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Hydrazides of clozapine: A new class of D_1 dopamine receptor subtype selective antagonists

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Abstract—Acylated and aroylated hydrazinoclozapines are highly potent dopamine D_1 antagonists that show remarkable selectivity over other dopamine receptors. The most potent compound in this series is the 2,6-dimethoxybenzhydrazide **33** with a D_1 K_i of 1.6 nM and 212-fold selectivity over D_2 receptor.

Dopamine (DA) receptors belong to the G-protein coupled receptor superfamily. Five DA receptor subtypes are known (D_1–D_5) belonging to two main subgroups, D_1-like and D_2-like. The D_1-like subgroup includes the D_1 and D_5 receptors, while the D_2-like subgroup includes the D_2, D_3, and D_4 receptor subtypes.[1] The prototypical partial D_1 selective agonist SKF 38393 and antagonist SCH 23390 have been invaluable tools with which to study the function of this receptor subtype.[2,3] A number of other highly selective ligands have been discovered and used for the understanding of neurodegenerative and psychiatric disorders involving the dopaminergic system.[4,5a–d]

Clozapine **1**, (8-chloro-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo[*b*,*e*][1.4]diazepine), is the prototype of a group of 'atypical' antipsychotic drugs exhibiting clinical efficacy similar to that of the classical antipsychotics but lacking most of their motor side effects.[6–8] Clozapine binds with moderate affinity to D_1 receptors but interacts with other dopaminergic (D_2), serotogenic (5-HT_{2A}), adrenergic (α1 and α2), histaminergic, muscarinic, and other receptors.[9–20] Closely related compound **2** was identified in our flash throughput screening and proved to have good affinity for the D_1 receptor (D_1 K_i = 25.6 nM) but modest selectivity over other DA receptors. Importantly, the activity of compound **2** differs from that of clozapine in the selectivity versus the D_2 receptor (Fig. 1).

The synthesis of hydrazinoclozapine **5** and further modifications of the hydrazine moiety as well as product affinity and selectivity at DA and other receptors will be described.

The synthesis of these compounds was carried out as described in Scheme 1. Commercially available aminobenzoic acid **3** was cyclized to the lactone **4** in 96% yield. Compound **4** was subsequently reacted with $TiCl_4$ and *N*-methylpiperazine to afford clozapine in 53% yield.[21] Clozapine was converted to the nitroso compound by diazotization using isoamyl nitrite and the resulting nitroso compound was reduced to the hydrazinoclozapine **5** in excellent yields.[22,23] Modifications of the hydrazine unit have been extensively investigated for

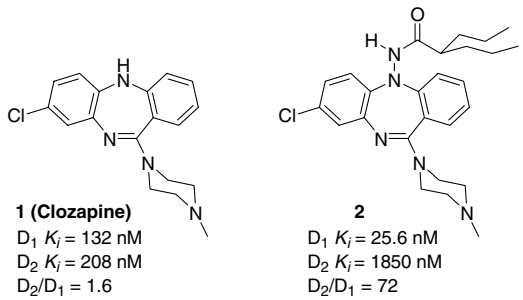

Figure 1. Clozapine and acylated hydrazinoclozapine.

Keywords: Clozapine; Hydrazinoclozapine; Dopamine D1.
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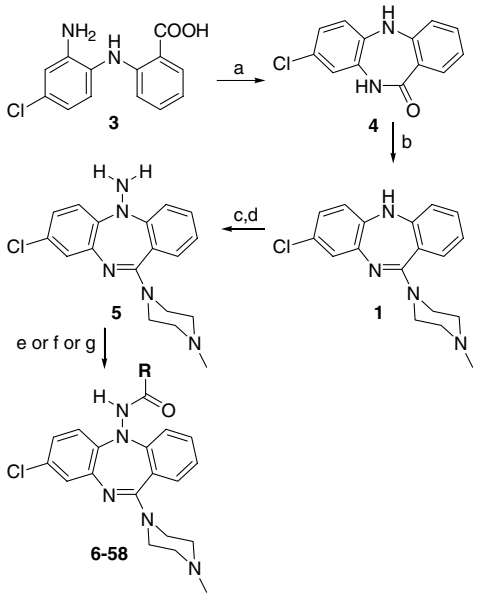


Scheme 1. Reagents and conditions: (a) xylene, reflux, 96%; (b) $TiCl_4$, *N*-methylpiperazine, 53%; (c) isoamyl-ONO, CH_2Cl_2; (d) Zn, HOAc, 95%; (e) RCOOH, DCC, DMAP, CH_2Cl_2; (f) EDCI, MeCN; (g) RCOCl, TEA, CH_2Cl_2.

