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Indoline and Piperazine Containing Derivatives as a Novel Class of Mixed D_2/D_4 Receptor Antagonists. Part 2: Asymmetric Synthesis and Biological Evaluation

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Abstract—A series of chiral benzylpiperazinyl-1-(2,3-dihydro-indol-1-yl)ethanone derivatives were prepared and examined for their affinity at dopamine D_2 and D_4 receptors. Three compounds having D_2/D_4 affinity ratios approximating that found for the atypical neuroleptic clozapine were further evaluated in behavioral tests of antipsychotic efficacy and motor side effects.
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About 1% of the general population suffers from schizophrenia at some point in their life, making this disease one of the most common CNS disorders in the world. Since (Arvid) Carlsson and his colleagues first found that antipsychotic agents increased dopamine turnover in rodent brain in 1963,[1] the dopamine hypothesis of schizophrenia has been used as the primary rationale for antipsychotic drug design. Indeed, most clinically effective antipsychotic drugs display a high correlation between their clinical potency and their affinity at the dopamine D_2 receptor, suggesting this site as the critical target for mediating therapeutic effects. Clozapine (**1**) is unique among neuroleptic drugs in that it shows a low propensity to induce the motor side effects common to the drug class. In fact, reports suggested that the clinical potency of clozapine was found to correlate better with its affinity for the dopamine D_4 than the D_2 receptor. This focused attention on D_4 receptor as a potential target for antipsychotic drug development and a variety of D_4 selective chemical entities were subsequently identified. Unfortunately, although several D_4 receptor specific antagonists were examined in clinical trials,[2–5] no positive efficacy data for these agents was reported.

In light of the clinical finding that selective D_4 antagonist properties alone provided insufficient efficacy in treating psychosis, we considered the possibility that the specific ratio of D_2 to D_4 receptor binding affinity found in clozapine may explain the drug's unique profile. We therefore set out to identify drug candidates having a more clozapine-like mix of affinities for these two dopamine receptor subtypes,[6–8] with a target of high D_4 (<10 nM) and moderate D_2 (<200 nM) affinities which maintained a similar binding ratio to that of clozapine. Literature reports of this ratio vary from approximately 10/1 to a lower limit of 2/1. For this study, we used our experimentally derived ratio of 10/1 as our target point. A secondary criteria of our search required lower binding affinity to α_1 (<1000 nM) in order to avert undesirable cardiovascular effects.

In the proceeding paper of this series, we identified two potent mixed D_2/D_4 antagonists 2-[-4-(4-chloro-benzyl)-piperazin-1-yl]-1-(2-methy-2,3-dihydro-indol-1-yl)-ethanone (**2**) and 2-[-4-(4-methyl-benzyl)-piperazin-1-yl]-1-(2-methy-2,3-dihydro-indol-1-yl)-ethanone (**3**) (Fig. 1) which, as racemates, closely fit the criteria. For further studies toward the identification of clinical candidates, we needed to obtain each enantiomer of these two compounds and closely examine the best compounds in behavioral models of antipsychotic efficacy and motor side effects. Herein we report the asymmetric synthesis of **2** and **3**, as well as other chiral 2-substituted indoline containing compounds for further SAR studies.

Scheme 1 depicts the chiral synthesis of (*R*)- 2-[4-(4-methylbenzyl)-piperazin-1-yl]-1-(2-methy-2,3-dihydro-

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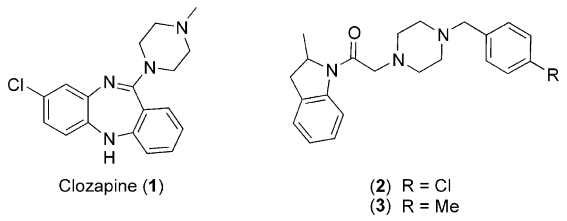
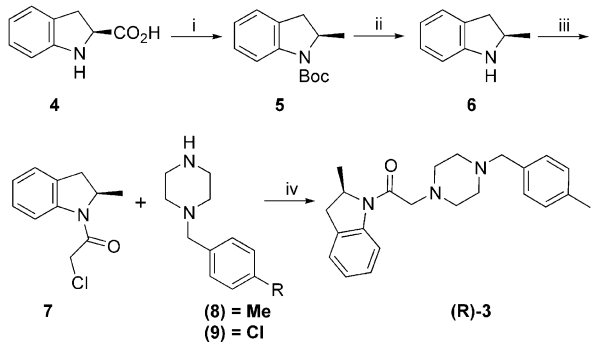


Figure 1.



