Identification of A Butyrophenone Analog as a Potential Atypical Antipsychotic Agent: 4-[4-(4-Chlorophenyl)-1,4-diazepan-1-yl]-1-(4-fluorophenyl)butan-1-one

Seth Y. Ablordeppey[a,*], **Ramazan Altundas**[a], **Barbara Bricker**[a], **Xue Y. Zhu**[a], **Suresh E. V. K. Eyunni**[a], **Tanise Jackson**[a], **Abdul Khan**[a], and **Bryan L. Roth**[b]

a*Division of Basic Pharmaceutical Sciences, Florida A&M University, College of Pharmacy and Pharmaceutical Sciences, Tallahassee, FL 32307, USA*

b*Department of Pharmacology, Medicinal Chemistry and Psychiatry, University of North Carolina at Chapel Hill School of Medicine, NC 27599, USA*

Abstract

The synthesis and exploration of novel butyrophenones have led to the identification of a diazepane analog of haloperidol, 4-[4-(4-Chlorophenyl)-1,4-diazepan-1-yl]-1-(4-fluorophenyl)butan-1-one (Compound **13**) with an interesting multireceptor binding profile. Compound **13** was evaluated for its binding affinities at DA subtype receptors, 5HT subtype receptors, H-1, M-1 receptors and at NET, DAT and SERT transporters. At each of these receptors, compound **13** was equipotent or better than several of the standards currently in use. In *in vivo* mouse and rat models to evaluate its efficacy and propensity to elicit catalepsy and hence EPS in humans, compound **13** showed similar efficacy as clozapine and did not produce catalepsy at five times its ED_{50} value.

Keywords

Haloperidol; antipsychotics; butyrophenone; dopamine receptor ligands; serotonin receptor ligands; D2-like receptor ligands; diazepane; atypical antipsychotics

The introduction of clozapine[1] (Clozaril, **1**) as a treatment option in the 1990s has opened the door to the use of atypical antipsychotics and the replacement of the prototypic agent, haloperidol (**2**) as a drug of choice for the treatment of schizophrenia. The superior therapeutic profile of clozapine and other atypical antipsychotic drugs and the long-term debilitating side-effects[2] associated with haloperidol treatment have conspired to diminish its use in the clinic. Among several hypotheses put forth to explain the long-term extrapyramidal side effects of haloperidol including tardive dyskinesia, is the fact that haloperidol undergoes biotransformations that lead to the formation of toxic metabolites.[3,4] Specifically, haloperidol has been shown to undergo CYP450-mediated oxidation to form pyridinium metabolites[5] which can potentially damage dopamine receptors in the nigrostriatum of the brain (Chart 1). Based on this hypothesis, we [6-9] proposed a drug design strategy that replaces the piperidine ring in haloperidol with bioisosteric equivalents which could not undergo *in vivo*

*Corresponding Author: Tel: 850-599-3867, Fax: 850-599-3934, e-mail: seth.ablordeppey@famu.edu.

biotransformation to pyridinium species known to be associated with neuronal toxicity. A similar strategy has more recently appeared in the literature.[10] The outcome of such a strategy was to enhance the pharmacological and side-effect profiles of the resulting agents.

Our laboratory has previously shown that modification in the piperidine ring of haloperidol results in significant changes in pharmacological activity.[6-7] We also identified new bioisosteric groups of the piperidine ring with binding profiles similar or better than those of haloperidol at the D2 dopamine receptor subtype.[7-9] Several of these compounds are shown in Chart 2, with new binding affinity data at selected receptors reported in Table 1.

Although compounds **5** and **6** have high affinity for $5HT_{1A}$ and $5HT_{2A}$ receptors, the demonstration that compound **5** elicits a higher catalepsy than haloperidol dampened our desire to pursue the tropane moiety as a piperidine bioisostere.[9] It is important to note that the alcoholic function in compounds **5** and **6** contributes significantly to affinity not only at the D2-like receptors but also at the $5HT_{1A}$ and $5HT_{2A}$ receptors (cf compounds **5**, **6** and **7**). Interestingly, the OH group does not appear to play a significant role in the binding of the tropane analogs to the $5HT_{2C}$ receptor.

The reaction mechanism by which haloperidol is metabolized *in vivo* to a pyridinium species or BCPP+ has been suggested to begin with dehydration of the tertiary alcoholic function.[5] A drug design strategy to prevent such a biotransformation is to eliminate the alcoholic function. Compound **3**, a haloperidol analog without its alcoholic function, was previously synthesized and evaluated at D2-like receptors.[7] The results supported the notion that the alcoholic function contributes to affinity but is not an absolute requirement for binding to dopamine receptors. Compound **4** in which the C-OH group was replaced with a nitrogen atom showed a lower binding affinity than haloperidol at the dopamine receptors.[8] Compounds **5** and **6** were designed on the premise that the tropane moiety could not form a pyridinium ring due to the fact that a double bond could not form at the ring junctions adjacent to the N atom.[9] In addition, bridging the ring restricts conformational mobility and holds the alcoholic function in a preferred conformation.[8] Binding evaluation showed that both compounds were as potent or more potent than haloperidol at D2-like receptors. It was further demonstrated that the OH group is in the axial position and this led to the suggestion that haloperidol most likely binds to the dopamine receptors with its OH in the axial position. Comparing **5** and **6** suggests that the chloro group contributes to binding but is also not an absolute requirement. Removal of the OH group from the tropane ring (Compound **7**) again decreased affinity but retained sufficient dopamine binding affinity as observed for compound **3**. Contraction of the piperidine ring to a pyrrolidine (**8**) resulted in a decrease in affinity but expansion to a 7-membered homopiperidine ring (**9**) retained affinity as in haloperidol, thus suggesting that homopiperidine ring is bioisosteric to piperidine ring.[7] Separation of the enantiomers of compound **8** and their subsequent evaluation at D2-like receptors revealed that (+)-**8** was the eutomer.[6]

To further explore these changes in the piperidine moiety of haloperidol in our search for new antipsychotic drugs with atypical pharmacological profiles, we have embarked on SAR studies with modifications primarily in the piperidine ring that could lead to the identification of new agents with clozapine-like binding profiles but without the associated weight gain and new onset type II diabetes.[11] Thus, it was desirable not only to evaluate the binding of the compounds at dopamine receptors but also at serotonergic receptors ($5HT_{1A}$ and $5HT_{2A}$) since binding to these receptors is often associated with therapeutic advantages observed in atypical antipsychotic drugs.[12] In addition, evaluation of the compounds at $5HT_{2C}$ and histamine H-1 receptors will be valuable indicators of whether these compounds have the potential to develop the weight gain side effect associated with atypical antipsychotic drugs.[13] In line with the multiple receptor targeting strategy in the development of new antipsychotic agents, we have focused our design efforts on obtaining agents that have the following receptor binding profiles:

a. Binding to DA D2 receptor with moderate affinity (30 < Ki < 150 nM)

b. Binding to DA D4 receptor with high affinity (Ki < 10 nM)

c. Binding to $5HT_{1A}$ and $5HT_{2A}$ receptors with high affinity (Ki < 50 nM)

d. Binding to $5HT_{2C}$ and H-1 receptors with very low affinity (Ki > 500 nM)

The above criteria (a – d) will also guide the selection of compounds for evaluation in *in vivo* animal models for efficacy and the propensity of the compounds to produce catalepsy, a condition associated with extrapyramidal side-effects in humans.[14] To achieve the drug design objectives, we have utilized observations from previous SAR studies to design compounds **10 - 14** (Chart 3) as probes for the dopamine, serotonin and histamine H-1 receptor subtypes in an attempt to obtain new prototype drugs.

Chemistry

Construction of the ethylene-bridged piperazine moiety was initiated using (2S, 5R)-diethyl 2,5-dibromohexanedioate (**16**) as the starting material (Scheme 1) as previously reported.[15, 16] Double alkylation of 4-chlorophenyl aniline with **16** yielded N-chlorophenyl pyrrolidine (**17**). Amidation of **17** with benzylamine produced the amide, **18** which underwent a ring closure with the remaining ester function to form the bridged piperazine, **19**. An attempt to reduce the lactam functions of **19** with $LiAlH_4$ also resulted in the removal of the 4-chloro group on the phenyl ring to form the di-substituted 3,8-diaza-bicyclo[3.2.1]octane (**20**). Debenzylation of **20** to form **22** was accomplished by reaction with ethyl chloroformate and the subsequent hydrolytic cleavage of the resulting carbamate, **21**. Compound **10** was obtained by alkylation of the secondary amine function of **22** with 4-chloro-4′-fluorobutyrophenone. Target compounds **11** and **12** were obtained by alkylating the corresponding starting materials, **23** and **24** respectively (commercially available from Sigma-Aldrich) with 4-chloro-4′-fluorobutyrophenone (Schemes 2 and 3). Scheme 4 describes the synthesis of target compound **13** in five steps. Initial starting material 4-chloroboronic acid (**26**) was synthesized from 4-chlorobromobenzene by lithiation with n-butyl lithium and treatment with trimethylborate. The chloroboronic acid was coupled to N-Boc protected 1,4-diazepane (**28**) under palladium-catalyzed amination condition to form **29**. Deprotection of **29** in trifluoroacetic acid delivered amine **30** which was alkylated in the usual way with 4-chloro-4′-fluorobutyrophenone to form **13**. An alternative synthesis of compound **13** was also explored in which the 4-chlorophenyl homopiperazine (**30**) was obtained in one step without the protection and deprotection steps. The reaction involved the coupling of 4-chloroiodobenzene directly with unprotected homopiperazine under CuI catalysis to form compound **30** in excellent yield. The same alkylation step delivered the desired compound **13** in 61% overall yield (Scheme 5).

8-Methyl-8-aza-bicyclo[3.2.1]octa-3-one (**31**) served as the starting material for the synthesis of target compound **14** (Scheme 5). Compound **31** was converted to the cyclic lactam (**32**) by insertion of a nitrogen atom followed by reduction using lithium aluminum hydride to form **33** in very good yield (80%). The free secondary amine in **33** was BOC protected prior to its treatment with ethyl chloroformate to form the carbamate protected amine, **35**. Compound **35** was BOC deprotected and then coupled with 4-chloroboronic acid in the presence of copper acetate to yield **37**. Decarbamylation of **37** was achieved using KOH in ethylene glycol and the resulting secondary amine was alkylated with 4-chloro-4′-fluorobutyrophenone under the usual alkylating condition to produce the desired compound **14**.

Results and Discussion

Our previous observation that bridging the piperidine ring to form the tropane analogs of haloperidol resulted in 7- and 3-fold increases in binding affinity at the D2 and D4 receptors

respectively informed the design of target compounds **10** and **14**. The rationale was to investigate bridged analogs to probe the effect of conformational restriction on piperazine and homopiperazine equivalents of haloperidol. Compound **10** can also be viewed as an analog of **7** with the carbon at position 4 of the ring replaced by a nitrogen atom. The ethylene bridge in compound **10** holds the piperazine ring in the chair conformation while in compound **11** the ring is held in the boat conformation by a methylene group. Binding affinity data for compounds **10 -14** are reported in Table 2.

