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Modification of the clozapine structure by parallel synthesis

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Abstract—A structure–activity study based on the core structure of clozapine **1b** was accomplished by utilizing high-throughput synthesis. Several focused libraries were designed and synthesized to quickly develop SAR. The results indicate that by varying different regions of clozapine, both D_1-selective and D_2-selective compounds can be obtained.

Dopamine receptors can be broadly classified into two subtypes: the D_1-like subtype (D_1 and D_5) that upon activation stimulates the production of cAMP and the D_2-like subtype (D_2–D_4) that inhibits cAMP production upon activation. Many D_2 antagonists are used to treat schizophrenia,[1] while D_2 agonists such as daverium are used to treat Parkinson's disease.[2] D_1/D_5 antagonists such as ecopipam have been studied for drug and alcohol abuse,[3] and D_1 agonists such as fenoldopam are used to treat hypertension.[4] Clozapine **1b** has been used to treat schizophrenia since 1982.[5,6] Unlike typical antipsychotics such as chloropromazine and haloperidol, which are effective only against positive symptoms of schizophrenia, clozapine is effective against both positive and negative symptoms. Furthermore, clozapine does not induce severe extrapyramidal side effects (EPS) at clinically effective doses.[7,8] The unique profile of clozapine, however, may not be attributed solely to its blockade of the D_2 receptor, as it also binds other dopaminergic serotonergic, adrenergic, histaminergic and muscarinic receptors.[5] This non-selective profile is believed to elicit the superior overall efficacy of clozapine.[5] Although some SAR investigations of clozapine have been reported in the literature, to our knowledge, a broader exploration of the clozapine SAR is not available.[9–20] Herein, we wish to report our results of modifications of the N-5 nitrogen region, the distal piperazine nitrogen region, and the tricyclic skeleton of clozapine using high-throughput parallel synthesis[21] (Scheme 1).

Our initial derivatization of the core clozapine N-5 nitrogen included sulfonylation, acylation, reductive alkylation, and urea formation. As expected, this hindered nitrogen was less reactive and only a few reactions provided the desired products. Some representative results are shown in Table 1. Significant among these results, the sulfonamides **2b** and **2c** retained D_1 affinity.[22] Introducing an alkylated nitrogen atom exocyclic to the core tricyclic ring provided the hydrazine **2e** which also retained the D_1 activity. Interestingly, hydrazide **3a** further improved the affinity for D_1 (K_i = 13 nM) and the compound possessed high selectivity against D_2 receptor (D_2/D_1 = 87). Compound **3a** was an ideal lead for SAR exploration using parallel synthesis. Both solution-phase and solid-phase synthesis could be used to prepare analogous targets. Thus, compound **4b**[23,24] was coupled with Argopore-CHO resin followed by acylation with acid chlorides.[21] The final product could be cleaved from the resin using 100% TFA (Scheme 2).[25] In addition, a solution-phase parallel synthesis was also developed to further expand the SAR.[26]

Selected results from our first 60-membered library by solid-phase synthesis are listed in Table 2. Overall, substitution on the phenyl ring improved the D_1 affinity, regardless of the electronic/steric nature of the

Keywords: Dopamine receptors; Clozapine; D_1-selective antagonist; D_2-selective antagonist; High-throughput synthesis.
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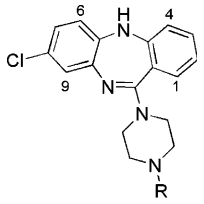
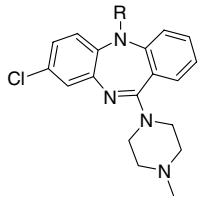


Scheme 1.

Table 1.



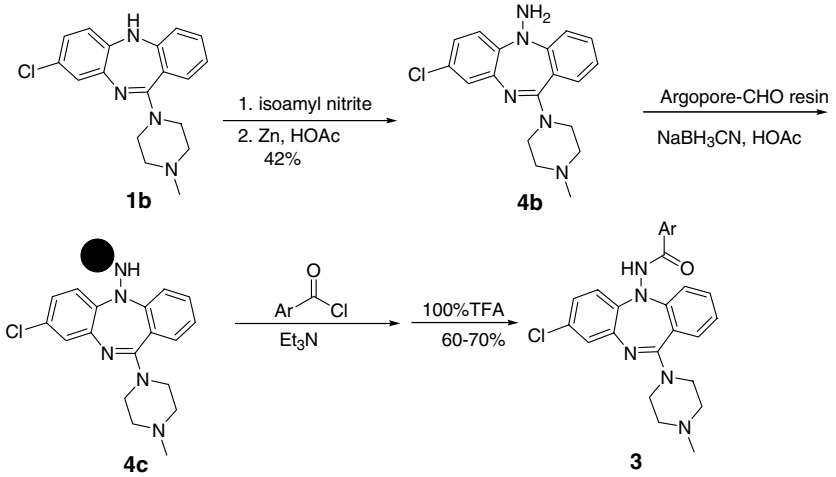
Compound	R	D_1 K_i (nM)	D_2 K_i (nM)	D_2/D_1
1b	H	132	208	1.6
2a	*o*-TolCO	1232	4927	4.0
2b	*p*-TolSO$_2$	65	338	5.2
2c	*o*-TolSO$_2$	71	63	0.9
2d	3,5-Cl$_2$PhNHCO	175	3896	22
2e	2-MeOPhCH$_2$NH	60	202	3.4
3a	2-MeOPhCONH	13	1126	87