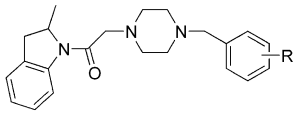
Scheme 1. Reagents and conditions. (i) ref 9; (ii) TFA/DCM (1/1), 0 °C to rt, 16 h, 100%; (iii) chloroacetyl chloride, TEA, $CHCl_3$, 0 °C to rt, 3 h, 87%; (iv) K_2CO_3, MeCN, reflux, 16 h, 92%.

indol-1-yl)ethanone **3**. The intermediate Boc protected indoline **(*R*)-5** was prepared in four steps starting from (*S*)-indoline-2-carboxylic acid **4** using the reported procedure.[9] Treatment of **(*R*)-5** with trifluoroacetic acid in dichloromethane provided (*R*)-2-methylindoline **6**, which then coupled with chloroacetyl chloride to give amide **7**. Reaction of compound **7** with (4-methylbenzyl)piperazine **8** in presence of potassium carbonate in acetonitrile generated the final product **(*R*)-3**.[10] Use of (4-chlorobenzyl)piperazine **9** gave **(*R*)-2**. **(*S*)-2** and **(*S*)-3** were also prepared in the same manner as depicted in Scheme 1 using N-Boc (*S*)-2-methylindoline **5** as the starting material.[9]

As shown in Table 1, the (*R*) configuration of both compounds **2** and **3** display a much higher affinity than the corresponding (*S*) enantiomers for D_2 and D_4 receptors. This encouraged us to investigate additional (*R*)-2-methylindoline containing compounds with various benzylic groups, which could be prepared using **(*R*)-7** as the precursor. Although most substitutions on the benzylic group decreased dopamine binding affinities relative to **(*R*)-2** and **(*R*)-3**, the 5-chloro-2-methoxy compound **(*R*)-23** showed a very promising pharmacological profile.

In order to further explore the effect of alternate groups at the 2 position, various chiral 2-substituted indoline containing compounds were prepared via the routes shown graphically in Scheme 2 and Scheme 3. In Scheme 2, the key intermediate (2*S*)-2-vinylindoline **34** was prepared starting from Boc protected amino alcohol **31**, followed by Swern oxidation, Wittig condensation and removal of the protecting group. Stepwise condensation of **34** with chloroacetyl chloride and 4-chlorobenzylpiperazine provided **35**, which could then hydrogenated