Both compounds **10** and **11** have only moderate to weak binding affinity at the D2-like receptors and do not meet the proposed criteria. Interestingly, compound **12**, previously reported by Yevich, J. P. et al.[17] with the 4-chlorophenyl ring in compound **4** replaced by 1,3-diazine moiety, met the four criteria set of interest (Table 3). The authors pharmacologically evaluated compound **12** and its ketone-reduced analog for their binding affinity for DAD2, sigma and cortical α_1 adrenergic receptors but not at the receptors of interest in this study. Thus, its synthesis provides an opportunity to compare the contributions of 4-chlorophenyl moiety in compound **4** with the 1,3-diazine ring in **12** to the binding affinities at the various receptors.

Compound **13**, which can be viewed as a homopiperazine analog of haloperidol and compound **9**, meets all the criteria except for its binding affinity at the $5HT_{1A}$ receptor. Compound **14**, a bridged analog of compound **13**, also showed low binding affinity for the receptors of interest (Table 2) and failed to meet the other stated criteria. Thus, it would appear that bridging does not necessarily result in favorable attributes for these compounds. Based on these observations, compound **13** was selected for further pharmacological evaluation.

Table 3 shows a head to head comparison of the binding affinities of compound **13** and several atypical antipsychotic drugs currently in use at several receptors known to have implications for the therapeutic value of atypical antipsychotic drugs. Compound **13** has moderate affinity for the DA D2 receptor and high affinity at the D4 receptor. The effect of compound **13** on apormophine-treated mice (see later) suggests D2 receptor antagonism and thus, would be expected to demonstrate antipsychotic properties in humans.[18] $5HT_{1A}$ receptor activation has been suggested to contribute to the improved activity of certain atypical antipsychotic drugs. [12b,19]

Compound **13** has moderate affinity for $5HT_{1A}$ (Ki = 117 nM). This binding affinity is higher than that for risperidone and quetiapine but similar to that of clozapine (Ki = 140 nM). Compound **13** also binds with high affinity at the $5HT_{2A}$ receptor (Ki = 23.6 nM), a receptor implicated in the therapeutic efficacy of atypical antipsychotic drugs in treating the negative symptoms of schizophrenia and preventing motor disturbances associated with antipsychotic treatment.[20] While this affinity is 10-fold better than that of quetiapine, other atypical antipsychotics have higher affinity for this receptor. $5HT_6$ receptors are implicated in the amelioration of the cognitive disturbances in schizophrenia and both clozapine and olanzapine have high affinity for this receptor.[21] While **13** has weak affinity for the $5HT_6$ receptor (Ki = 295.8 nM), it is over 13-fold more potent at this receptor than quetiapine and over 6-fold better than risperidone.

Treatment of schizophrenia with atypical antipsychotic drugs has been associated with weight gain and the onset of type II diabetes mellitus. Two receptors, histamine H-1 and $5HT_{2C}$, have been suggested to be involved in this adverse event. Several papers[22] have demonstrated that there is significant correlation between affinity for H-1 receptor and weight gain and hence the observation that compound **13** has much lower affinity (Ki = 186 nM) for H1 receptor than risperidone (19 nM), quetiapine (8.7 nM), ziprasidone (47 nM), olanzapine (2.8 nM) and clozapine (1.8 nM) is an indication that compound **13** may have a lower propensity to induce weight gain. In addition, the low affinity of **13** for the $5HT_{2C}$ receptor (Ki = 1425 nM) compared

to ziprasidone (Ki = 0.72 nM), risperidone (Ki = 10 nM) and olanzapine (Ki = 10 nM) suggests a possible decrease in the propensity of **13** for treatment-emergent weight gain. The observation that 5-HT_{2B} receptor agonists are associated with increased risk for valvular heart disease[23] will require that antipsychotics that interact with 5HT receptors be evaluated for binding to the $5HT_{2B}$ receptor. Thus, it is encouraging to note that compound **13** does not bind appreciably to $5HT_{2B}$ receptor (Ki = 495). Also encouraging is the observation that compound **13** lacks affinity for the cholinergic muscarinic M_3 receptor (Ki = 3000 nM), a receptor reported to have a contributing role in new-onset diabetes in schizophrenic patients treated with atypical antipsychotics.[12a]

Compound **13** was also evaluated at neurotransmitter uptake transporters. Compound **13** has very weak affinity for both the dopamine (DAT) and norepinephrine (NET) transporters but has similar affinity for the serotonin transporter (SERT) (Ki = 56.6 nM) compared to aripiprazole (Ki = 98 nM).[24] This binding profile at the transporters suggests that while it has potential for antidepressant properties, it has a much lower potential to induce cocaine-like adverse properties since it has a very weak binding affinity for DAT.

Animal behavioral studies were initiated to provide preliminary evidence on efficacy and to evaluate the propensity of compound **13** to induce catalepsy. The ability to block apomorphine induced stereotypy is an indication of a drug's *in vivo* capacity to antagonize dopamine D2 receptors while induction of catalepsy is considered to be an indication of a drug's propensity to produce extrapyramidal symptoms in humans. The ED_{50} values of compound **13**, clozapine and haloperidol are estimated from the dose-response curves in Figs 1–3. The ED_{50} value of compound **13** in inhibiting apomorphine-induced stereotypy was estimated to be 35.8 μmole/kg compared to clozapine's 42.4 μmole/kg suggesting that compound **13** is slightly more potent at blocking the D2 receptors *in vivo* than clozapine on a molar basis. This is also consistent with their estimated Ki values at the D2 receptor. The ED_{50} values comparing compound **13**, clozapine and haloperidol is reported in Table 4. Compound **13** and clozapine have $ED_{50}s$ (μmole/kg) that are about 500 times that of haloperidol. The results of these behavioral studies support the radioligand binding data that compound **13** does not bind to D2 receptors as well as haloperidol, but does bind to D2 receptors with about the same affinity as clozapine.

Fig 4 depicts plots of mean catalepsy scores for compound **13** with clozapine and haloperidol as controls. The vehicle served as a negative control in the study. The ED_{50} value for each compound was used as the basis for the dose used for the first set of comparative evaluations. It is clear that haloperidol showed the highest propensity to induce catalepsy while clozapine and compound **13** had no significant difference between them. The extent of the absence of catalepsy was evaluated by increasing the dose of compound **13** up to 5 times the ED_{50} value at which point it was impossible to retain the drug in solution for intraperitoneal delivery. At $5\times$ the ED_{50} value, there was no appreciable cataleptogenic activity observed. Taken together, the combined receptor binding profile and the absence of catalepsy suggest that compound **13**, a butyrophenone analogue which does not induce catalepsy up to $5\times$ its ED_{50} value may serve as an atypical antipsychotic agent.

Experimental

Melting points were determined on a Gallenkamp (UK) apparatus and are uncorrected. NMR spectra were obtained on a Varian 300 MHz Mercury Spectrometer. Elemental analyses were carried out by Atlantic Microlab, Inc., Norcross, GA and are within 0.4% of theory unless otherwise noted. Flash chromatography was performed with Davisil grade 634 silica gel. N,N-Dimethylformamide was distilled from CaSO4 and stored over 4 Å molecular sieves. 4-Chloro-4′-fluorobutyrophenone was obtained from Sigma-Aldrich but was purified by

distillation under reduced pressure to a colorless liquid prior to use. Other starting materials were used without further purification.

Synthesis of compound 10

The synthesis of compound **10** followed the methods previously reported by Cignarella et al.[15] and Thompson et. al.[16] as depicted in Scheme 1.

Cis-2,5-dicarboethoxy-1-[4-chlorophenyl]pyrrolidine, 17

To a solution of (2S, 5R)-diethyl 2, 5-dibromo-hexanedioate (**16**) (10g, 27.8 mmol) in anhydrous DME (80 mL), was added 4-chloroaniline (14.2g, 111.1 mmol) and KI (1g, 6.02 mmol). The resulting mixture was refluxed under N_2 with stirring for 16 h. and allowed to cool to room temperature. Excess DME was removed under vacuum and the residue was triturated in Et_2O (100 mL) to yield a solid. The solid obtained was filtered, washed with Et_2O and the organic phase was treated with 100 mL of ice cold 5 N HCl and H_2O, dried over $MgSO_4$ and solvent removed in vacuo. The crude compound was purified by chromatography on silica gel (8:2 hexane :EtOAc) to give compound **17** (8.5g, 93.5%) as a mixture of trans-2,5-dicarboethoxy-1-[4-chlorophenyl]pyrrolidine and cis-2,5-dicarboethoxy-1-[4-chlorophenyl] pyrrolidine.

2-Benzylcarbamyl-5-carboethoxy-1-[4-chlorophenyl]pyrrolidine, 18

A mixture of *cis-* and *trans-*2,5-dicarboethoxy-1-[4-chlorophenyl]pyrolidine, **17** (8.5g, 26 mmol) was dissolved in 10 mL of *o*-xylene. Benzylamine (3.61 g, 33.74 mmol) was added to the reaction mixture and refluxed for 18 h. under N_2, cooled and solvent evaporated. The residue was subjected to column chromatography (silica gel) eluting with (1:1) hexane: EtOAc to give compound **18** (4.4 g, 39.8 %). ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.19 (t, *J* = 7.13 Hz, 3H), 2.01-2.45 (m, 4H), 4.11-4.37 (m, 4H), 4.40-4.46 (dd, *J* = 6.0, 3.2 Hz, 2H), 6.36-6.44 (m, 2H), 7.09-7.24 (m, 7H), 8.52 (m, 1H).

3-Benzyl-8-[4-chlorophenyl]-3,8-diazabicyclo[3.2.1]octane-2,4-dione, 19

The solution of compound **18** (4.0 g, 10.35mmol) in 30 mL of 1N NaOH (75% *aq.* ethanol) was stirred at room temperature for 2 h. The reaction was quenched with HCl gas to the end point of phenolphthalein and the solid was filtered. The solvent was removed in vacuo and the solid material was re-dissolved in absolute alcohol. The solution was partially removed in vacuo and the residue was treated with Ac_2O (5 mL) and NaOAc (1 g). After refluxing overnight under N_2, the excess Ac_2O was removed and the reaction mixture was quenched with aq. Na_2CO_3, extracted with CH_2Cl_2 (3×100 mL) and dried over anhydrous $MgSO_4$. The solvent was removed in vacuo and separated on a silica gel column (7:3 hexane : EtOAc) to give compound **19** (2.56 g, 56.7%, white solid, mp 163-164 °C). ^{1}H NMR (270 MHz, CDCl$_3$) δ 2.04-2.12 (m, 2H), 2.46-2.55 (m, 2H), 4.63-4.66 (dd, *J* = 5.1, 2.7 Hz, 2H), 4.70 (s, 2H), 6.70-6.82 (m, 4H), 7.03-7.12 (m, 5H).

3-Benzyl-8-phenyl-3, 8-diazabicyclo[3.2.1]octane, 20

To a solution of compound **19** (0.58 g, 1.70 mmol) in anhydrous THF (3 mL), was added slowly, 1 M LiAlH$_4$ (10.24 mL, 10.24 mmol) and refluxed for 2 days under N_2. The reaction was quenched in ice bath with 10% NaOH (20 mL) and extracted with CH_2Cl_2. The organic phase was separated, dried over $MgSO_4$ and removed in vacuo. The residue was subjected to column chromatography (silica gel, 9:1 hexane:ethyl acetate) to give compound **20** (0.4 g, 84.6%, white crystals, mp 115-117 °C). ^{1}H NMR (270 MHz, CDCl$_3$) δ 1.87-2.08 (m, 4H), 2.51-2.52 (d, *J* = 2.0 Hz, 4H), 3.41 (s, 2H), 4.13-4.15 (m, 2H), 6.65-6.78 (m, 3H), 7.15-7.30 (m, 7H).