substituents and their position on the phenyl ring. Polysubstitution also improved the D_1 affinity (compounds **3j**–**m**). In general, compounds with 2-substitution displayed lower K_i values than 3- or 4-substituted ones (compounds **3i** vs **3d** and **3f**).[27] This trend was in agreement with our previous observation that 2-substitution, especially 2,6-disubstitution, afforded both strong binding to the D_1 receptor and high D_2/D_1 selectivity (compounds **3o**–**p**).[27] Interestingly, 3,4,5-trimethoxyphenyl amide (compound **3m**) also provided significant D_2/D_1 selectivity. Furthermore, introduction of the pyridyl group also significantly improved D_2/D_1 selectivity (compounds **3q**–**r**).

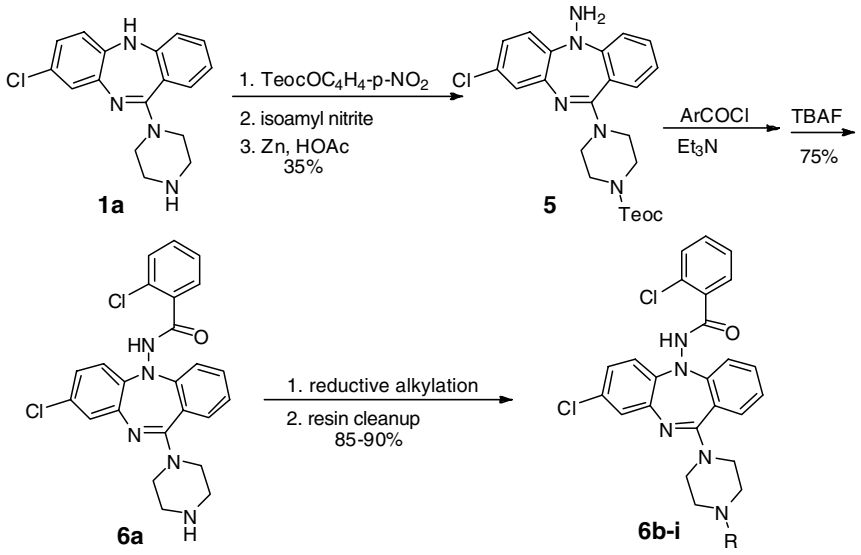
Table 2.

Compound	Ar	D_1 K_i (nM)	D_2 K_i (nM)	D_2/D_1
3b	Ph	157	1150	7.3
3c	4-CNPh	68	>2000	>29
3d	4-MeOPh	47	>3000	>63
3e	3-CNPh	62	3648	59
3f	3-CF$_3$Ph	148	1362	9.2
3g	2-BrPh	16	792	50
3h	2-ClPh	4.1	181	44
3i	2-MeOPh	13	1126	87
3j	2,3-F$_2$Ph	16	533	33
3k	3,4-OCH$_2$OPh	56	828	15
3l	2-CF$_3$, 4-FPh	49	1616	33
3m	3,4,5-(MeO)$_3$Ph	31	5561	180
3n	2,3,4,5-F$_4$Ph	32	1280	40
3o	2,6-Me$_2$Ph	3.0	287	96
3p	2,6-(MeO)$_2$Ph	1.6	340	210
3q	3-Pyridyl	85	12710	150
3r	4-Pyridyl	86	28425	330

Since little was known about the SAR on the distal piperazine nitrogen in this particular series, we synthesized compound **6a** and used reductive alkylation to introduce various alkyl groups on this nitrogen in a high-throughput fashion. At the time of our investigation, synthesis of such analogs in a clozapine or clozapine-like series using this synthetic strategy had not been reported.[28] Subsequently, Capuano et al. reported synthesis of *N*-arylmethyl clozapine analogs using this strategy.[19] In our experiment, the reaction was conducted in solution phase with 75 aldehydes and ketones, followed by resin cleanup as shown in Scheme 3.[29] Selected binding data are shown in Table 3. The data strongly suggested that small and unhindered alkyl groups were preferred on this distal nitrogen. The *N*-methyl analog **3h** was the best compound from this series.[27] Larger alkyl groups decreased D_1 affinity, leading to lower D_2/D_1 selectivity. Our efforts to modify the



Scheme 2.



