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Chlorophenylpiperazine analogues as high affinity dopamine transporter ligands

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ABSTRACT

Selective σ_2 ligands continue to be an active target for medications to attenuate the effects of psychostimulants. In the course of our studies to determine the optimal substituents in the σ_2-selective phenyl piperazines analogues with reduced activity at other neurotransmitter systems, we discovered that 1-(3-chlorophenyl)-4-phenethylpiperazine actually had preferentially increased affinity for dopamine transporters (DAT), yielding a highly selective DAT ligand.

Drugs that have the capability to suppress the stimulant and toxic activities of psychomotor stimulants are urgently required, yet little success has been achieved.[1–6] Our studies have shown that selective sigma (σ) receptor antagonists can attenuate stimulant and neurotoxic effects of cocaine and methamphetamine in rodents.[7–11] However, elucidation of the specific receptor subtypes (σ_1 and σ_2) involved are hampered by the paucity of σ_2-selective ligands.[12]

In earlier studies, simple piperazine analogues demonstrated anti-psychostimulant activity with affinity for both σ receptor subtypes.[13–16] We expanded the library of piperazine analogues through the exploration of an electron withdrawing pyridyl ring linked to the piperazine, yielding *N*-(2-pyridyl)piperazine analogues, which favors the σ_2 receptor subtype over the σ_1 receptor subtype.[17,18] To further explore σ_2-selective piperazine analogues, we introduced electron withdrawing chloro-substituents into the σ_2-selective 1-(3-phenylpropyl)-4-(2-pyridyl)piperazine (**UMB38**) scaffold to create a series of ligands that were pharmacologically evaluated at both the σ_1 and σ_2 receptor subtypes in addition to commonly tested neurotransmitter receptors and transporters that have historically been of concern for off-target interactions of σ ligands.

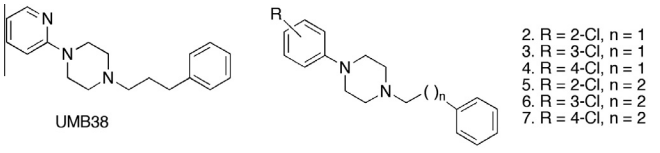

Figure 1. Template compound UMB38 and chloro-substituted analogues.

Table 1
Competition binding study results for σ receptors and dopamine transporters

Compound	σ_1	σ_2	σ_1/σ_2	DAT	σ_1/DAT
UMB38	82 ± 0.2*	4.9 ± 0.8*	16.73	9148 ± 1008	0.0090
2	0.15 ± 0.01	56.32 ± 4.16	0.003	0.13 ± 0.01	1.15
3	6.47 ± 0.48	82.31 ± 6.77	0.079	0.04 ± 0.01	161.75
4	1.85 ± 0.20	33.30 ± 2.02	0.056	6 ± 0.33	0.31
5	0.56 ± 0.06	7.79 ± 0.52	0.072	482 ± 24	0.0012
6	4.93 ± 0.52	12.59 ± 1.17	0.39	>10,000	n.d.
7	0.12 ± 0.003	10.89 ± 0.97	0.011	2647 ± 158	0.000045

Values are reported as K_i ± S.E.M. (nM). A value of >10,000 indicates less than 30% of the radioligand was displaced at the 10,000 nM ligand concentration. DAT = dopamine transporter; n.d. = not determined. σ_1/σ_2 and σ_1/DAT are selectivity ratios, respectively.
* Data from previously reported compound Ref. 17

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Table 2
Competition binding study results for neurotransmitter receptors and transporters

Compound	5-HT_2	D_2	SERT	NET	Opioid	PCP/NMDA
UMB38	1238 ± 61	940 ± 87	>10,000	>10,000	>10,000	>10,000
2	484 ± 6	67 ± 6	>10,000	790 ± 33	4830 ± 391	>10,000
3	53 ± 7	327 ± 23	802 ± 68	1107 ± 35	>10,000	>10,000
4	518 ± 52	834 ± 56	372 ± 13	>10,000	>10,000	>10,000
5	952 ± 98	77 ± 7	409 ± 29	>10,000	4600 ± 495	>10,000
6	498 ± 50	128 ± 9	488 ± 36	>10,000	>10,000	>10,000
7	391 ± 48	660 ± 19	1104 ± 92	>10,000	>10,000	>10,000