3-Carboethoxy-8-phenyl-3, 8-diazabicyclo[3.2.1]octane, 21

To a solution of compound **20** (0.77 g, 2.8 mmol) in anhydrous toluene (15 mL), was added ethyl chloroformate (1.6 mL, 16.6 mmol) and refluxed for 16 h under N_2. The resulting solution was allowed to cool to RT and excess solvent was removed under vacuum. The resulting residue was subjected to column chromatography (silica gel, 8:2 hexane:ethyl acetate) to give compound **21** (0.65 g; 90.5%) as white crystals, mp 124.4 °C. ^{1}H NMR (270 MHz, CDCl$_3$) δ 1.22 (t, J = 8.1 Hz, 3H), 1.79-1.84 (m, 2H), 1.98-2.04 (m, 2H), 3.29 (t, J = 13.5 Hz, 2H), 3.60-3.76 (dd, J = 13.5 Hz, 2H), 4.07-4.18 (m, 4H), 6.70-6.80 (m, 3H), 7.18-7.26 (m, 2H).

8-Phenyl-3,8-diazabicyclo[3.2.1]octane, 22

To a solution of compound **21** (0.73 g, 2.48 mmol) in EtOH (5 mL), was added KOH [3 g in H_2O (3 mL)], EtOH (25 mL) followed by NH_2NH_2 (3 mL). The resulting mixture was refluxed for 16 h. Excess alcohol was removed and the residue was extracted with EtOAc (4×50 mL) and H_2O (20 mL) to give compound **22** (0.4 g 86%). ^{1}H NMR (270 MHz, CDCl$_3$) δ 1.88-2.07 (m, 4H), 2.51-2.57 (dd, J = 13.5, 2.7 Hz, 2H), 3.19-3.24 (d, J = 13.5 Hz, 2H), 4.09 (br s, 2H), 6.67-6.78 (m, 3H), 7.17-7.24 (m, 2H).

8-Phenyl-3,8-diazabicyclo[3.2.1]octan-1-yl-1-(4-fluorophenyl)butan-1-one, 10

To a mixture of compound **22** (0.56 g, 2.96 mmol), K_2CO_3 (1.4g, 10 mmol) and KI (0.3 g) in DME (10 mL), was added 4-chloro-4-fluorobutyrophenone (2.0 g, 10.01 mmol) and stirred. The mixture was refluxed for 16 h under N_2 atmosphere and the reaction mixture was allowed to cool to room temperature. Excess DME was removed in vacuo and the residue was extracted with CH_2Cl_2 (3×50 mL) followed by brine (50 mL). The organic phase was collected and dried over anhydrous $MgSO_4$ and the solvent evaporated. The resulting crude product was subjected to column chromatography (silica gel, 8:2 EtOAc:MeOH) to give an oily residue (1.21 g, 68.2 %) and subsequently converted to an oxalate salt as a white solid, mp 194.7-195.5 °C. ^{1}H-NMR (270 MHz, CDCl$_3$) δ 1.89-1.99 (m, 6H), 2.39 (t, J = 6.8, 2H), 2.47-2.65 (m, 4H), 3.06 (t, J = 7.3, 2H), 4.21 (m, 2H), 6.73-6.85 (m, 3H), 7.17-7.33 (m, 4H), 8.05-8.10 (m, 2H). Anal. ($C_{24}H_{26}CFN_2O_5$:H_2O) calcd. C 64.62, H 6.19, N 6.28. Found C 64.66, H 6.11, N 6.14.

4-[5-(4-Chloro-phenyl)-2,5-diaza-bicyclo[2.2.1]hept-2-yl]-1-(4-fluoro-phenyl)-butan-1-one, 11

A mixture of compound **23** (0.5g, 2.4 mmol), 4-chloro-4-fluorobutyrophenone (1.9g, 9.6 mmol) KI (50mg) and K_2CO_3 (138 mg) in DME (10 ml) was refluxed in an atmosphere of N_2 for 60 hours. Excess DME was removed under vacuum and residue was partitioned between CH_2Cl_2 (3×75 ml) and brine (50 ml). The organic phase was pooled, dried over anhydrous Na_2SO_4 and the solvent removed in vacuo to afford a residue as the crude product. The crude product was purified by column chromatography using 0 - 20% MeOH in EtOAc to yield compound **11** (755mg, 84.5%), which was subsequently converted to the oxalic acid salt as a white crystal, mp 116.4 -116.8 °C. ^{1}H-NMR (300 MHz, DMSOd_6) δ 1.86-1.93 (m, 4H), 2.09-2.21 (AB system, J=10.7, 2H), 3.00-3.37 (m, 6H), 3.11 (t, J=6.7, 2H), 3,37-3.58 (AB system, J=9.3, 2H). 4.42 (m, 1H), 4.60 (m, 1H), 6.65-6.68 (Part of AAXX, 2H), 7.32-7.38 (Part of AAXX, 2H), 8.00-8.04 (Part of AAXX, 2H). Anal. $C_{21}H_{22}ClFN_2O.1.5C_2H_2O_4$ calcd: C 56.73, H 4.96, N 5.52; Found: C 56.49, H 4.91, N 5.42

4-[4-(2-Pyrimidine)piperazin-1-yl]-1-(4-fluorophenyl)butan-1-one, 12

A mixture of 2-(piperazin-1-yl)pyrimidine dihydrochloride (0.5g, 2.1 mmol), 4-chloro-4′-fluorobutyrophenone (1.3 g, 6.3 mmol), KI (25 mg), K_2CO_3 (1.5 g, 10.9 mmol), in DME (10 mL) was refluxed with stirring under N_2 for 12 h. After cooling to room temperature, the mixture was diluted with EtOAc (200 mL) and washed with H_2O. The organic layer was dried (Na_2SO_4) and filtered. The filtrate was concentrated in vacuo to dryness and the residue was purified by chromatography on silica gel to give compound **12** (563 mg, 78%), mp 109 -110

°C. ^{1}H NMR (CDCl$_3$): 8.29 (2H, d, J = 4.5 Hz), 8.02 (2H, dd, J = 5.1, 8.7 Hz), 7.13 (2H, t, J = 8.7 Hz), 6.47 (1H, t, J = 4.5 Hz), 3.76 (4H, m), 3.01 (2H, t, J = 7.5 Hz), 2.46 (6H, m), 1.99 (2H, m). Calcd for C$_{18}$H$_{21}$FN$_4$O: C, 65.84; H, 6.45; N, 17.06. Found: C, 65.91; H, 6.51; N 16.86.

4-Chlorophenylboronic Acid, 26

To a solution of 4-bromochlorobenzene, **25** (4 g, 20.9 mmol) in THF (80 mL) was added *n*-BuLi (22 mL, 1.6 M) at −78 °C under N$_2$ and stirred for 1h at the same temperature. The reaction mixture was treated with B(OMe)$_3$ (398.8 ml, 78.3 mmol) at −78 °C, allowed to warm up to room temperature and then stirred overnight. The pH of the mixture was then adjusted with a solution of NaHCO$_3$ to pH 6.5 and extracted with CH$_2$Cl$_2$ (3 × 150 mL). The pooled organic phase was dried (MgSO$_4$), the solvent was removed in vacuo and the crude product was column chromatographed (silica gel, 7:3, hexane:EtOAc) to yield compound **26** (4-chlorophenyl-boronic acid) (2 g, 61 %) as a white solid. ^{1}H-NMR (270 MHz, CD$_3$OD) δ 7.30-7.77 (m, 4H).

1-*t*-Butyl homopiperazine carboxylate, 28

To a solution of homopiperazine, **27** (10g, 0.1 mol) in H$_2$O (104 mL) and t-BuOH (119 mL) containing 2.5 N NaOH (16 mL) was added di-*tert*-butyl dicarbonate (8.55 g, 0.039 mol) at 0 °C for 40 min. The resulting mixture was stirred for 1 h at room temperature and the solution was concentrated in vacuo. The crude material was extracted with CH$_2$Cl$_2$ (3 × 100 mL), dried over MgSO$_4$ and the solvent evaporated. The crude product was subjected to column chromatography (silica gel, 9:1:1 MeOH : CH$_2$Cl$_2$: NH$_4$OH to give 1-*t*-Butyl homopiperazine carboxylate, **28** (5 g, 64 %). ^{1}H-NMR (270 MHz, CD$_3$OD) δ 1.46 (s, 9H), 1.79 (q, *J*=5.9, 2H), 2.78-2.87 (m, 4H), 3.41-3.49 (m, 4H).

1-*t*-Butyl-4-(4-chlorophenyl)homopiperazine carboxylate, 29

The mixture of 4-chlorophenyl-boronic Acid, **26** (1.65 g, 10.54 mmol), **28** (1.05g, 5.27 mmol), Cu(OAc)$_2$ (1.43 g, 7.87 mmol), triethylamine (0.726 mL) and molecular sieves (4 g) in CH$_2$Cl$_2$ (50 mL) was stirred at room temperature under air. The reaction was quenched with a 4 ml of NH$_4$OH in 4 mL of MeOH. The mixture was filtered through celite and purified by column chromatography using hexane:EtOAc (7:3) as eluent on silica gel to give compound **29** (1.2 g, 73%) ^{1}H-NMR (300 MHz, CDCl$_3$) δ 1.35 (s, 4H), 1.42 (s, 5H), 1.91-1.98 (m, 2H), 3.18 (t, 1H, J = 6.2 Hz), 3.29 (t, 1H, J = 6), 3.48-3.7 (m, 6H), 6.58 (d, 2H, J = 8.7 Hz), 7.12 (m, 2H, J = 9 Hz)

1-(4-Chlorophenyl)homopiperazine, 30

Trifluoroacetic acid (5 mL) was slowly added in a drop-wise manner to a solution of compound **29** in CH$_2$Cl$_2$ (5 mL) at ambient temperature and the resulting mixture was allowed to stir overnight. The reaction was quenched with aq. NaOH and extracted with EtOAc (3 × 100 mL), washed with NaHCO$_3$ solution (50 mL) and brine (50 mL). The organic phase was collected and dried over MgSO$_4$ and evaporated in vacuo. The crude product was subjected to column chromatography (silica gel, 9:1:1 MeOH: CH$_2$Cl$_2$:NH$_4$OH to yield 1-(4-chlorophenyl) homopiperazine, **30** (0.720 g, 88%). ^{1}H-NMR (300 MHz, CDCl$_3$) δ 1.92-1.98 (m, 2H), 2.86 (t, 2H, *J* = 5.7), 3.04 (t, *J* = 5.4 Hz), 3.53 (q, 4H, *J* = 6.4 Hz), 5.50 (br s, 1H), 6.58 (d, 2H, *J* = 9), 7.14 (d, 2H, *J* = 9.2 Hz).

