Ki ± SEM) in nM at Relevant CNS Receptors							
Compound	**5-HT$_{1A}$**	**5-HT$_{2A}$**	**5-HT$_7$**	**D$_1$**	**D$_2$**	**D$_3$**	**D$_4$**	**D$_5$**
Haldol (1)	3600	120	ND	120	0.89	2.5	10	ND
2	658±50	302±23	1042±45	130±11.0	24±2.0	668±39	1.5±0.1	414±34
3	317±29	72±7	656±29	22.0±1.0	8.8±1.0	49.0±1.7	4.3±0.2	103±5
4	74.0±6.0	93±10	1044±52	27.0±2.0	5.3±0.3	182±22	2.4±0.1	113±9
a_5	27.7±8.0	30.9±6.0	ND	ND	0.31	0.71	12.0	ND
6	102±0.7	317±2	472±5	>10K	33±0.26	9.7±0.1	9.4±0.1	>10K
7	123±2	236±1	420±4	>10K	22±0.17	4.10±0.03	49.0±0.5	>10K
8	20.0±0.2	89.0±1.4	97.0±1.0	347±5	2.9±0.31	0.70±0.004	8.9±0.04	MT
9	13.0±0.1	509±7	917±11	MT	27±0.36	11.0±0.1	28.0±0.3	MT
10	8.2±0.1	115±1	882±9	4093±68	4.8±0.04	1.3±0.01	27.0±0.2	MT
bOlanzapine	2100	3.3	NA	52	20	45	50	NA

ND = Not determined; NA = Not available; MT = Missed assay threshold of 50% inhibition.

aPreviously reported in ref. 9 except at 5-HT$_{1A}$R & 5-HT$_{2A}$R;

bData from reference 12.

Table 2

Binding affinities expressed as Mean Ki ± SEM (nM) for the compounds at Off-Target Receptors22

Compound	Binding Affinities (Ki ± SEM) in nM						
	5-HT$_{2B}$	**5-HT$_{2C}$**	**H$_1$**	**M$_1$–M$_3$**	**DAT**	**NET**	**SERT**
Haldol (1)	NA	4700	440	ND	ND	5500	1800
2	723±65	MT	128.5±17.5	MT	MT	1225±87	1867±146
3	1622±134	>10K	698.6±91.7	MT	>10K	3959±471	2228±187
4	790±68	MT	115.0±9.7	MT	497±28	2249±239	160±24
5	ND	872±178	8780±1625	ND	ND	ND	ND
6	1381±23	2434±44	1719±30	MT	778±10	MT	414±6
7	898	MT	4149±14	MT	620±9	1791±28	255±2
8	1057±14	1498±26	977±16	MT	1081±11	1409±19	316±4
9	270±2	1861±20	1207±16	MT	285±4.38	510±5	227±3
10	520±5	1122±15	1009±10	MT	354±11	462±5	102±0.6

ND = Not determined; MT = Missed assay threshold of 50% inhibition.

[a] Previously reported in ref. 9 except at 5-HT$_{1A}$R and 5-HT$_{2A}$R. Ki values without the associated SEM, are within 20% of the mean value.

Table 3

In Vivo Data on Haloperidol, compounds **8** and **9**

Compound	APO-Reversal ED50		Bar Test/Catalepsy MAED		CLP/Catalepsy MAED	
	mg/kg	μmole/kg	mg/kg	μmole/kg	mg/kg	μmole/kg
Haloperidol	0.122	0.325	0.240	0.640	0.480	1.28
Compound **8**	0.165	0.373	0.578	1.31	0.964	2.18
Compound **9**	3.14	7.10	12.7	28.7	12.7	28.7

MAED = Minimum Adverse Effective Dose.