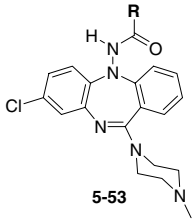
the purpose of improving the overall pharmacokinetic profile. Acylations were carried out either by using acid or acid chloride with hydrazinoclozapine under standard reaction conditions.[24]

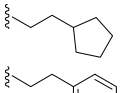
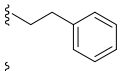
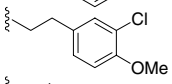
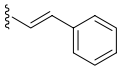
Initial SAR results are shown in Table 1. Clozapine is moderately active in the dopamine D_1 assay with a K_i of 132 nM (average value of three K_i determinations on human D_1).[25] Hydrazine **5** showed 280 nM K_i at the D_1 dopamine receptor. The effect of the acylated hydrazine moiety was investigated. Acetylation of **5** decreases the dopaminergic activity by 2-fold, whereas *tert*-butyl and trifluoroacetylated derivatives showed improved potency. The cyclohexyl analog **15** demonstrated dopamine D_1 K_i of 42 nM. Generally, alkanoyl analogs showed moderate dopaminergic activity.

Next we turned our attention to the aromatic substitution on the acyl hydrazine group. The aromatic hydrazides showed excellent D_1 dopaminergic affinity. Simple benzoyl derivative **16** showed only moderate dopamine D_1 activity, however substitution on the benzene ring greatly enhanced the D_1 affinity. The 4-methyl, 3-methyl, 3-chloro, 3-iodo, and 2-chloro groups are very well tolerated. The D_2 selectivity for compound **22** is noteworthy. High D_1/D_2 selectivity is common among this series (Table 2).

An extension of the aromatic SAR that clearly indicates the preference for 2,6-disubstitution on the benzene ring for high dopamine D_1 affinity is shown in Table 3. We sought to explore 2,6-disubstitution in detail. These compounds display excellent dopamine D_1 affinity with high selectivity over dopamine D_2 receptors. Dichloro compound **30** has $K_i = 3.4$ nM at D_1 receptor with a selectivity of 70-fold over D_2. The 2,6-dimethoxy compound **33** is one of the best compounds in this series

Table 1. Dopamine D_1 and D_2 affinities of acyl hydrazides



Compound	R	K_i[a] (nM)		D_2/D_1
		D_1	D_2	
1	—	132	208	1.6
5	—	280	647	2
6		494	na	na
7		98	na	na
8		86	1900	22
9		154	400	3
10		111	1950	18
11		79	621	8
12		56	582	10
13		103	1600	16
14		150	na	na
15		42	1776	42

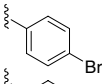
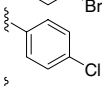
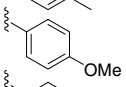
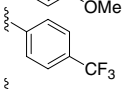
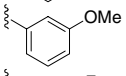
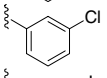
na, not available.
[a] Mean value of at least three separate K_i determinations.

(1.6 nM D_1 K_i and 212-fold selectivity over D_2). This compound is 16-fold more potent than our initial lead at D_1. A dramatic increase in D_2 selectivity was observed by the introduction of amino group at the ortho position as seen for compound **37** (D_1 $K_i = 3.2$ nM, $D_2/D_1 = 317$).[26]

Several of these compounds showed an excellent pharmacokinetic profile as evidenced from a 'rapid rat' PK experiment.[27] Compound **28** has a rapid rat AUC of 2656 ng h/mL with C_{max} of 821 ng/mL and a T_{max} of 1 h. Compound **33** has an AUC of 2667 ng h/mL with C_{max} of 802 ng/mL and a T_{max} of 0.5 h.

