Synthesis and Pharmacology of 6-Substituted Benztropines: Discovery of Novel Dopamine Uptake Inhibitors Possessing Low Binding Affinity to the Dopamine Transporter

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A series of 6α- and 6β-substituted benztropines were synthesized. A marked enantioselectivity was observed for the 6β-methoxylated benztropines, the (1R)-isomers being more potent than the corresponding (1S) compounds. The racemic 6α-methoxy-3-(4′,4″-difluorodiphenyloxymethyl)-tropane (5g) was the most potent compound. It has been found that modifications at the 6-position of benztropine might reduce the DAT binding affinity, maintaining otherwise a significant dopamine uptake inhibitory activity. A reinvestigation of the absolute configuration of 6β-methoxytropinone proved the 6R configuration for the (+)-enantiomer.

Introduction

Cocaine (1) is a potent stimulant of the central nervous system, and its widespread abuse has an extremely negative impact in our society. The development of therapeutic agents that will assist addicted individuals during detoxification programs is therefore particularly desirable.

Cocaine is a nonselective drug that interacts with a variety of pharmacologically distinct sites. It binds with high affinity to the transporter sites for the neurotransmitters dopamine (DA), serotonin (5-hydroxytryptamine, 5-HT), and noradrenaline (NA), thereby inhibiting the reuptake of these amines into the presynaptic neurons.1-4 Since the dopamine transporter (DAT) is considered to be the main target of the biochemical action of cocaine as well as of its behavioral effects, many intervention strategies have focused on the dopaminergic pathway.1-6

The search for potential anti-cocaine medications has led to an extensive study of the structure–activity relationships (SAR) of 1 at the dopamine transporter, and 2-substituted 3-aryltropanes have been extensively studied as cocaine congeners and developed as tools to explore the DAT.1,2,6 This broad class of compounds has provided interesting insight into the nature of the dopamine transporter pharmacophore.1,2,6 Additionally, 6- and 7-substituted tropanes have been widely inves-
tigated in order to discover appropriate molecular modifications that may lead to the discovery of cocaine antagonists or partial agonists.7-13

Among the structural classes of compounds interacting with DAT, which have provided interesting results as potential medications to treat cocaine abuse, the benztropine group has also received particular attention.5,6 The introduction of a 2-carbomethoxy group into benztropine (2) by Meltzer14 et al. resulted in a new class of compounds with the (1S) derivative exhibiting significant DAT affinity and the most potent representative being difluoropine (4). Hydroxylation at the 6–7 positions of difluoropine, produced, on the contrary, a significant decrease in binding affinity.7 Meltzer et al. have hypothesized that this class of dopamine uptake inhibitors are more like the GBR series in their mode of binding to the DAT.15 Benztropine is an anticholinergic DAT inhibitor, equipotent to cocaine, which is clinically used for the treatment of movement disorders that accompany Parkinson’s disease. Structurally, benztropine possesses a tropane ring, as found in cocaine, and a diphenylmethane ether group, as found in the GBR series.14 Drug design strategies have focused mainly on the substitution pattern in the aromatic moiety and the replacement of the N-methyl group by other substituents.5,17-20 Unlike cocaine, the benztropine analogues, despite their high affinity for the DAT, generally have not demonstrated a cocaine-like behavioral profile in animal models. After considerable research in this area, Newman and co-workers have hypothesized that this class of dopamine uptake inhibitors may access a DAT binding site distinct from that of cocaine, so explaining their discrepant behavioral profile.5,17 In preclinical studies aimed at gauging its possible use in the treatment of cocaine abuse, 4′,4″-
difluorobenzopine (3) has shown some promise in the attenuation of cocaine effects in animal studies. 21