Table 1. Effect of chirality and benzylic group substitution

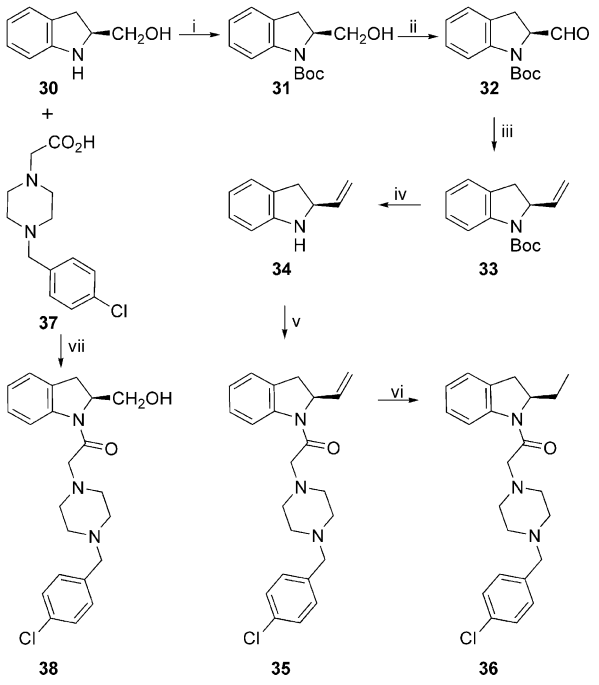


Compd	Config.	R′	K_i (nM)		
			D_2	D_4	α_1
Clozapine	—	—	138	9	—
Haloperidol	—	—	1	5	—
2	*R*	4-Cl	191	6	1899
2	*S*	4-Cl	9395	139	1325
3	*R*	4-Me	113	2	1118
3	*S*	4-Me	2364	64	906
10	*R*	4-F	286	5	2471
11	*R*	4-CF_3	5940	5	> 10,000
12	*R*	4-Et	758	3	1357
13	*R*	4-OMe	673	9.9	724
14	*R*	3-Cl	747	6.1	1685
15	*R*	3-F	1000	6	—
16	*R*	2-Cl	1149	4	> 10,000
17	*R*	2-F	> 1000	27	> 10,000
18	*R*	4-Cl-2-OMe	273	3	1063
19	*R*	3,5-diCl	271	7	> 10,000
20	*R*	2,5-diCl	95	12	2280
21	*R*	3,4-diCl	43	43	305
22	*R*	2,5-diOMe	59	29	1661
23	*R*	5-Cl-2-OMe	28	5	980
24	*R*	5-Cl-2Oet	134	11	1093
25	*R*	5-Cl-2-isoPr	218	10	—
26	*R*	2-Cl-3,4-diF	199	12	> 1000
27	*R*	4-Cl-2,5-diF	2583	6	> 10,000
28	*R*	3-F-4-Me	713	2	807
29	*R*	3,4-OCH_2O–	1434	3	1133

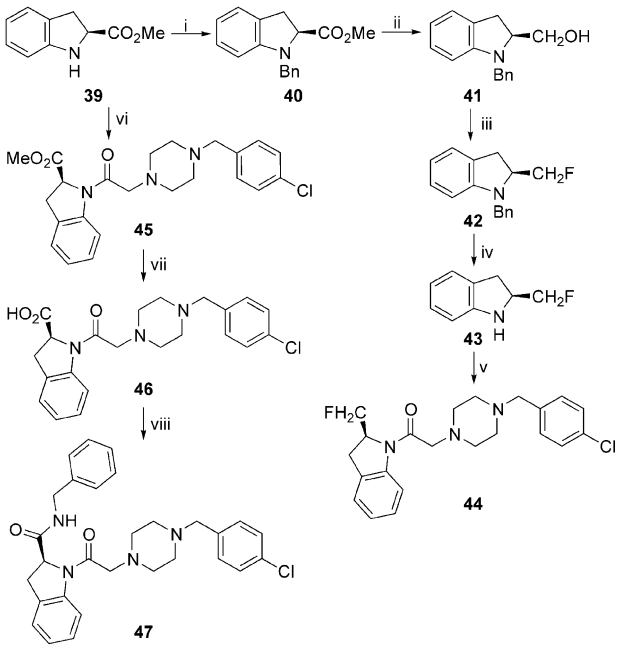
to **36** with 5% Pd/C at atmosphere pressure without effecting the 4-chlorobenzyl group. Coupling of alcohol **30** and acid **37** in the presence of EDCI provided product **38**.

Attempts to prepare (*S*)-2-fluoromethylindoline **43** by treatment of Boc amino alcohol **31** with DAST and then removal of protecting group were not successful. Instead, the intramolecular cyclization compound (*S*)-9,9a-dihydro-1*H*-oxazolo[3,4-*a*]indol-3-one was produced in high yield.[11] Therefore, we selected (*S*)-indoline-2-carboxylic acid methyl ester **39** as the starting material (Scheme 3), which was then protected with a benzyl group. After reduction of **40** with LAH, the hydroxyl group of alcohol **41** was fluorinated by treatment with DAST to give **42**. Hydrogenation of **42** provided the important intermediate (*S*)-2-fluoromethylindoline **43**,[12] which was then converted to **44** following similar reactions to those shown in Scheme 1. In addition, compounds **45**, **46** and **47** were prepared by the general functional transformations shown in Scheme 3.