The dopamine D_1 binding affinity of heterocyclic hydrazides is shown in Table 4. The 2-furyl and 2-thienyl analogs showed good affinity ($K_i = 15$ and 14 nM, respectively) with moderate selectivity, whereas 3-thienyl analog **41** exhibited 9 nM affinity and 333-fold selec-

Table 2. Dopamine D_1 and D_2 affinities of benzoyl hydrazides

Compound	R	K_i[a] (nM)		D_2/D_1
		D_1	D_2	
16		157	1150	7
17		14	2386	170
18		35	9251	262
19		7.7	1521	197
20		47	3000	63
21		68	na	na
22		3	517	172
23		13	1126	86
24		47	921	19
25		4	181	45
26		3.6	350	97
27		12	374	31
28		4	181	45

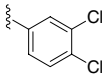
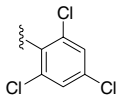
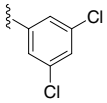
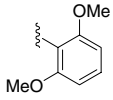
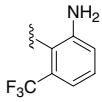
na, not available.
[a] Mean value of at least three separate K_i determinations.

tivity over D_2. Other thiophene derivatives such as **42**–**44** showed good affinity for D_1 with low selectivity over D_2. Other heterocyclic analogs such as **45** and **46** also showed reasonably good D_1 affinity. The 2-chloro-3-pyridyl analog **47** gives a D_1 K_i of 11 nM with 55-fold selectivity over D_2 receptor.

The influence of the polycyclic aromatic substitution on the acyl hydrazine moiety was also investigated. Naphthyl and quinolyl groups are tolerated as shown in Table 5. Notable compounds in this series are **48** (D_1 K_i = 3.8 nM, D_2/D_1 = 121), **49** (D_1 K_i = 7.4 nM, D_2/D_1 = 178), and **52** (D_1K_i = 10 nM, D_2/D_1 = 89). Compound **48** showed exceptional PK profile. The rat AUC for this compound was 12930 ng h/mL with a C_{max} of 3567 ng/mL and a T_{max} of 1 h. Introduction of a simple ethoxy group at 2-position of the naphthyl ring reduces the D_1 affinity by 17-fold (compound **50**).

The possibility of displacing the fluoro substitution on **27** was explored. The fluoro group could be replaced with piperidine without losing any D_1 activity. Introduction of bulky substituents such as *N*-methylpiperazinyl, phenoxy, etc. resulted in decreased D_1 binding affinity. The results are shown in Table 6.

Table 3. Dopamine D_1 and D_2 affinities of di- and tri-substituted benzoyl hydrazides

Compound	R	K_i[a] (nM)		D_2/D_1
		D_1	D_2	
29		12	6394	515
30		3.4	240	70
31		4	390	95
32		24	608	25
33		1.6	340	212
34		2.5	383	153
35		2.6	496	190
36		3	287	95
37		3.2	1015	317
38		7	92	13

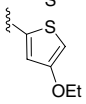
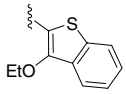
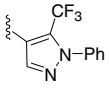
[a] Mean value of at least three separate K_i determinations.

We also prepared a wide variety of sulfonamides and ureas on the hydrazine moiety and found that those compounds are significantly less potent than the aroyl hydrazides.

Compounds with good dopamine D_1 binding were evaluated for functional antagonism in the cAMP assay. The K_b values correlated well with their corresponding K_i data. All these compounds functioned as full D_1 antagonists. Selectivity for D_1 was confirmed by assaying affinity at numerous receptors as shown in Table 7. Compounds **33**–**37** and **48** showed remarkable selectivities for D_1 over D_2 and D_4. Like most known D_1 ligands, these compounds displayed significant affinity at the D_5 receptor. These compounds have high affinity for 5-HT_{2A}, but were less active in the α2a binding assay.