We have recently undertaken a structural investigation at the two-carbon bridge region of benzopine, and we communicated that 6-methoxy-4',4''-difluoro- and 4',4''-chlorobenzopine possess high binding affinity (IC50 = 25–32 nM) to DAT, as compared to benzopine (IC50 = 118 nM) and cocaine (IC50 = 150 nM), when evaluated under identical assay conditions. 22 Herein, we report the synthesis and pharmacological characterization of a novel series of 6-substituted benzopines (Figure 2). 22 A reinvestigation of the absolute configuration of the (+)-6β-methoxytropinone (+)-7 and (-)-7, possessing the known configuration at the carbon atom bearing the methoxy functionality, would constitute appropriate starting materials for their preparation (Scheme 1). Previous investigations had indicated the 6S configuration of the (+)-6β-methoxytropinone. 23 Consequently, we referred to literature indications to assign, incorrectly, stereochemistry in our communications. 12,22 In contrast, we have now proved unequivocally that (+)-6a-c and their synthetic precursor (+)-6β-methoxytropinone (+)-7 and (-)-7, possessing the known configuration at the carbon atom bearing the methoxy functionality, would constitute appropriate starting materials for their preparation (Scheme 1). Previous investigations had indicated the 6S configuration of the (+)-6β-methoxytropinone. 23 Consequently, we referred to literature indications to assign, incorrectly, stereochemistry in our communications. 12,22 In contrast, we have now proved unequivocally that (+)-6a-c and their synthetic precursor (+)-6β-methoxytropinone (+)-7 and (-)-7, possessing the known configuration at the carbon atom bearing the methoxy functionality, would constitute appropriate starting materials for their preparation (Scheme 1). Previous investigations had indicated the 6S configuration of the (+)-6β-methoxytropinone. 23 Consequently, we referred to literature indications to assign, incorrectly, stereochemistry in our communications. 12,22

Chemistry. Regarding the synthesis of stereoisomers (+)-6a-c and (-)-6a-c, we considered, as previously communicated, 22 that the chiral methoxytropinones (+)-7 and (-)-7, possessing the known configuration at the carbon atom bearing the methoxy functionality, would constitute appropriate starting materials for their preparation (Scheme 1). Previous investigations had indicated the 6S configuration of the (+)-6β-methoxytropinone. 23 Consequently, we referred to literature indications to assign, incorrectly, stereochemistry in our communications. 12,22 In contrast, we have now proved unequivocally that (+)-6a-c and their synthetic precursor (+)-6β-methoxytropinone (+)-7 and (-)-7, possessing the known configuration at the carbon atom bearing the methoxy functionality, would constitute appropriate starting materials for their preparation (Scheme 1). Previous investigations had indicated the 6S configuration of the (+)-6β-methoxytropinone. 23 Consequently, we referred to literature indications to assign, incorrectly, stereochemistry in our communications. 12,22

In the following step (Scheme 1), the stereoselective reduction (H2 and PtO2 as catalyst) of the ketones (+)-7 and (-)-7 produced the corresponding alcohols derivatives (-)-8 and (+)-8 in optically pure form. These were reacted with the appropriate benzhydrols in the presence of p-toluenesulfonic acid and refluxed with a
Kishner conditions, was effected in two steps by Mitsunobu chemistry. Thus, the 6β-hydroxy derivative 21 was reacted with p-nitrobenzoic acid and triphenylphosphine in the presence of diethyl azodicarboxylate to give the ester intermediate 22, which was in turn hydrolyzed with LiOH/THF/H2O to provide the 6α-hydroxy analogue 23. Finally, the benzhydryl derivatives (±)-24 and (±)-25 were obtained as described above for 5g,h.

**Biological Results and Discussion**

Binding affinities of all novel synthesized compounds were evaluated in radiolabeled ligand displacement assays for DAT in the brain. Compounds were examined for their ability to displace [3H]WIN 35,428 and [3H]paroxetine from the dopamine and serotonin transporters in rat caudate putamen. Additionally, all compounds were tested for their ability to inhibit high-affinity uptake of [3H]dopamine into striatal nerve endings (synaptosomes). Biological data are reported in Table 1. There was a good correlation between uptake inhibition and binding values ($r = 0.949, P < 0.001$), as shown in Figure 5.