4-[4-(4-Chlorophenyl)-1,4-diazepan-1-yl]-1-(4-fluorophenyl)butan-1-one, 13

A mixture of compound **30** (709 mg, 3.4 mmol) in dry DME (15 mL) and K$_2$CO$_3$ (1.9 g, 13.9 mmol) was heated for 20 min. after which 4-chloro-4-flourobutyrophenone (2.8 g, 13.9 mmol) was added followed by KI (0.3 g). The reaction mixture was refluxed for 18 h under an

atmosphere of N_2. Excess DME was removed in vacuo and the residue was extracted with CH_2Cl_2 (3×100 mL) and brine (50 mL). The organic phase was collected and dried over $MgSO_4$ and solvent removed under vacuum. The crude product was subjected to column chromatography (silica gel, 9:1 EtOAc:MeOH) to yield compound **13** (1.19 g, 87%) as an oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.84-1.95 (m, 4H), 2.53-2.62 (m, 4H), 2.75 (t, 2H, *J* = 5.1 Hz), 2.93 (t, 2H, *J* = 7.2 Hz) 3.39-3.48 (m, 4H), 6.58 (d, 2H, *J* = 9.6 Hz), 7.05-7.13 (m, 4H), 7.96-7.92 (dd, 2H, *J* = 9, 5.1 Hz). Subsequently, compound **13** was converted to the oxalate salt, crystallized from MeOH/Et_2O as a white solid, mp 146.6-146.4 °C. Anal. ($C_{23}H_{26}ClFN_2O_2$) calcd. C 59.42, H 5.64, N 6.03 found C 59.27, H 5.70, N 6.05.

Alternative Method for Compound 13

A mixture of homopiperazine (2 g, 20 mmol), CuI (380 mg, 2 mmol), K_2CO_3 (5.5 g, 40 mmol), ethylene glycol (2.4 g, 40 mmol), 1-chloro-4-iodobenzene (4.76 g, 20 mmol) in ^{i}PrOH (50 mL) was refluxed for 12 h under N_2. The mixture was diluted with EtOAc (500 mL) and washed with water. The organic phase was dried (Na_2SO_4), and filtered. The filtrate was concentrated in vacuo to dryness followed by chromatography on silica gel (EtOAc then MeOH/EtOAc =10/90) to afford 1-(4-chlorophenyl)-1,4-diazepane (3.8 g, 90%).

A mixture of 1-(4-chlorophenyl)-1,4-diazepane (**30**) (540 mg, 2.57 mmol), 4-chloro-4-fluorobutyrophenone (2 g, 10.28 mmol), KI (25 mg), K_2CO_3 (880 mg, 6.38 mmol) in DME (20 mL) was refluxed under N_2 for 12 h. The mixture was diluted with EtOAc (500 mL) and washed with H_2O. The organic phase was dried (Na_2SO_4), and filtered. The filtrate was concentrated in vacuo to dryness followed by column chromatography on silica gel (pure EtOAc) to afford 4-[4-(4-chlorophenyl)-1, 4-diazepan-1-yl]-1-(4-fluorophenyl)butan-1-one, **13**. The product was converted to the salt form using ethereal HCl and then crystallized from MeOH/Et_2O to give the pure HCl salt (715 mg, 68%) mp 165-166 °C, ^{1}H NMR (DMSO): δ 10.48 (1H, brs), 8.04 (2H, dd, *J* = 5.7, 9.3 Hz), 7.35 (2H, t, *J* = 8.7 Hz), 7.19 (2H, d, *J* = 9.3 Hz), 7.76 (2H, d, *J* = 9.3 Hz), 3.78 (2H, m), 3.42 (4H, m), 3.15 (6H, m), 2.38 (1H, m), 2.15 (1H, m), 2.03 (2H, m). *Calcd for* $C_{21}H_{25}Cl_2FN_2O$: C, 61.32; H, 6.13; N, 6.81. *Found*: C, 61.20; H, 5.98; N, 6.78.

9-Methyl-3,9-diazabicyclo[4.2.1]nonan-4-one, 32

Using the method reported by Michaels and Zaugg,[25] conc. H_2SO_4 (37.5 mL) was added drop wise at −20 °C, to a mixture of tropinone, **31** (16.7g, 32 mmol) in anhydrous $CHCl_3$ (120 mL). This was followed by a slow addition of sodium azide (15.6 g) for 30 minutes while maintaining the temperature below −5 °C. After completing the addition, the reaction mixture was stirred at room temperature for 15 min, and then heated at 50 °C overnight under nitrogen. The reaction mixture was then neutralized with $NaHCO_3$, extracted with CH_2Cl_2, and the organic layer was collected, dried over Na_2SO_4 and evaporated in vacuo to afford a light yellowish substance as **32** (13.8 g, 78 %); MS: 155 (M+1)$^+$, IR (KBr): 3190 (-NH), 1620 (C=O).

3,9-Diazabicyclo[4.2.1]nonane-3,9-dicarboxylic acid 3-tert-butyl ester 9-ethyl ester, 35

To a solution of compound **32** (15.2g, 30 mmol) in anhydrous THF (100 mL), was added very slowly under nitrogen powdered LAH (14.1 g, 8 eq). The mixture was allowed to stir at room temperature overnight before being quenched with MeOH, followed by H_2O, filtered, washed twice with CH_2Cl_2, extracted, dried over Na_2SO_4 and evaporated to yield 9-Methyl-3,9-diazabicyclo[4.2.1]nonane, compound **33** (11 g, 80 %). TLC analysis showed a single spot and was used as such without characterization. Compound **33** (12.0 g, 24 mmol) was dissolved in 15 mL of *t*-BuOH and added a solution of Boc-anhydride (5.8g, 28mmol) in *t*-BuOH (10 mL) for 5 min. and the reaction mixture was stirred overnight. The reaction was then neutralized with $NaHCO_3$, extracted with CH_2Cl_2, dried over Na_2SO_4 and evaporated in vacuo to yield 9-Methyl-3,9-diazabicyclo[4.2.1]nonane-3-carboxylic acid t-butyl ester, **34** (13.8 g, 67 %).

Compound **34** (5.8 g, 32 mmol) was dissolved in anhydrous toluene (20 mL) and ethyl chloroformate (3.6 g, 33.3 mmol) was slowly added in a drop-wise manner. The reaction mixture was refluxed overnight and the solvent was evaporated as an azeotrope with alcohol. The resulting product, 3,9-diazabicyclo[4.2.1]nonane-3,9-dicarboxylic acid 3-tert-butyl ester 9-ethyl ester, **35** was dried and used as such without further purification for the next reaction.

3-(4-Chlorophenyl)-3,9-diazabicyclo[4.2.1]nonane-9-dicarboxylic acid ethyl ester, 37

Compound **35** (5.5 g, 18.4 mmol) was dissolved in CF_3COOH (3 mL) and stirred for 4h at room temperature. The reaction mixture was then neutralized with $NaHCO_3$, extracted with CH_2Cl_2, dried over Na_2SO_4 and evaporated to get a light yellowish oil, 3,9-diazabicyclo[4.2.1] nonane-9-carboxylic acid ethyl ester, **36** (2.85 g, 78 %). Compound **36** (5 g, 25.6 mmol), 4-chlorophenylboronic acid (5.6 g, 35.8 mmol), $Cu(OAc)_2$ (5.7g, 31.4 mmol) and Et_3N (2 mL) in CH_2Cl_2 (50 mL) with molecular sieves (2 g) was stirred in open air for 48 h. The reaction mixture was then quenched with methanolic NH_3 solution, filtered over celite, extracted with CH_2Cl_2 (100 mL), dried and solvent evaporated under vacuum. The crude residue was subjected to column chromatography (silica gel, 4:1 CH_2Cl_2:MeOH) to give a yellowish compound **37** (4.5 g, 58 %). ^{1}H NMR (300MHz, $CDCl_3$): δ 1.20-1.26 (m, 3H), 1.39-1.6 (m, 2H), 1.75-1.95 (m, 2H), 2.0-2.29 (m, 2H), 3.0-3.30 (m, 2H), 3.54-3.68 (m, 1H), 3.70-3.8 (m, 1H), 4.05-4.20 (m, 2H), 426-4.43 (m, 2H), 6.62-6.66 (dd, 2H, J = 7.8, 3.3 Hz), 7.06-7.12 (m, 2H).

3-(4-Chlorophenyl)-3,9-diazabicyclo[4.2.1]nonane, 38

Compound **37** (2.2 g, 7.12 mmol), 50 % aq. KOH (2 ml) in ethylene glycol (2 mL) was heated at 90 °C overnight. The reaction mixture was then extracted with CH_2Cl_2 (2 × 80 mL), dried over Na_2SO_4 and evaporated under vacuum. The crude product was subjected to column chromatography (silica gel, 4:2 CH_2Cl_2:MeOH) to yield compound **38** (0.99 g, 59 %). ^{1}H NMR (300MHz, $CDCl_3$): δ 1.32-1.76 (m, 4H), 1.90-2.25 (m, 4H), 3.15-3.35 (m, 4H), 6.63 (d, 2H, J = 9 Hz), 7.09 (d, 2H, J = 9 Hz).

4-[3-(4-Chlorophenyl)-3,9-diazabicyclo[4.2.1]non-9-yl]-1-(4-fluorophenyl)butan-1-one, 14

A mixture of compound **38** (0.45g, 1.9 mmol) and K_2CO_3 (1g, 7.6 mmol) in dry DME (15 mL) was heated at 80 °C for 20 minutes and 4-chloro-4-fluorobutyrophenone (2.2 ml, 7.6 mmol) was added in a drop-wise manner and refluxed overnight under nitrogen. Excess DME was removed in vacuo and the residue was extracted with CH_2Cl_2 (3 × 60 ml). The pooled organic phase was collected and dried over Na_2SO_4 and evaporated under vacuum. The crude product was subjected to column chromatography (silica gel, 4:1 CH_2Cl_2-EtOAc) to yield compound **4** (0.55 g, 68.2 %) as an oil which was converted into the oxalate salt. (White crystalline solid, mp:182-184 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20 -1.66 (m, 4H), 1.80-2.00 (m, 4H), 2.50-2.75 (m, 2H), 2.90-3.05 (m, 2H), 3.35-3.60 (m, 6H), 6.58 (m, 2H), 7.05-7.15 (m, 4H), 7.91-7.97 (m, 2H), Anal. Calcd. for $C_{25}H_{26}N_2O_5ClF$: $0.25H_2O$: C, 60.61; H, 5.80; N, 5.65. Found C, 60.53; H, 5.75; N, 5.53.

