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Bioorganic & Medicinal Chemistry Letters 15 (2005) 4915–4918
Bioorganic & Medicinal Chemistry Letters

The synthesis and biological evaluation of dopamine transporter inhibiting activity of substituted diphenylmethoxypiperidines

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Received 16 May 2005; revised 11 July 2005; accepted 9 August 2005
Available online 13 September 2005

Abstract—The synthesis of potent 4-aryl methoxypiperidinol inhibitors of the dopamine transporter is described. Symmetrical *para* substituents of the benzene rings are important for high potency in binding to the dopamine transporter. 4-[Bis(4-fluorophenyl) methoxy]-1-methylpiperidine has an IC_{50} of 22.1 ± 5.73 nM and increases locomotor activity in mice.

The dopamine transporter (DAT) is an integral membrane protein located in dopamine (DA) neuron terminals. It is responsible for regulating the extracellular concentrations of DA by taking up released DA into the presynaptic terminal. It is also the biological target for cocaine.[1] The psychoactive effects of DAT inhibitors like cocaine are associated with fast changes in the uptake of DA.[2] For example, within 4 s after intravenous cocaine administration there is significant inhibition of DA uptake that reaches a plateau in 20 s.[3] The development of potent long-acting inhibitors of the DAT has provided a great deal of information about the cocaine pharmacophore.[4,5] Moreover, such compounds may be useful as pharmacotherapeutic agents in the treatment of cocaine addiction.[2] Recent reports have shown that potent DAT inhibitors such as benzotropine (BZT) and GBR analogs have potential as pharmacotherapies for treatment of cocaine abuse.[4–6] Some of these analogs exhibit a slow onset and long duration of stimulant action that is evident as increased locomotor activity in animal models.[7] Both of these classes of DAT inhibitors have two common structural features: a diphenylmethoxy moiety and a nitrogen-based six-membered ring (Fig. 1).

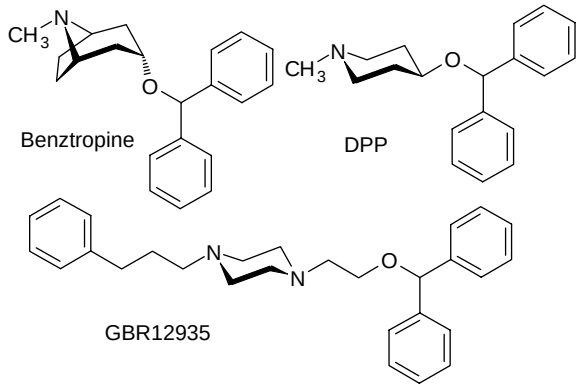

Figure 1. Chemical structures of known DAT inhibitors.

Diphenylpyraline (DPP), a well known H_1-histamine receptor antagonist, also has these structural features.[8] A flexible piperidine ring and short ether bond allow DPP to be considered a flexible analog of BZT and a structurally constrained derivative of GBR, respectively. Recently, we have shown that DPP is an effective DAT inhibitor.[9] This suggests that compounds structurally similar to DPP could also be DAT inhibitors.

We have explored the structure–activity relationships (SAR) of a series of piperidinol derivatives to develop a more potent DAT inhibitor than DPP. In the current study, we report the synthesis and in vitro biological evaluation of a series of piperidinols structurally

Keywords: Dopamine transporter; Inhibitor; Piperidinol-4; Locomotor activity.
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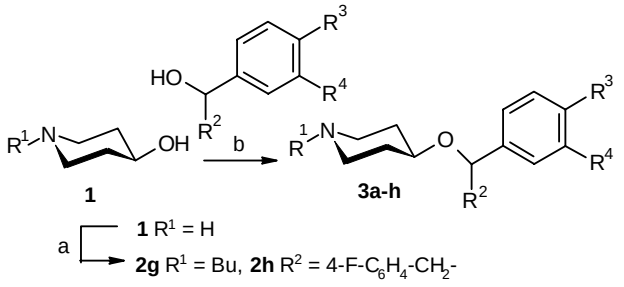
doi:10.1016/j.bmcl.2005.08.028