The effects of various 2-substitutions of indoline ring on receptor binding affinity are summarized in Table 2. Vinyl compound **(*S*)-35** shows similar potency with ethyl compound **(*R*)-36**. The 4-methylbenzylic group of **(*R*)-48** made it about two times potent than 4-chlorobenzylic **(*R*)-36** for D_2 receptor. This is identical with the comparison between **(*R*)-3** and **(*R*)-2**. All other substitutions show a significant loss for D_2 binding affinity.



Scheme 2. Reagents and conditions: (i) ref. 9; (ii) Swern oxidation, 84%; (iii) Methytriphenylphosphonium bromide, *n*-BuLi, argon, 0 °C, 2 h, 89%; (iv) TFA/DCM (1/1), 0 °C to rt, 16 h, 100%; (v) As per Scheme 1,step iii, 82%, and step iv, 90%; (vi) H_2, 5% Pd/C, EtOH, 1 atm, rt, 16 h, 96%; (vii) EDCI, TEA, DMF, rt, 16 h, 72%.

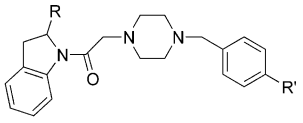


Scheme 3. Reagents and conditions: (i) BnBr, K_2CO_3, CH_3CN, reflux, 8 h, 99%; (ii) LAH, THF, N_2, 0 °C to rt, 5 h, 95%; (iii) DAST, DCM, argon, −70 °C, 2 h, temp to rt over 2 h, 94%; (iv) H_2, 10% Pd/C (Degussa type E101 NE/W), EtOH, 45 psi, rt, 24 h, 76%; (v) As Scheme 1, step iii, 88%, and step iv, 92%; (vi) As per Scheme 1, step iii, 89%, and step iv, 82%; (vii) LiOH hydrate, MeOH/H_2O (v/v = 3/1), rt, 16 h, 75%; (viii) $BnNH_2$, EDCI, TEA, DMF, rt, 16 h, 70%.

Affinities at D_2 and D_4 receptors were determined via standard competitive displacement assays using human D_2 and D_4 clones with [^{3}H]YM 09151 as the competitive ligand. Affinity at the α_1 receptor was determined via standard competitive displacement assays using rat brain homogenate with [^{3}H]prazosine as the competitive ligand. Compounds were also assessed as to their functional activity both at the D_2 and D_4 receptors. D_2 functional activity was assessed via compound reversal of quinpirole inhibited, forskolin stimulated cAMP production from whole cells, while D_4 functional activity was assessed via inhibition of quinpirole stimulated GTPγ^{35}S binding from cell membranes. Functional assessment of compounds **(*R*)-2**, **(*R*)-3** and **(*R*)-18** at both the D_2 and D_4 receptors indicated no agonist properties up to 10 μM, while demonstrating functional K_i values of 580, 120, and 140 nM at D_2 receptor, and 4.5, 3.2 and 3.1 nM.

Drugs with short half-lives create problems in maintaining steady-state concentrations in the therapeutic range. Drugs with long half-lives are favored in terms of efficacy but can lead to accumulation. Considering human microsomal metabolism (data not shown) and binding affinities, we selected **(*R*)-2**, **(*R*)-3** and **(*R*)-18** for behavior studies. When male Sprague–Dawley rats were injected subcutaneously with clozapine and haloperidol 30 min prior to receiving amphetamine (0.5 mg/kg), the agents produced a significant and dose-dependent decline in stimulated locomotor activity ($p < 0.05$, Fig. 2). The minimum effective doses (defined as the lowest dose tested that produced a significant effect relative to