Biology

Receptor Binding Studies

Binding affinities reported in Tables 1 – 3 were conducted by the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP) unless otherwise stated. Details of the methods and radioligands used for the binding assays were previously reported.[26]

Apomorphine Induced Climbing Stereotypy

A modified climbing test by Needham *et al.* was used[27] and provides the extent a potential antipsychotic agent inhibits the effect of apomorphine induced stereotypy. Swiss Webster male mice (~30 g) in groups of 5 were administered an intraperitoneal (IP) injection of 0.3 mL of vehicle (filtered 1% lactic acid) or increasing doses of the dopamine antagonists haloperidol (0.001, 0.01, 0.02, 0.05, 0.1, 0.2 mg/kg), compound **13** (12, 15, 17, 20, 25, 50, 80 mg/kg), and clozapine (3, 10, 20, 30, 60 mg/kg). Animals were then challenged at 30 minutes post injection with 1.5- 1.6 mg/kg of the agonist apomorphine in 0.9% NaCl + 0.1% ascorbic acid, placed in cylindrical wire cages (12 cm in diameter, 14 cm in height), and observed for climbing behavior at 10 and 20 minutes post dose. Climbing behavior was assessed as follows: 3-4 paws on the cage floor = 0 score; 2 and 3 paws on the cage = 1 score; 4 paws on the cage = 2 score. Scores were expressed as mean % climbing inhibition, and plotted in figures 1-3. ED50s in Table 4 were calculated using Graph Pad Prism non-linear regression software with sigmoidal dose-response, variable slope curve-fitting.

Bar Test for Catalepsy

The bar test used by Hoffman and Donovan[28] was modified for our use. Male Sprague-Dawley rats (88-207 g) in groups of 5-8 were dosed by IP injection with 1-23 mL/kg of vehicle (filtered 1% lactic acid), haloperidol (0.3 mg/kg), compound **13** (28, 42, 70 mg/kg), or clozapine (30 mg/kg). Catalepsy severity was assessed at 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) up to a maximum of 120 seconds. This was repeated 2 more times and the average (n=3 tries) seconds recorded for each rat. The mean of all the average seconds for one dose for one time point (n=5-8 rats) was divided by 5 and a mean catalepsy score assigned and plotted in figure 4 with a maximum possible score of 24.

Acknowledgements

References

1. Crilly J. The history of clozapine and its emergence in the US market: a review and analysis. Hist Psychiatry 2007;18:39–60. [PubMed: 17580753]

2. a) Peabody CA, Brody D, Warner MD. Tardive dyskinesia after low-dose haloperidol. Biol Psychiatry 1987;22:111–112. [PubMed: 3790633] b) Riddle MA, Hardin MT, Towbin KE, Leckman JF, Cohen DJ. Tardive dyskinesia following haloperidol treatment in Tourette's syndrome. Arch Gen Psychiatry 1987;44:98–99. [PubMed: 3467661] c) 64 Chirita V, Boisteanu P, Pirozynski T. Tardive dyskinesia syndrome in neuroleptic therapy. General considerations and clinical observations on forty cases investigated. Rev Med Chir Soc Med Nat Iasi 1987;91:53. [PubMed: 2889249] d) Munyon WH, Salo R, Briones DF. Cytotoxic effects of neuroleptic drugs. Psychopharmacol (Berl) 1987;91:182–188. e) Petty LK, Spar CJ. Haloperidol-induced tardive dyskinesia in a 10-year-old girl. Am J Psychiatry 1980;137:745–746. [PubMed: 7377407] f) Gerlach J, Simmelsgaard H. Tardive dyskinesia during and following treatment with haloperidol, haloperidol + biperiden, thioridazine, and clozapine. Psychopharmacol (Berl) 1978;59:105–112.

3. Ablordeppey SY, Borne RF. Detection of a quaternary pyridinium metabolite in the liver of a haloperidol-treated rat. Pharmacol Biochem Behavior 1993;46:739–744.

4. a) Subramanyam B, Rollema H, Woolf T, Castagnoli N Jr. Identification of a potentially neurotoxic pyridinium metabolite of haloperidol in rats. Biochem Biophys Res Commun 1990;166:238–244.

[PubMed: 2302206] b) Subramanyam B, Pond SM, Eyles DW, Whiteford HA, Fouda HG, Castagnoli N Jr. Identification of potentially neurotoxic pyridinium metabolite in the urine of schizophrenic patients treated with haloperidol. Biochem Biophys Res Commun 1991;181:573. [PubMed: 1755839] c) Subramanyam B, Woolf T, Castagnoli N Jr. Studies on the in-vitro conversion of haloperidol to a potentially neurotoxic pyridinium metabolite. Chem Res Toxicol 1991;4:123–128. [PubMed: 1912294]

5. a) Fang J, Gorrod JW. Dehydration is the first step in the bioactivation of haloperidol to its pyridinium metabolite. Toxicol Lett 1991;59:117–123. [PubMed: 1755019] b) Eyles DW, McLennan HR, Jones A, McGrath JJ, Stedman TJ, Pond SM. Quantitative analysis of two pyridinium metabolites of haloperidol in patients with schizophrenia. Clin Pharmacol Ther 1994;56:512–520. [PubMed: 7955815] c) Eyles DW, Avent KM, Stedman TJ, Pond SM. Two pyridinium metabolites of haloperidol are present in the brain of patients at post-mortem. Life Sci 1997;60:529–534. [PubMed: 9042387] d) Fang J, Zuo D, Yu PH. Comparison of cytotoxicity of a quaternary pyridinium metabolite of haloperidol with neurotoxin N-methyl-4-phenylpyridinium towards cultured dopaminergic neuroblastoma cells. Psychopharmacology 1995;121:373–378. [PubMed: 8584620] e) Rollema H, Skolnik M, D'Engelbronner J, Igarashi K, Usuki E, Castagnoli N Jr. MPP+-like neurotoxicity of a pyridinium metabolite derived from haloperidol: in vivo microdialysis and in vitro mitochondrial studies. J Pharmacol Exp Ther 1994;268:380–387. [PubMed: 8301579]

6. a) Ablordeppey SY, Lyles-Eggleston M, Bricker B, Zhang W, Zhu XY, Goodman C, Roth BL. 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. Bioorg Med Chem Lett 2006;16:3219–3223. [PubMed: 16621538] b) Ablordeppey SY, Borne RF. Design and Synthesis of Novel analogues of haloperidol incapable of forming MPP+-like metabolites. Med Chem Res 1993;3:459–467.

7. Lyles-Eggleston M, Altundas R, Xia J, Sikazwe DMN, Fan P, Yang Q, Li S, Zhang W, Zhu X, Schmidt AW, Vanase-Frawley M, Shrhkande A, Villalobos A, Ablordeppey SY. Design, Synthesis, and Evaluation of Metabolism-Based Analogues of haloperidol Incapable of Forming MPP+-like Species. J Med Chem 2004;47:497–508. [PubMed: 14736232]

8. Sikazwe DMN, Li S, Mardenborough L, Cody V, Roth BL, Ablordeppey SY. Haloperidol: towards further understanding of the structural contributions of its pharmacophoric elements at D2-like receptors. Bioorg Med Chem Lett 2004;14:5739–5742. [PubMed: 15501032]

9. Sikazwe DMN, Li S, Lyles-Eggleston M, Ablordeppey SY. The Acute EPS of haloperidol May be Unrelated to Its Metabolic Transformation to BCPP+ Bioorg Med Chem Lett 2003;13:3779–3782. [PubMed: 14552778]

10. Tacke R, Popp F, Müller B, Theis B, Burschka C, Hamacher A, Kassack MU, Schepmann D, Wünsch B, Jurva U, Wellner E. Sila-Haloperidol, a Silicon Analogue of the Dopamine (D(2)) Receptor Antagonist haloperidol: Synthesis, Pharmacological Properties, and Metabolic Fate. ChemMedChem 2008;3:152–164. [PubMed: 18022977]

11. a) McIntyre RS, McCann SM, Kennedy SH. Antipsychotic metabolic effects: weight gain, diabetes mellitus, and lipid abnormalities. Can J Psychiatry 2001;46:273–81. [PubMed: 11320682] b) Taylor DM, McAskill R. Atypical antipsychotics and weight gain--a systematic review. Acta Psychiatr Scand 2000;101:416–32. [PubMed: 10868465] c) Wirshing DA, Spellberg BJ, Erhart SM, Marder SR, Wirshing WC. Novel antipsychotics and new onset diabetes. Biol Psychiatry 1998;44:778–83. [PubMed: 9798083]

12. a) Seeger TF, Seymour PA, Schmidt AW, Zorn SH, Schulz DW, Lebel LA, McLean S, Guanowsky V, Howard HR, Lowe JA, Heym J. Ziprasidone (CP-88,059): a new antipsychotic with combined dopamine and serotonin receptor antagonist activity. J Pharmacol Exp Ther 1995;275:101–113. [PubMed: 7562537] b) Schmidt AW, Lebel LA, Howard HR Jr, Zorn SH. Ziprasidone: a novel antipsychotic agent with a unique human receptor binding profile. Eur J Pharmacol 2001;425:197–201. [PubMed: 11513838] c) McCreary AC, Glennon JC, Ashby CR Jr, Meltzer HY, Li Z, Reinders JH, Hesselink MB, Long SK, Herremans AH, van Stuivenberg H, Feenstra RW, Kruse CG. SLV313 (1-(2,3-dihydro-benzo[1,4]dioxin-5-yl)-4- [5-(4-fluoro-phenyl)-pyridin-3-ylmethyl]-piperazine monohydro-chloride): a novel dopamine D2 receptor antagonist and 5-HT1A receptor agonist potential antipsychotic drug. Neuropsychopharmacol 2007;32:78–94.

13. Matsui-Sakata A, Ohtani H, Sawada Y. Receptor occupancy-based analysis of the contributions of various receptors to antipsychotics-induced weight gain and diabetes mellitus. Drug Metab Pharmacokinet 2005;20:368–78. [PubMed: 16272755]

14. Sanberg PR, Bunsey MD, Giordano M, Norman AB. The catalepsy test: its ups and downs. Behav Neurosci 1988;102:748–759. [PubMed: 2904271] b) Crocker AD, Hemsley KM. An animal model of extrapyramidal side effects induced by antipsychotic drugs: Relationship with D2 dopamine receptor occupancy. Prog Neuro-Psychophamachol & Biol Psychiat 2001;25:573–590. c) Hoffman DC, Donovan H. Catalespy as a rodent model for detecting antipsychotic drugs with extrapyramidal side effect liability. Psychopharmacology 1995;120:128–133. [PubMed: 7480543]

15. Cignarella G, Giangiacomo N, Emilio O. Bicyclic homologs of piperazine. II. Synthesis of 3,8-diazabicyclo[3.2.1]octane. New synthesis of 8-methyl-3,8-diazabicyclo[3.2.1]octane. J Org Chem 1961;26:2747.

16. Thompson PE, Zeigler JB, McCall JW. Antifilarial Agents. Diazabicyclooctanes and diazabicycloheptanes as bridged analogs of diethylcarbamazine. J Med Chem 1974;17:481–487. [PubMed: 4830249]

17. Yevich JP, New JS, Lobeck WG, Dextraze P, Bernstein E, Taylor DP, Yocca FD, Eison MS, Temple DL Jr. Synthesis and biological characterization of α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol and analogs as potential atypical antipsychotic agents. J Med Chem 1992;35:4516–25. [PubMed: 1361578]

18. a) Seeman P, Lee T, Chau-Wong M, Wong K. Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature 1976;261:717–9. [PubMed: 945467] b) Seeman P, Chau-Wong M, Tedesco J, Wong K. Brain receptors for antipsychotic drugs and dopamine: direct binding assays. Proc Natl Acad Sci U S A 1975;72:4376–80. [PubMed: 1060115] c) Seeman P, Corbett R, Van Tol HHM. Atypical neuroleptics have low affinity for dopamine D2 receptors or are selective for D4 receptors. Neuropsychopharmacol 1997;16:93–110.