similar to DPP. In all recent reviews, the group of very potent DAT inhibitors has bis-*para*-halogen substituted diphenylmethoxy moieties like BZT, GBR, and their piperidine analogs.[4,5,10] These compounds are more lipophilic than their unsubstituted counterparts. In addition, one of the most reliable methods in medicinal chemistry for improving in vitro activity is to incorporate a properly positioned lipophilic group[11] (e.g., by increasing the length of *N*-alkyl groups). As a result, we synthesized known[8,12] DPP-related structures and new compounds. This study also evaluates the in vivo effects of novel inhibitors with high DAT affinity on locomotor activity in mice. DAT inhibitors are known to elevate striatal extracellular DA levels and this effect is associated with behavioral hyperactivity.[7]

The compounds were synthesized as previously described for the series of tropane compounds[13,14] (Scheme 1). Briefly, N-substituted piperidin-4-ols **2g**, **h** were prepared by alkylation with alkyl halides in *N*,*N*-dimethylformamide in the presence of anhydrous potassium carbonate[13] in yields of approximately 90%. Commercially available substituted benzophenones were reduced to the benzhydrols using sodium borohydride in isopropanol in nearly quantitative yield. The ethers were synthesized by condensation of benzhydrols with 25% molar excess of N-substituted piperidin-4-ols or *N*-methylpiperidin-4-ol in benzene with a Dean–Stark trap.[14] *para*-Toluenesulfonic acid was used as a catalyst. It was removed by extraction with sodium hydroxide and final mixtures were purified by flash chromatography.[15] The yields ranged from 60 to 100% based on recovered starting material. Another described method resulted in poor yields.[12]



Scheme 1. Reagents and conditions: (a) Hal-R, K_2CO_3, DMF, 40 °C; (b) *p*-TSA, benzene/DMF (25:1), reflux.

The in vitro IC_{50} values (Table 1) of compounds **3a**–**h** at DAT were determined by displacement of [^{125}I]RTI-55 binding in rat striatal membranes, as described previously.[16] The most potent compounds, **3a**–**c**, **g**, were tested in vivo for effects on locomotor activity in mice as described previously.[9] Figure 2 shows the time course effects of these compounds on locomotor activity over the entire 3 h session in 5 min bins. Table 1 reports locomotor activity over the entire 3 h sessions. Statistical analyses were carried out using a two-way ANOVA.

We used the ''rule of 5'' to select compounds for in vivo experiments.[11] ''Molinspiration'' is an on-line software tool that allows us to rapidly calculate all parameters of this rule and have an N_v descriptor.[17] The N_v-molecular violating parameter is an integral number which reflects the importance of each parameter of the rule of 5

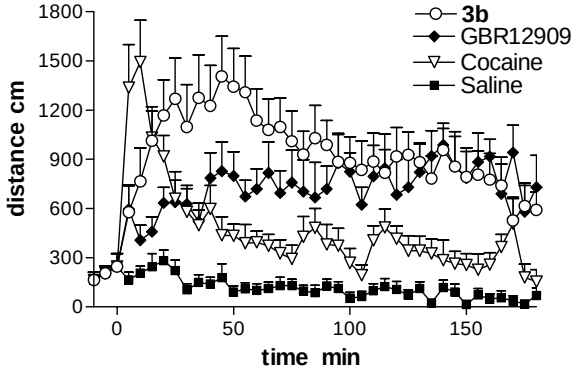


Figure 2. Effects of **3b**, GBR12909, cocaine and saline on locomotor activity. Mice were injected intraperitoneally with equivalent doses (17.8 μmol/kg) of each drug. Horizontal activity was monitored for 3 h as described previously,[9] and data are expressed as cm/5 min. All compounds significantly increased locomotor activity levels compared with saline, $P < 0.001$. Fifty minutes after injection, there were significant differences in locomotor activity produced by each compound compared with the others, $P < 0.001$.