Scheme 3.

Table 3.

Compound	R	D_1 K_i (nM)	D_2 K_i (nM)	D2/D1
3h	Me	4.0	181	44
6b	Et	54	709	13
6c	*n*-Pr	26	409	16
6d	*i*-Pr	191	998	5.2
6e	CH_2Pr-*i*	81	>3000	>37
6f	CH_2Bu-*t*	447	>3000	>6.7
6g	CH_2CHEt_2	505	>3000	>5.9
6h	CH_2cyclohexyl	294	>3000	>10
6i	2-FBn	503	>2000	>4.0

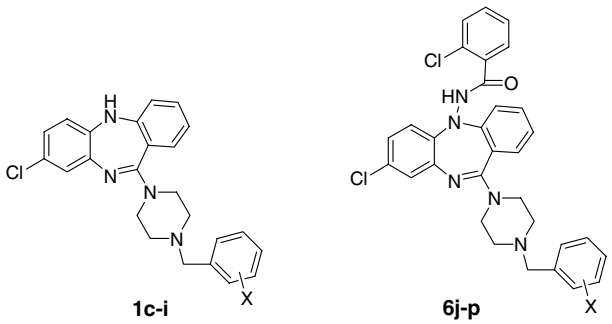
piperazine conformation by introducing a methyl group alpha to the distal piperazine nitrogen provided similar SAR trends.[30] Other efforts to explore this region included replacing the piperazine ring with homopiperazine or *N*,*N*,*N*′-trimethylethylenediamine and these modifications resulted in at least 100-fold loss of D_1 and D_2 activity.[31]

For direct comparison of the hydrazine and clozapine series, the reductive alkylation chemistry was applied to *N*-desmethyl clozapine **1a** and 43 compounds were synthesized. A previous study by Capuano et al. focused on reductive alkylation of **1a** with a few substituted benzaldehydes and it was concluded that introduction of an *N*-arylmethyl group into the clozapine structure did not have a significant effect on D_2 binding.[19] Our results, in Table 4, were in sharp contrast to Capuano's. We found that bigger alkyl groups led to diminished D_1 affinity for compounds **1c**–**i** as we had previously found for hydrazide **6b**–**i**. However, it was quite interesting to see that the D_2 affinity of **1c**–**i** was dramatically improved to the extent that they became D_2-selective antagonists. The best compound **1i** had a D_2 K_i of 3 nM with greater than 1000-fold selectivity over D_1, whereas the corresponding *N*-acylhydrazino analog **6p** had D_2 K_i of >3000 nM. Although D_2-selective antagonists such as amisulpride have been reported as effective antipsychotic drugs,[32] to our knowledge, this is the first report of a highly selective D_2 antagonist with a clozapine-like structure. This finding is of particular relevance to antipsychotic drug research.

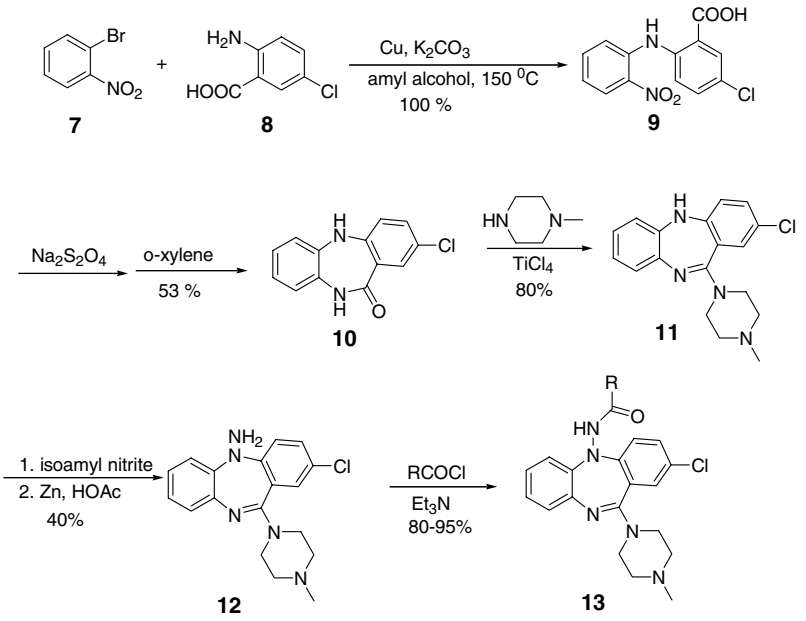
Our exploration of the tricyclic skeleton SAR started with relocating the 8-Cl group to the 2-position based on literature reports that such a manipulation to clozapine improved D_1 and D_2 affinity by 10-fold.[10,11] The synthesis of the building block **12** is depicted in Scheme 4.[33] Using the solution-phase chemistry described above, modification of the top part of the molecule produced 51 compounds. Some representative examples from this library are shown in Table 5. The anticipated improvement of D_1 affinity did not occur in this amide series, whereas improvement of D_2 affinity was observed in some cases, resulting in lower D_2/D_1 selectivity (compounds **13j** vs **3p**). In general, the SAR of this series was consistent with that of compound **3** in Table 2. The best compounds were still those bearing 2-substituted and 2,6-disubstitutedbenzoyl amides (compounds **13i**–**j**). Interestingly, 3,4,5-trimethoxyphenyl amide **13g** still held a D_2 selectivity greater than 100-fold (Scheme 5).