19. a) Bardin L, Auclair A, Kleven MS, Prinssen EP, Koek W, Newman-Tancredi A, Depoortère R. Pharmacological profiles in rats of novel antipsychotics with combined dopamine D2/serotonin 5-HT1A activity: comparison with typical and atypical conventional antipsychotics. Behav Pharmacol 2007;18:103–18. [PubMed: 17351418] b) Cuisiat S, Bourdiol N, Lacharme V, Newman-Tancredi A, Colpaert F, Vacher B. Towards a new generation of potential antipsychotic agents combining D2 and 5-HT1A receptor activities. J Med Chem 2007;50:865–76. [PubMed: 17300168]

20. a) Meltzer HY, Matsubara S, Lee JC. Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin-2 pKi values. J Pharmacol Exp Ther 1989;251:238–246. [PubMed: 2571717] b) Rasmussen K, Aghajanian GK. Potency of antipsychotics in reversing the effects of a hallucinogenic drug on locus coeruleus neurons correlates with 5-HT2 binding affinity. Neuropsychopharmacol 1988;1:101–107.

21. Meltzer HY, Li Z, Kaneda Y, Ichikawa J. Serotonin receptors : their key role in drugs to treat schizophrenia. Progress in Neuro-Psychopharmacology and Biological Psychiatry 2003;27:1159–1172. [PubMed: 14642974]

22. a) Kroeze WK, Hufeisen SJ, Popadak BA, Renock SM, Steinberg S, Ernsberger P, Jayathilake K, Meltzer HY, Roth BL. H1-histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. Neuropsychopharmacol 2003;28:519–526. b) Matsui-Sakata A, Ohtani H, Sawada Y. Receptor occupancy-based analysis of the contributions of various receptors to antipsychotics-induced weight gain and diabetes mellitus. Drug Metab Pharmacokinet 2005;20:368–378. [PubMed: 16272755]

23. a) Setola V, Dukat M, Glennon RA, Roth BL. Molecular determinants for the interaction of the valvulopathic anorexigen norfenfluramine with the 5-HT2B receptor. Mol Pharmacol 2005;68:20–33. [PubMed: 15831837] b) Rothman RB, Baumann MH, Savage JE, Rauser L, McBride A, Hufeisen SJ, Roth BL. Evidence for possible involvement of 5-HT(2B) receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. Circulation 2000;102:2836–41. [PubMed: 11104741]http://cardiology.jwatch.org/cgi/content/full/2007/103/2)

24. http://www.rxlist.com/cgi/generic/abilify_cp.htm

25. Michaels RJ, Zaugg HE. Synthesis of 9-Methyl-3,9-diazabicyclo[4.2.1]-nonane, 25. J Org Chem 1960:637–638.

26. Shapiro DA, Renock S, Arrington E, Chiodo LA, Liu LX, Sibley DR, Roth BL, Mailman R. Aripiprazole, a novel atypical antipsychotic drug with a unique and robust pharmacology. Neuropsychopharmacology 2003;28:1400–1411. [PubMed: 12784105]b)See references in ref 25a

27. a) Needham PL, Atkinson J, Skill MJ, Heal DJ. Zotepine: preclinical tests predict antipsychotic efficacy and an atypical profile. Psychopharmacol Bull 1996;32:123. [PubMed: 8927661] b) Protais P, Costentin J, Schwartz JC. Climbing Behavior Induced by Apomorphine in Mice: a Simple Test for the Study of Dopamine Receptors in Striatum. Psychopharmacology 1976;50:1–6. [PubMed: 827755]

28. a) Hoffman DC, Donovan H. Catalepsy as a rodent model for detecting antipsychotic drugs with extrapyramidal side effect liability. Psychopharmacol (Berl) 1995;120:128–33. b) Hoffman DC, Donovan H. D1 and D2 dopamine receptor antagonists reverse prepulse inhibition deficits in an animal model of schizophrenia. Psychopharmacol (Berl) 1994;115:447–53.

Apomorphine Challenge Test of Haloperidol
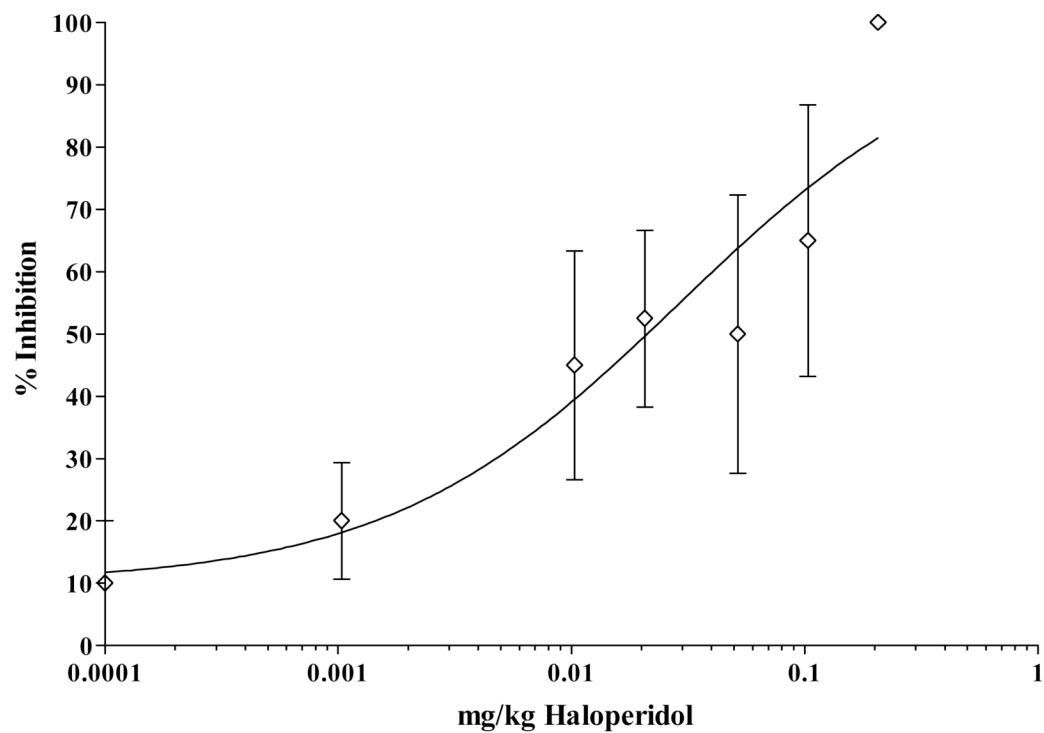
◇ Haloperidol; (error bars are +/- SEM); (n=5)

Fig 1.

Apomorphine Challenge Test of Compound 13

Compound 13 ; (error bars are +/- SEM); (n=5)

Fig 2.

Apomorphine Challenge Test of Clozapine

○ Clozapine ; (error bars are +/- SEM); (n=5)

Fig 3.