Table 2. Effect of various 2-substitutions of indoline



Compd	Config.	R	R′	K_i (nM)		
				D_2	D_4	α_1
35	*S*	–CH=CH_2	Cl	271	5	2177
36	*R*	–Et	Cl	219	4	1605
38	*S*	–CH_2OH	Cl	3027	10	>1000
44	*S*	–CH_2F	Cl	1096	21	1260
45	*S*	–CO_2Me	Cl	1178	5	>1000
45	*S*	–CO_2Me	Cl	>1000	1760	453
46	*S*	–CO_2H	Cl	>1000	8464	>1000
47	*S*	–CONBn	Cl	>1000	>1000	—
48	*R*	–Et	Me	96	3	1330
49	*S*	–CO_2Me	Me	491	8	100

the amphetamine alone control group using a Fisher's LSD post hoc, $P < 0.05$) were 1.0 and 0.06 mg/kg for clozapine and haloperidol, respectively. Both **(*R*)-3** and **(*R*)-18** displayed significant dose dependent reductions in amphetamine-induced locomotor activity for doses greater than 4 mg/kg (Fig. 3). Although **(*R*)-2** did display a trend toward reduction in amphetamine-induced activity, it did not reach significance over the dose range tested (2–8 mg/kg). In the catalepsy experiments (Fig. 3), haloperidol displayed a minimum effective dose of

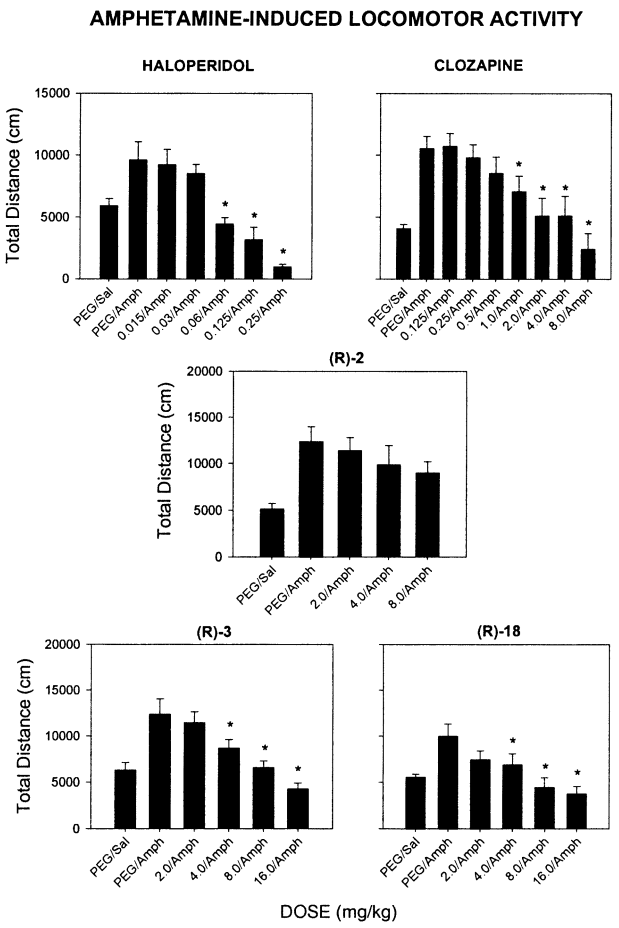


Figure 2. The effects of haloperidol, clozapine, **(*R*)-2**, **(*R*)-3** and **(*R*)-18** on amphetamine-stimulated locomotor activity. $*p<0.05$ vs. PEG/Amph; PEG: 50% PEG 400 in distillated water.