In order to further evaluate the combination effect of introducing an additional chlorine atom and a hydrazide on dopamine receptor affinity, we used the above chemistry to synthesize the dichloroclozapine building block **16** and a 54-membered library synthesis of compounds **17** was accomplished (Table 6). Similar to their analogs in Table 5, the 2-substituted and 2,6-disubstituted phenyl amides provided the best D_1 affinity (compounds **17h**–**j**), with D_2/D_1 selectivity generally being low except for **17h**. A comparison of the results in Tables 2 and 6 demonstrated that the chlorine atom at the 2-position on the tricyclic skeleton significantly improves the D_2 activity but not the D_1 activity.

Table 4.



X	Compound	D_1 K_i (nM)	D_2 K_i (nM)	Compound	D_1 K_i (nM)	D_2 K_i (nM)
2,4-F_2	**1c**	835	177	**6j**	886	>3000
2,3-(OCH_2O)	**1d**	637	80	**6k**	571	>2000
2,4-$(Dime)_2$	**1e**	482	65	**6l**	1097	>3000
3,4-(OC_2H_4O)	**1f**	583	29	**6m**	238	>2000
4-*t*-Bu-Cyclohexyl	**1g**	1734	11	**6n**	2273	>3000
2,3-(OCF_2O)	**1h**	3106	8	**6o**	4749	>3000
3-CF_3O	**1i**	3799	3.4	**6p**	2568	>3000

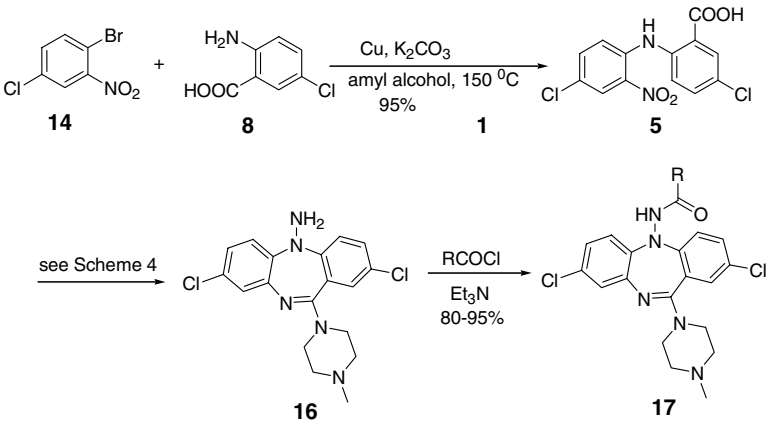


Scheme 4.

Table 5.

Compound	R	D_1 K_i (nM)	D_2 K_i (nM)	D_2/D_1
13a	4-CNPh	64	1969	31
13b	3-CNPh	99	1186	12
13c	3-OMePh	86	1379	16
13d	2-CF_3, 5-FPh	22	421	19
13e	2,3,4,5-F_4Ph	156	759	4.9
13f	3,4-OCH_2OPh	83	1261	15
13g	3,4,5-$(MeO)_3$Ph	20	3230	160
13h	2-MePh	48	1571	33
13i	2-IPh	7.0	231	33
13j	2,6-$(MeO)_2$Ph	6.0	197	33

In summary, we have utilized a parallel synthesis strategy to develop SAR of three different regions of clozapine **1b**. Modification of the aryl group on the top region of the molecule resulted in high affinity D_1 antagonists such as **3h**, which was also selective against D_2. Modification of the tricyclic skeleton provided D_1 antagonists such as **13j** and **17h** with similar affinity but lower D_2 selectivity. While installation of bigger alkyl groups on the distal piperazine nitrogen resulted in loss of D_1 and D_2 activity in hydrazide series **6e**–**6p**, highly D_2-selective compounds such as **1i** were discovered when the same chemistry was applied to *N*-desmethyl clozapine **1a**.