Catalepsy of Haloperidol, Compound 13, Clozapine & Vehicle

Error bars are +/- SEM, n = 5-8
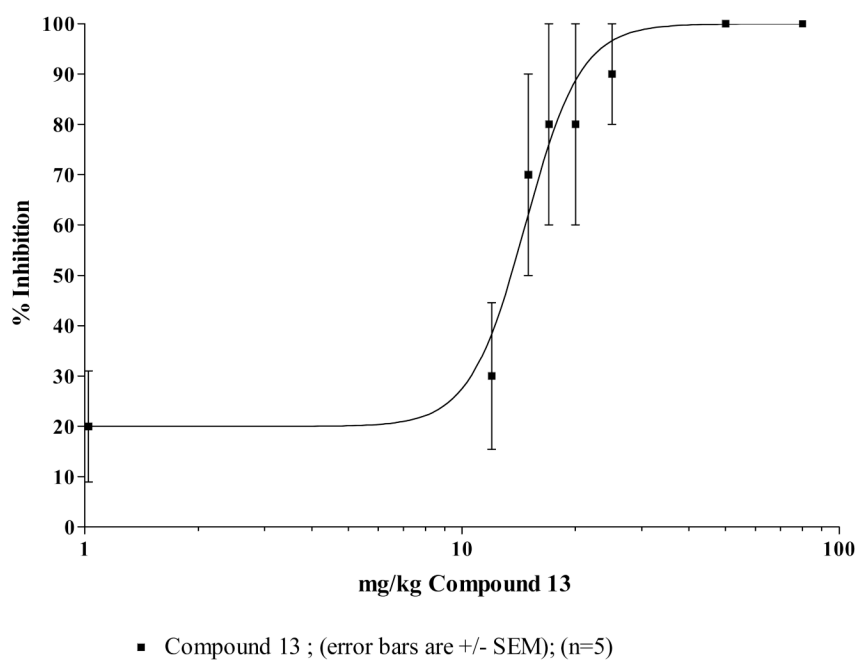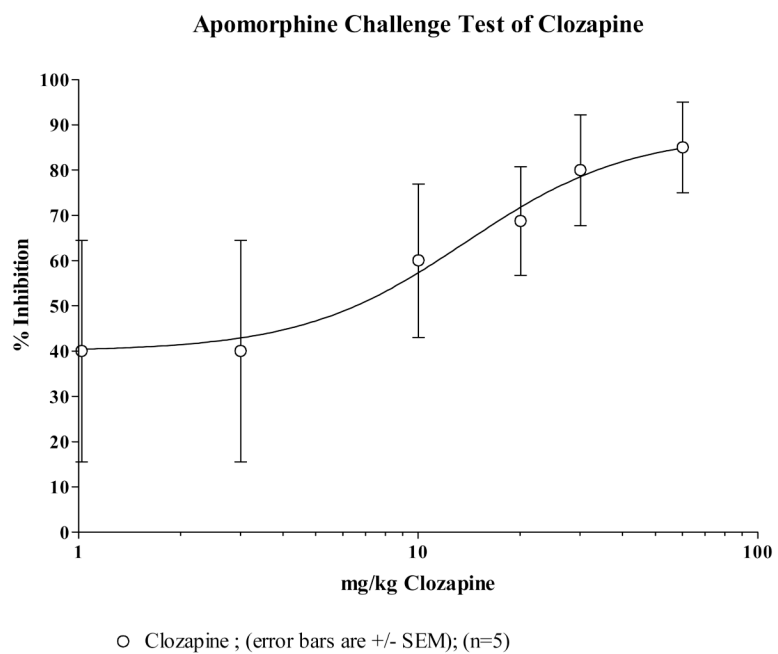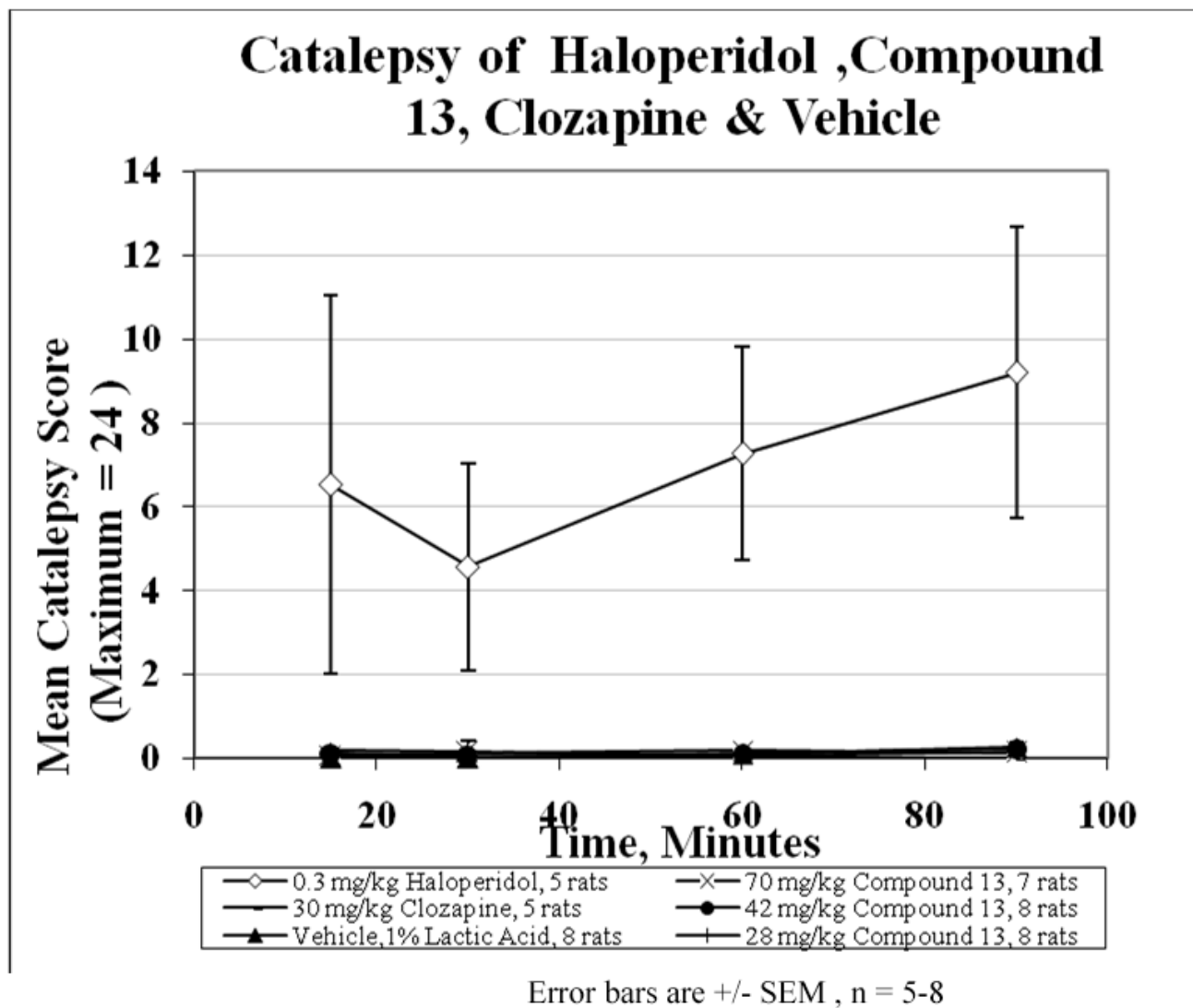
Fig 4.
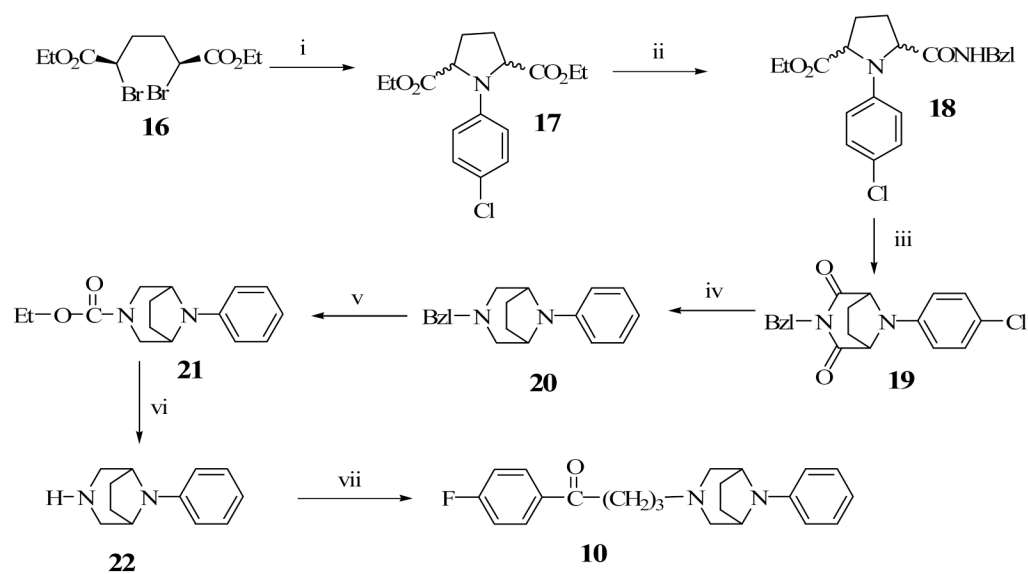
Scheme 1a.

[a] Reagents: (i) 4-Chloroaniline, DME, KI; (ii) Benzylamine, o-Xylene; (iii) (a) NaOH, EtOH; (b) Ac$_2$O, NaOAc; (iv) LiAlH$_4$; (v) ClCO$_2$Et, Toluene; (vi) KOH, EtOH, NH$_2$NH$_2$; (vii) 4-Chloro-4'-fluorobutyrophenone, KI, K$_2$CO$_3$, DME.
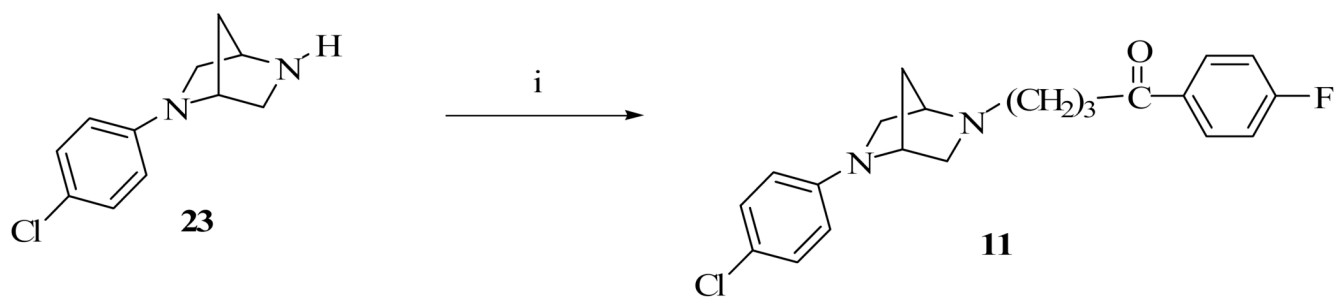
Scheme 2a.
a Reagents: (i) 4-Chloro-4′-fluorobutyrophenone, KI, K_2CO_3, DME.
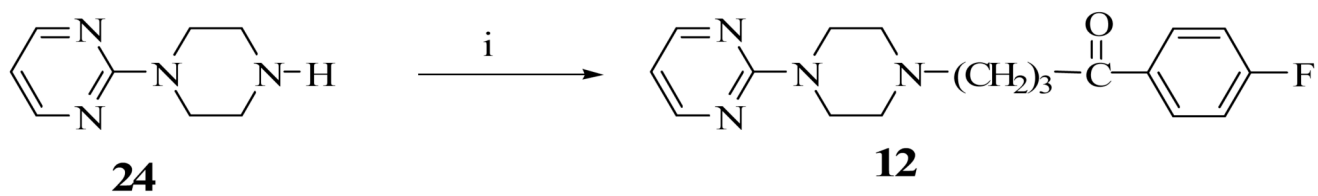
Scheme 3a.
a Reagents: (i) 4-Chloro-4′-fluorobutyrophenone, KI, K_2CO_3, DME.
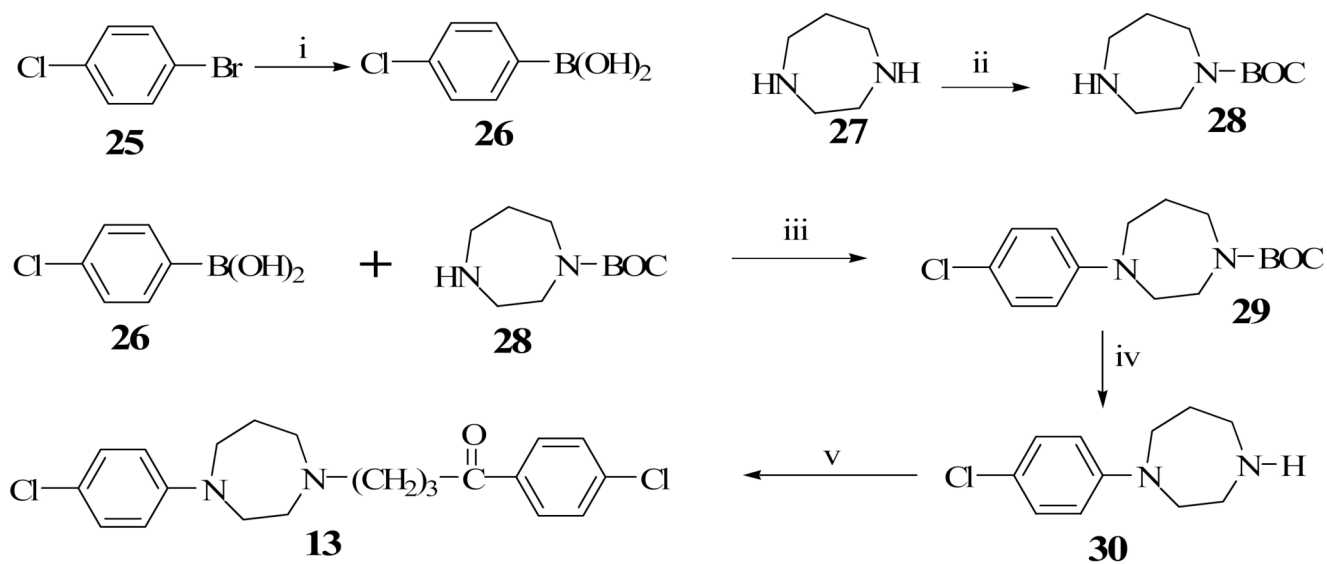
Scheme 4a.

[a] Reagents: (i) (a) n-BuLi, -78 °C, THF; (b) $B(OMe)_3$; (ii) t-BuOH, NaOH, (t-BuOCO)$_2$O; (iii) $Cu(OAc)_2$, NEt$_3$, Mol sieves (4Å), rt; (iv) TFA, CH_2Cl_2; (v) 4-Chloro-4′-fluorobutyrophenone, KI, K_2CO_3, DME.
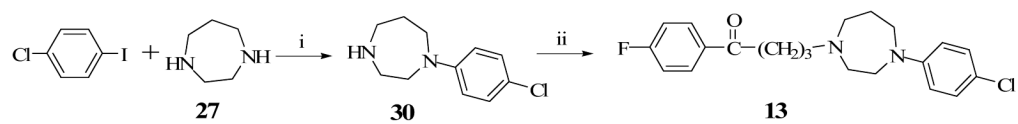
Scheme 5a.