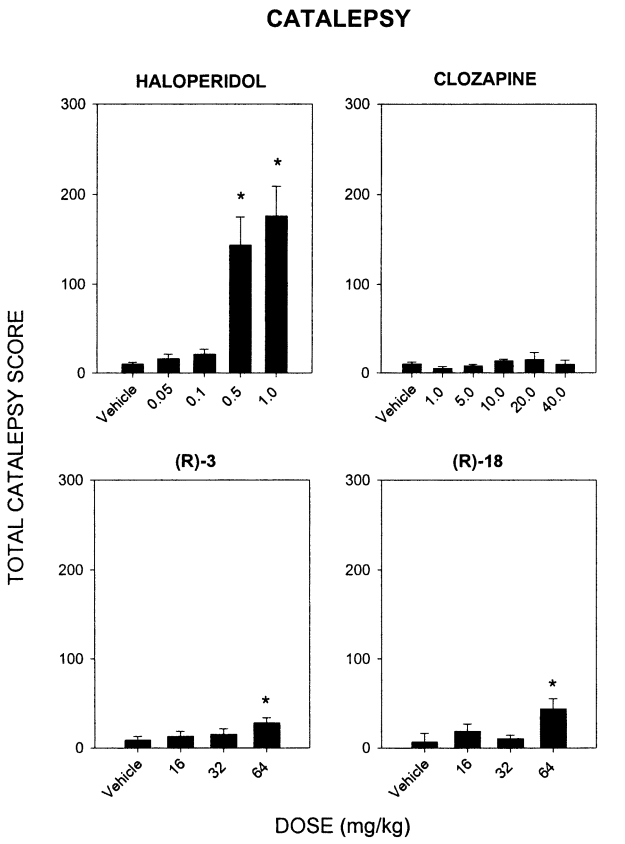


Figure 3. The effects of haloperidol, clozapine, **(*R*)-3** and **(*R*)-18** on catalepsy in rats. $*p<0.05$ versus vehicle.

0.5 mg/kg. Clozapine showed no significant cataleptic effects over the dose range tested (1–40 mg/kg). **(*R*)-3** and **(*R*)-18** showed minimal cataleptic effects which reached significance only at the highest dose tested.

The larger receptor affinity profile of **(*R*)-3** was determined using standard ligand displacement assays against a battery of 45 other receptor and ion channels. Significant affinity ($K_i > 1$ μM) was observed only for the serotonin transporter (532 nM) and the σ1 binding site (976 nM). These results strongly suggest that the behavioral profile of **(*R*)-3**, and by inference clozapine, results from the unique affinity ratio between the D_2 and D_4 receptor.

In our examination of indolinylglycinamides as potential antipsychotic agents, we have attempted to approximate the D_2/D_4 ratio of clozapine on the theory that this ratio might be a key to its atypical nature. Although the small size of the data set make it difficult to draw firm conclusions, the behavioral results obtained for two compounds which met these in vitro criteria lend support to this postulate. All behavioral tests for antipsychotic efficacy have their limitations, particularly in light of the suggestion by many researchers that the assays of antipsychotic efficacy were developed using D_2 ligands and results thus obtained may therefore be largely D_2 mediated. Thus, the true value of D_2/D_4 ratio hypothesis will rest upon clinical evaluation of compounds, like **(*R*)-3** and **(*R*)-18**, which display this dopaminergic profile.

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10. Colorless oil; $[\alpha]_D = -47.3°$ ($c = 1.02$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.28 (d, $J = 4.4$ Hz, 3H), 2.33 (s, 3H), 2.53 (br s, 4H), 2.59 (m, 2H); 2.65 (br s, 4H), 3.21–3.25 (m, 1H), 3.33–3.42 (m, 1H), 3.49 (s, 2H), 4.75 (m, 1H), 7.03 (t, $J = 8.0$ Hz, 1H), 7.12 (d, $J = 8.4$ Hz, 2H), 7.18–7.21 (m, 5H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 20.8, 21.5, 36.3, 52.6, 53.2, 54,5, 61.3, 62.4, 117.8, 123.7, 124.7, 127.1, 128.6, 128.9, 130.0, 134.6, 136.3, 141.4, 167.0; LC-MS (APCI, m/z) 364 (M + 1). A portion of the product was converted to the dihydrobromide salt, mp 241–243 °C (isopropanol/ethyl acetate).
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