Table 1. Binding and horizontal locomotor activity of 4-arylmethoxypiperidinols

		R^1	R^2	R^3	R^4	Calculated log *P*	IC_{50} (DAT) nM[c]	Locomotor activity (m/3 h)[c]
GBR 12909	—	—	—	—	—	—	0.43 ± 0.15[b]	26.3 ± 4.4
Cocaine	—	—	—	—	—	—	104 ± 49[b]	16.7 ± 2.8
DPP[a]	$C_{19}H_{23}NO$	CH_3	Ph	H	H	3.95	420 ± 9	12.2 ± 2.1
3a	$C_{19}H_{21}Cl_2NO$	CH_3	4-Cl–C_6H_4–	Cl	H	5.20	44.0 ± 10.9	5.18 ± 0.87
3b	$C_{19}H_{21}F_2NO$	CH_3	4-F–C_6H_4–	F	H	3.99	22.1 ± 5.7	34.8 ± 5.2
3c	$C_{19}H_{22}ClNO$	CH_3	Ph	Cl	H	4.58	155 ± 10	5.89 ± 1.01
3d	$C_{19}H_{22}FNO$	CH_3	Ph	F	H	3.97	277 ± 10	—
3e	$C_{20}H_{25}NO$	CH_3	Ph	H	CH_3	4.27	293 ± 10	—
3f	$C_{14}H_{20}FNO$	CH_3	CH_3	F	H	3.11	264 ± 9	—
3g	$C_{22}H_{27}Cl_2NO$	C_4H_9	4-Cl–C_6H_4–	Cl	H	6.55	12.5 ± 7.5	3.87 ± 0.64
3h	$C_{25}H_{24}Cl_2FNO$	4-F–C_6H_4–CH_2–	4-Cl–C_6H_4–	Cl	H	6.53	50.6 ± 2.8	—

[a] Purchased from Aldrich.
[b] See Ref. 16a.
[c] The data are means; ± SEM; 0.9% NaCl was used as a vehicle. Locomotor activity produced by vehicle was −3.92 m/3 h.

for the structure of interest.[17] On the basis of experimental IC_{50} values, we developed a simple quantitative structure–activity relationship (QSAR) model to predict IC_{50} values for new compounds.[17] We chose a linear type of regression equation which allowed us to reach a reasonable value for r^2. Our equation shows that more active compounds should be more lipophilic than DPP in in vitro assays.[17] This requirement may be because our target protein, the DAT, is an integral membrane protein, and the binding site may be on the transmembrane domains. We predicted the IC_{50} values for **3g** and **3h** to be 17.55 and 26.06 nM, respectively. Their actual IC_{50} values were 12.5 and 50.6 nM, respectively.

All of our compounds are more potent than DPP. Compound **3e** is more lipophilic than DPP and has increased affinity for the DAT. The results of the binding assay show that replacing the aromatic ring of DPP with a methyl group, in order to reduce lipophilicity, leads to a higher affinity of **3f** than DPP for the DAT. Perhaps a substituent on the aromatic ring is important for binding, in addition to lipophilicity. Symmetrically disubstituted halogen compounds **3a** and **3b** were more active than their monosubstituted counterparts **3c** and **3d**, respectively. Chlorosubstituted compound **3a** was a twofold less potent inhibitor of DAT than fluorosubstituted **3b**. The most lipophilic compound **3g** exhibited higher potency in the binding assay, as was predicted. Perhaps an increase in the molecular volume of substituents on the nitrogen atom of the piperidine ring led to the stabilization of the biologically active conformer. Aliphatic substitution on the nitrogen of **3g** led to a more potent DAT inhibitor than with the fluorobenzyl fragment in **3h**. This is in accord with the previous statement concerning a properly positioned lipophilic group.[11] Our binding results agree with the trends reported for halogenated benztropine analogs.[18]

In our previous research, we found that DPP was fairly effective at increasing locomotor activity at a dose of 5.0 mg/kg (17.8 μmol/kg).[9] All compounds were tested at this dose. Compound **3b** was most active in this assay (see Table 1 and Fig. 2). All synthesized compounds had a longer duration of effect on locomotor activity (approximately 3 h) than cocaine, but shorter than GBR (more than 3 h). DPP and **3b** are more lipophilic than cocaine but less than GBR or BZT.[17] This fact may explain the slow onset and long duration of effects of these compounds on locomotor activity in mice. We made a prediction of the bioavailability of our compounds using the rule of 5 to better understand the extremely low activity of **3a**, **3c**, and **3g** in vivo.[11] Calculations of parameters of the rule of 5 showed that compounds **3a** and **3g** with heavy substituents like chlorine atoms should have poor bioavailability (see the value of log *P* in Table 1). These compounds violate Lipinski's rule of 5.[11,17] Compounds **3a**, **3c**, and **3g** have a lower solubility in the assay buffers. Thus, it is likely that they have poor penetration through the blood–brain barrier.