Values are reported as K_i ± S.E.M. (nM). A value of >10,000 indicates less than 30% of the radioligand was displaced at the 10,000 nM ligand concentration. 5-HT_2 = serotonin receptor subfamily; D_2 = dopamine receptor subtype; SERT = serotonin transporter; NET = norepinephrine transporter; PCP/NMDA = phencyclidine/*N*-methyl-D-aspartate.

The analogues (**2**–**7**, Fig. 1) were prepared by the reaction of the appropriate phenyl piperazine and the appropriate brominated phenyl alkane in DMF in the presence of $NaHCO_3$ at room temperature for 24 h. The product was extracted from water into diethyl ether and concentrated. Yields of pure free bases were observed between 87% and 97%. After removal of the solvent, all amines were converted into water-soluble oxalic acid salts. All free bases displayed NMR and mass spectral data consistent with the assigned structures, and combustion analysis of all salts were ±0.4% of the theoretical calculation (Atlantic Microlabs, Norcross, GA, USA). A more detailed chemical description can be found in the Supplementary data section. The synthesized ligands were evaluated using competition binding assays as previously described.[9,16,19]

This series of piperazine analogues showed high-to-moderate affinity for both σ_1 and σ_2 receptor subtypes (Table 1). σ Binding affinities have previously been published for **3**, **6** and **7**; however, said binding affinities were obtained using [^{3}H]di-*o*-tolylguanidine (DTG) without the inclusion of (+)-pentazocine, conditions that do not differentiate affinities between the σ_1 and σ_2 receptor subtypes.[20] While the primary objective of this research was to identify electron withdrawing chloro-substituents that optimize σ_2-selectivity, unfortunately, all selectivity was lost upon removal of the 2-pyridyl functional group. As such, the 2-, 3- and 4-chloro moieties were unable to sustain or improve σ_2-selectivity (see Table 2).

Of particular interest was the dopamine transporter (DAT) binding affinities for the chloro-substituted phenylethyl ligands. Compounds **2**, **3** and **4** displayed high affinity for DAT, whereby **3** displayed the greatest selectivity for DAT (>160-fold) at all sites tested compared to other ligands herein. Psychostimulant use, including both cocaine and methamphetamine, has been shown to affect DAT function.[21] Previous studies have identified phenyl-substituted piperazine derivatives classified as DAT inhibitors, including 1-(2-(bis(4-fluorphenyl)-methoxy)-ethyl)-4-(3-phenyl-propyl)piperazine (**GBR12909**),[22] that have been extensively studied as anti-cocaine therapeutics; however, problematic side-effect liabilities associated with **GBR12909** have halted future clinical studies.[23]

With respect to our initial research intent as well as unexpected findings, ligands depicting dual interactions with DAT and σ receptors can potentially attenuate the behavioral effects of psychostimulants.[24,25] Simultaneous DAT/σ receptor inhibition can effectively reduce cocaine self-administration,[26] an effect not seen with σ receptor antagonists alone.[27,28] Furthermore, structure–activity relationships (SAR) of dual DAT/σ_1 ligands have been investigated with the intent of curbing psychostimulant abuse,[29,30] with the σ_1 receptor subtype more definitely established as a potential therapeutic target for psychostimulant abuse to that of the σ_2 receptor subtype.[31,32] Ligands herein that are σ_1-selective (**5** and **7**), DAT-selective (**3**) or demonstrate dual σ_1/DAT activity (**2**) offer potential for future anti-psychostimulant medication development.

Although not the result expected, the SAR herein uncovered one of the highest affinity and most selective ligands for DAT (**3**) to date. This compound has the potential to increase our understanding of DAT, while informing that such substituents are not appropriate for σ_2 selective ligands.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.09.038.

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