Alternative Synthetic Scheme for Compound **13**

[a] Reagents and conditions: (i) CuI, ethylene glycol, K_2CO_3, [i]PrOH, 80 °C, reflux, 12 h; (ii): KI, K_2CO_3, DME, 4-chloro-4′-fluorobutyrophenone, reflux, 12 h.
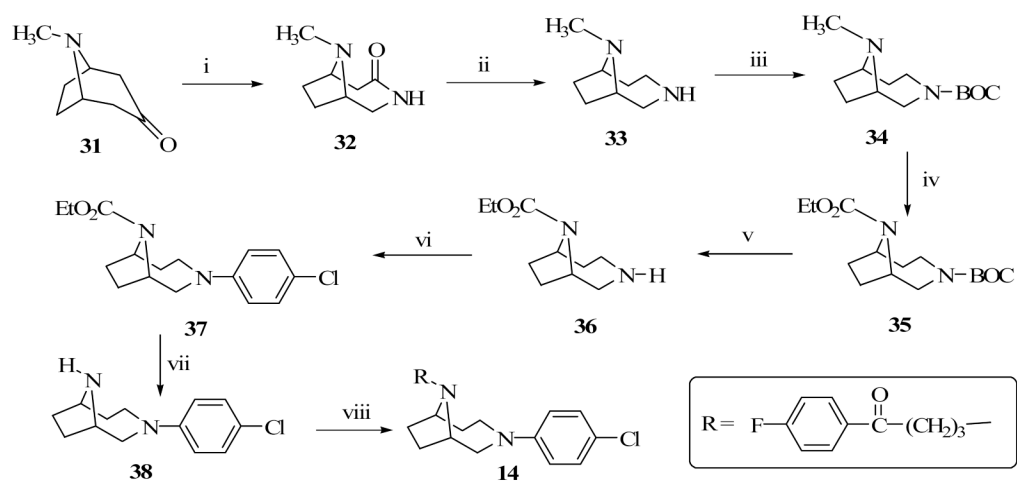
Scheme 6a.
[a] Reagents: (i) H_2SO_4, NaN_3; (ii) $LiAlH_4$, THF; (iii) t-BuOH, (t-BuOCO)$_2$O; (iv) $ClCO_2Et$, Toluene; (v) TFA (vi) 4-Chlorophenylboronic Acid, $Cu(OAc)_2$, Et_3N, CH_2Cl_2, Mol sieves (4Å), RT, 24 – 48 hr; (vii) KOH, Ethylene glycol; (viii) 4-Chloro-4′-fluorobutyrophenone, KI, K_2CO_3, DME.
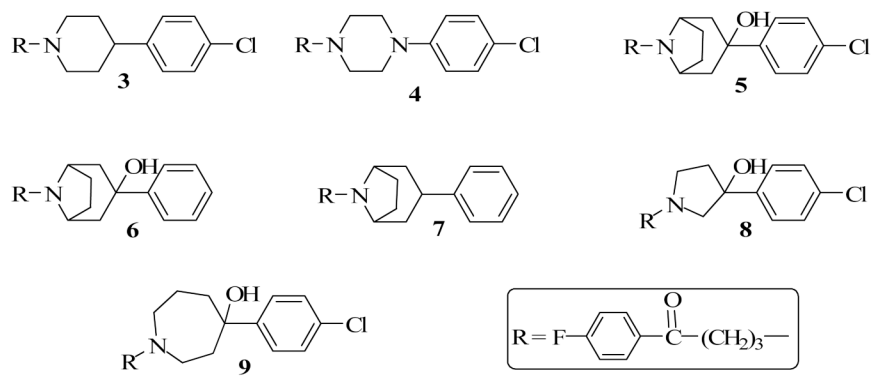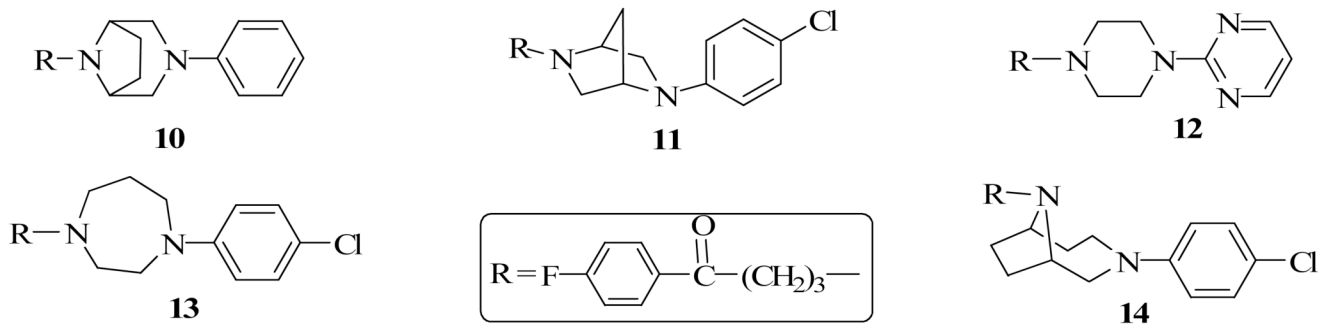
Chart 1.

Chart 2.

10

11

12

13

R = F—⟨⟩—C(=O)—(CH₂)₃—

14
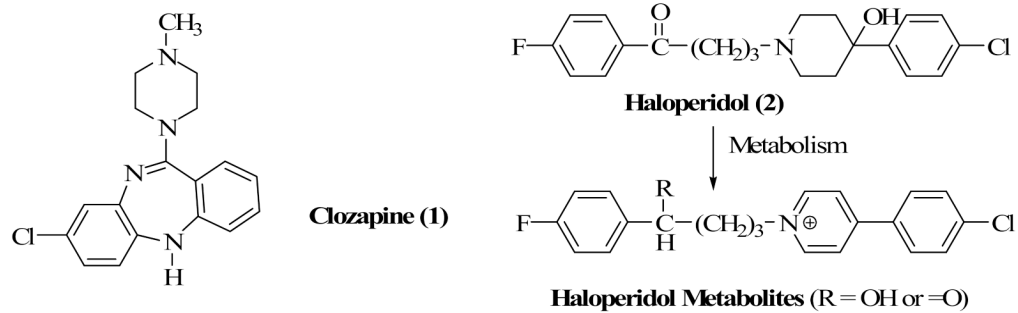
Chart 3.

Table 1

Binding Affinity Constants of Compounds to Selected 5-HT and H-1 Receptors.

Compds	Binding Data of Compounds, Ki ± SEM (nM)							Ki D_2/Ki D_4 Ratio
	DAD_2	DAD_4	$5HT_{1A}$	$5HT_{2A}$	$5HT_{2C}$	H-1	
Cloz (**1**)[a]	130	54	140	8.9	17	1.8	2.4
Hal (**2**)[a]	0.89	10	3600	120	4700	440	0.09
4[b]	253.5±38.9	17.5±2.0	90.9±21.0	109.6±16.0	3552±943	157.6±36.0	2.8
5[b]	1.6±0.14	5.3±1.0	27.7±8.0	30.9±6.0	872.1±178.0	8780±1625	0.30
6[b]	2.3±0.28	19.2±2.3	37.6±6.0	12.3±3.0	>10,000	635.4±96.0	0.12
7[b]	124.4±16.5	176.8±36.0	414.4±101.0	106.0±21.0	1065±244	973.5±207.0	0.70
(+)-**8**[b]	51.1±6.0	3.6±0.48	772.3±90.0	75.8±12.0	3598±1162	1467±473	14.2
(-)-**8**[b]	489.4±119.4	245.5±29.2	831.5±126.0	241.5±39.0	1252±461	259.6±34.0	2.0

[a]Binding data obtained from reference 12b.

[b]The synthesis and/or DA binding data of compounds previously reported in ref. 7 - 9

Table 2

Binding Affinity Constants of Compounds to Dopamine, 5HT and Histamine Receptors.

Compds	Binding (Ki±SEM) Data of Synthetic Compounds at Selected Receptors (nM)							
	D2	D3	D4	5HT$_{1A}$	5HT$_{2A}$	5HT$_{2C}$	H1
Cloz(**1**)[a]	130	240	54	140	8.9	17.0	1.8
Hal (**2**)[a]	1.4	2.5	3.3	3600	120	4700	440
10[b]	155	600	2100	ND	ND	ND	ND
11[b]	170	220	520	ND	ND	ND	ND
12	98.0±15.3	244.1±106.0	6.53±0.76	30.5±5.0	22.0±4.0	4132±1081	911.8±152.0
13	43.3±13.3	158.8±35.1	6.6±0.6	117.4±32.6	23.6±2.7	1425±207	188.6±16.0
14	178.4±29.2	548.1±246.0	41.8±9.0	2332±470	194.8±53.0	3513±912	1014±206

ND = Not Determined.; Hal = Haloperidol; Cloz = Clozapine

[a]Data for compounds **1** & **2** were obtained from ref. 12b.

[b]Data from Pfizer Global Research and Development Laboratories by A. W. Schmidt.

Table 3

Comparative Binding Affinity Data for Compound **13** and Agents currently in use at Human Receptors and Rat Transporters.

[a] Receptor	Binding (Ki±SEM) Data of Compd 13 and standard agents at Selected Receptors (nM)[b]							
	Comp 13	[b]Clozapine	[b]Risperidone	[b]Ziprasidone	[b]Olanzapine	[b]Quetiapine	[b]Haldol
DA D1	162.7±33.1	290	580	130	52	1300	120
DA D2	43.3±13.3	130	2.2	3.1	20	180	1.4
DAD3	158.8±35.1	240	9.6	7.2	45	320	2.5
DAD4	6.6±0.6	54	8.5	32	50	2200	3.3
$5HT_{1A}$	117.4±32.6	140	210	2.5	2100	230	3600
$5HT_{2A}$	23.6±2.7	8.9	0.29	0.39	3.3	220	120
$5HT_{2B}$	495.2±94.0	NR	NR	NR	NR	NR	NR
$5HT_{2C}$	1425.0±207	17	10	0.72	10	1400	4700
$5HT_6$	295.9±48.6	11	2000	76	10	1400	6000
Hist H1	188.6±16.0	1.8	19	47	2.8	8.7	440
M1	871.6±75.6	1.8	2800	5100	4.7	100	1600
DAT	1150±133	NR	NR	NR	NR	NR	NR
NET	850.7±175.0	390	28000	48	2000	680	5500
SERT	56.6±6.0	3900	1400	53	>15,000	>18,000	1800

[a] Human cloned receptors were used in all cases except for the NT transporters which are from rats.

[b] Data for standard drugs were obtained from Schmidt et al., reference 12b.

NR = Not Reported.

Table 4

Comparison of ED50 values for Apomorphine Test

Test Compound	FW	ED50, mg/kg	95% Confidence Interval, mg/kg	ED50, μmoles/kg
Compound **13**	411.3	14.7	13.4 to 16.1	35.8
Clozapine	326.8	13.8	7.57 to 25.3	42.4
Haloperidol	375.9	0.0293	0.0112 to 0.0768	0.0779