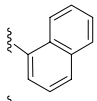
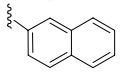
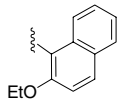
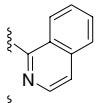
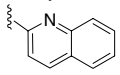
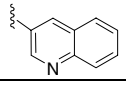
In summary, from our study on a new class of clozapine hydrazide derivatives emerged a series of important

Table 4. Dopamine D_1 and D_2 affinities of heteroaryl acyl hydrazides

Compound	R	K_i[a] (nM)		D_2/D_1
		D_1	D_2	
39		15	837	54
40		14	1474	105
41		9	2997	333
42		10	180	18
43		39	78	2
44		28	196	7
45		22	460	21
46		30	2084	69
47		11	630	55

[a] Mean value of at least three separate K_i determinations.

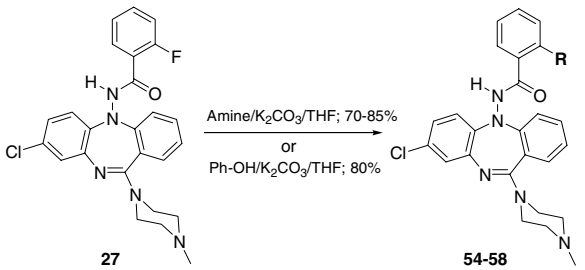
Table 5. Dopamine D_1 and D_2 affinities of polyaryl acyl hydrazides

Compound	R	K_i[a] (nM)		D_2/D_1
		D_1	D_2	
48		3.8	458	121
49		7.4	1314	178
50		65	274	4
51		23	484	21
52		10	931	89
53		28	na	na

na, not available.
[a] Mean value of at least three separate K_i determinations.

dopamine D_1 antagonists. The most significant results were obtained by introducing a 2,6-disubstituted benzoyl moiety to the clozapine hydrazine nucleus.

Table 6. Dopamine D_1 and D_2 affinities of benzoyl hydrazides



27 54-58

Compound	R	K_i[a] (nM)		D_2/D_1
		D_1	D_2	
54		62	3818	61
55		7.8	215	28
56		237	3772	16
57		88	3000	34
58		51	431	9

[a] Mean value of at least three separate K_i determinations.

Table 7. Additional selectivity data for compounds **28**, **33**–**37**, and **48**[a]

Compound	D_1 K_i	D_1 K_b	D_2 K_i	D_4 K_i	D_5 K_i	5-HT_{2A} K_i	α2a K_i
28	4.0	2.8	181	870	73	1.0	455
33	1.6	0.7	340	1810	38	13.8	169
34	2.5	2.3	383	2000	7.0	1.2	2000
35	2.6	1.7	496	808	53	0.9	327
36	3.0	2.8	287	1870	31	2.2	791
37	3.2	6.7	1015	4846	58	2.9	456
48	3.8	2.0	458	977	105	1.0	882

[a] K_i and K_b in nM (mean value of at least three separate determinations).

Acknowledgments

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24. Two methods have been generally employed for the formation of hydrazide link. The hydrazine **5** was treated with the corresponding acid chloride in presence of TEA followed by silica gel purification affording the hydrazide. In the case of acids, we used EDCI or DCC coupling strategy. (In all cases, the yield of the product was 60–80%.)
25. 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 nonspecific 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 nonspecific 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. LC–MS 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, and 9 min—stop. Chromatography was performed with Selecto Scientific flash silica gel, 32–63 μM.
26. A parallel synthesis method was developed for SAR determination; see Su, J. et al., following paper.
27. Korfmacher, W. A.; Cox, K. A.; Ng, K. J.; Veals, J.; Hsein, Y.; Wainhaus, S.; Broske, L.; Prelusky, D.; Nomeir, A.; White, R. E. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 335, Data are from pooled samples from two rats in cassette-accelerated rapid rat protocol as described in the above reference. Briefly, two male Sprauge–Dawley rats were dosed orally at a dose of 10 mg/kg. Blood samples were collected at different time points and analyzed according to reference 27. Compound plasma levels for individual animals used to calculate PK parameters were generally within ±25% of average values.