Scheme 5.

Table 6.

Compound	R	D_1 K_i (nM)	D_2 K_i (nM)	D_2/D_1
17a	4-CNPh	51	194	3.8
17b	3-CNPh	47	175	3.7
17c	3-OMePh	44	139	3.2
17d	2-CF_3, 5-FPh	24	69	2.9
17e	2,3,4,5-F_4Ph	98	270	2.8
17f	3,4-OCH_2OPh	130	286	2.2
17g	3,4,5-$(MeO)_3$Ph	16	267	17
17h	2-MePh	2.0	175	88
17i	2-IPh	12	36	3.0
17j	2,6-$(MeO)_2$Ph	11	97	8.8

Acknowledgments

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22. For experimentals: Ltk-cells stably expressing D_1 and D_2 receptors at a density of 4–7 pmol/mg protein were lysed in hypotonic buffer and centrifuged at 48,000*g*. Membrane pellets were frozen and stored at −80 °C for use in binding assays. Receptor affinities were determined by equilibrium binding experiments in which bound and free radioligands were separated by rapid filtration, and bound counts were quantified by liquid scintillation counting. For D_1 binding, the radioligand was [^{3}H] SCH 23390 (0.3 nM), and non-specific binding was defined by addition of 10 μM unlabeled SCH 23390. For D_2 binding, the radioligand was [^{3}H]methylspiperone (0.5 nM) and non-specific binding was defined using 10 μM (−)-sulpride. Test compounds, radioligand, and membrane homogenates prepared from CHO cells expressing each receptor subtype were incubated in a 200 μL volume for 1 h at room temperature prior to filtration on GF-C plates. Competition binding data were analyzed using Graphpad Prism, in which curves fit a one-site competition model with a Hill Slope equal to or approximately 1. Mean K_i values from four separate determinations are reported. The SEM was below 15% in each case. LCMS analysis was performed on

an Applied Biosystems API-100 mass spectrometer and Shimadzu SCL-10A LC column: Altech platinum C18, 3 micron, 33 mm × 7 mm ID; gradient flow: 0 min, 10% CH_3CN; 5 min, 95% CH_3CN; 7 min, 95% CH_3CN; 7.5 min, 10% CH_3CN; 9 min, stop. Chromatography was performed with Selecto Scientific flash silica gel, 32–63 μM.

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24. The conversion of clozapine **1b** to **4b** was based on Ref. 12 with modifications. Two grams of clozapine **1b** was dissolved in 80 mL DCM and 40 mL isoamylnitrite at rt for 3 h. The solvent was removed and the crude was dissolved in 40 mL HOAc. This solution was added dropwise to Zn (10 g)/HOAc (150 mL) over 1 h at 10–15 °C. Additional Zn (1 g) was periodically added to keep the green color of the solution. After 3 h, the solution was filtered and the solvent evaporated. Four hundred milliliters of DCM was added along with 50 mL of water. The pH was adjusted to 11 and extraction was done with 3×100 mL DCM. The crude was recrystallized with DCM and hexane to give 0.84 g of the hydrazine **4b** (42% yield). ^{1}H NMR (CDCl3): δ 2.40 (s, 3H), 2.70 (br m, 4 H), 3.60 (br m, 4H), 4.40 (br s, 2H) 6.77 (d, 1H, J = 8.2 Hz), 6.83 (s, 1H), 7.10–7.40 (m, 4H) 7.79 (d, 1H, J = 8.2 Hz).
25. 100% TFA for cleavage was necessary likely due to the presence of the basic functional group. Novabiochem-CHO resin gave similar results.
26. The general procedure for the solution-phase library synthesis is the following: To 10 mg of **4b** (0.029 mmol) in 0.9 mL DCE were added acid chloride (1.2 equiv) and Et_3N (2 equiv). After stirring for 6 h, resin-bound trisamine (6 equiv, Argonaut™), resin-bound isocyanate (3 equiv, Argonaut™) were added to absorb the excess acid chloride and unreacted hydrazine for 2 h. After filtration, the sample was dried with no further purification.[29]
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