We established that the *para*-substituents of the benzene rings of **3a**–**c**, **3g**, and **3h** are important for more effective binding to the DAT. Among our synthesized DAT inhibitors only **3b** had a greater effect than DPP, both in binding and on locomotor activity in mice. This compound is a good candidate for further QSAR study. We can conclude that the short inflexible link of the diphenylmethyloxy fragment in this series of piperidines can still adopt the bioactive conformation for high affinity for DAT in vitro. However, DPP and **3b** were less long-lasting than BZT or GBR12909 but more long-lasting than cocaine in live animal locomotor tests.

Acknowledgments

This work was supported by Wake Forest University School of Medicine Venture Funds, National Center for Minority Health and Health Disparities Grant MD00232 and National Institutes of Health Grant AA014091.

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15. For all compounds the mass spectra contained weak $(M-H)^+$ ions characteristic of piperidines and the typical isotope patterns for one or more halogen atoms in the structures. Characteristic ions that occurred for compounds **3a**–**3f** were *m*/*z* 99 (methylpiperidine fragment) as the base peak and *m*/*z* 114 (methylpiperidinol fragment) at approximately 50% R.A. in each mass spectrum. Significant diarylmethane fragments also occurred for all compounds except **3f**. For compounds with very weak $(M-H)^+$ ions in the EI mass spectrum, electrospray ionization mass spectra from a methanol solution were taken to confirm the molecular weight as the $(M+H)^+$ ion. The relative response of all compounds was from 95.8% up to 99.1%. The ^1H NMR (300 MHz, $CDCl_3$) spectra of *N*-methylpiperidines showed typical signals: 1.30–1.35 (m, 2H, H-2,6 ax), 1.85–1.90 (m, 2H, H-2,6 equiv.), 2.00–2.05

(m, 2H, H-3,5 ax), 2.40–2.45 (s, 3H, N-CH3), 2.90–3.00 (m, 2H, H-3,5 equiv.), 3.50–3.55 (m, 1H, H-4 ax), 5.35–5.45 (s, 1H, Ar-CH-Ar), 7.20–7.60 (m, aromatic C–H).

16. (a) Letchworth, S. R.; Smith, H. R.; Porrino, L. J.; Bennett, B. A.; Davies, H. M. L.; Sexton, T.; Childers, S. R. *JPET* **2000**, *293*, 686; (b) Potencies were calculated from displacement curves using 6–8 concentrations of unlabeled compounds. All data are mean values ± SEM of at least three separate experiments, each of which was conducted in triplicate. Potencies of all unlabeled compounds in displacing [^{125}I]RTI-55 binding are expressed as IC_{50} values because the biphasic nature of [^{125}I]RTI-55 binding to striatal membranes makes determination of accurate K_i values difficult. For a detailed description, see the publication 16a.
17. (a) Ertl, P.; Rohde, B.; Selzer, P. *J. Med. Chem.* **2000**, *43*, 3714; (b) Free online service: http://www.molinspiration.com; (c) QSAR model was developed on the basis of calculation of the molecular properties of compounds DPP, **3a**–**3h**. The equation of correlation was log (IC_{50}) = $-0.3403(\log P + N_a + pK_a) + 7.5292$ (where log *P*—calculated octanol/water partition coefficient, N_a—the number of hydrogen bond acceptors, pK_a— calculated dissociation constant of bases in water). R^2 value was 0.7533, $r^2 = 0.8699$ and $q^2 = 0.7855$. The prediction of bioavailability as an N_v—molecular violating parameter was performed on the basis of 'rule of 5' on the same web site. The compounds might have poor bioavailability if N_v is more then 0.
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