The enantiomers (1S,6R)-(+)â–6a-c and (1R,6S)-(−)-6a-c were prepared to investigate whether stereochemistry could play a role in binding and uptake activity of chiral benztropines. In general, the 6β-methoxylated chiral benztropines retain activity relative to their parent structures, benztropine and cocaine, in both binding and functional assays (Table 1). The enantiomers (−)-6a-c, bearing the (1R)-configuration, were more potent than the corresponding (1S) stereoisomers (+)-6a-c. All the newly synthesized methoxylated benztropines lacked SERT activity. In particular, a marked enantioselectivity is seen for (−)-6b being 23 times more potent than (+)-6b. Thus, while a 2-carbalkoxy-benzotropine series showed a strong biological preference for the (1S)-configuration,14,19,20 the above-described chiral 6-methoxylated benztropines show preference for the (1R)-configuration.

Interestingly, the (±)-6α-methoxy-4′,4″-difluorobenzotropine 5g is the most potent analogue in the reported series of methoxylated benztropines. Unlike the 6β-methoxylated derivatives, 5g demonstrates, moreover, a notable binding affinity for SERT. On the contrary, an increased bridge-steric bulk, as in the MOM-protected compounds 5a-c and 5h, resulted in a reduced binding affinity as compared with the methoxy derivatives.

Remarkable results were obtained when a hydroxyl group was introduced into the benztropine two-carbon bridge. Introduction of functionalities at the cocaine 6,7-bridge has attracted the attention of a number of research groups.7–13 In general, steric bulk at both positions has reduced the affinity of these compounds for the dopamine transporter, but the 6- or 7-hydroxyl group in 2-carbomethoxy-3-aryltropanes is tolerated when an appropriate substituent is present at the 3-position, with comparable potency and better selectivity for DAT.6,7,9 As reported in Table 1, the hydroxylated benztropines 5d,e demonstrated binding affinities in the micromolar range. It is remarkable that the hydroxylated 4′,4″-difluorobenzotropine 5f and 5i24 showed binding and dopamine uptake activity similar to the parent 3.5 Of interest, while the binding affinity of 5d,e is...
considerably diminished in comparison with benz-

tropine, their potency in inhibiting [3H]dopamine uptake (DAUI) is higher than cocaine; in fact, their dopamine uptake activity is 7–15 times higher than the binding activity. Contrary to our expectations, the binding activities of 24 and 25 were not particularly interesting; however, their uptake activity was also quite surprising, being higher than their binding values. To the best of our knowledge these differences in binding vs DAUI, as found for 5d,e 24, and 25, have not been previously encountered in benzotropine analogues lacking a 2-posi-

**Scheme 3.** Synthesis of 6a-Hydroxylated Benztropines

Reagents and conditions: (a) DIBAL-H, CH\(_2\)Cl\(_2\), –78 °C to 0 °C, 1 h; (b) aq HCl, MeOH, 65 °C, 10 h; (c) dimethoxymethane, CH\(_2\)Cl\(_2\), 4 Å molecular sieves, p-TsOH, 40 °C; (d) PtO\(_2\), H\(_2\), EtOH, 40 psi, 12 h; (e) 4,4'-difluorobenzhydryl chloride, Bu\(_3\)N, DMF, 160 °C; (f) 4, 4'-difluorobenzhydryl chloride, Bu\(_3\)N, DMF, 160 °C; (g) HCl, MeOH, 65 °C, 3 h.

**Scheme 4.** Synthesis of benzotropines 24 and 25

Reagents and conditions: (a) NH\(_2\)NH\(_2\)H\(_2\)O, EtOH, KOH, 130 °C/190 °C; (b) DEAD, 4-nitrobenzoic acid, P(Ph)\(_3\), toluene, 70 °C; (c) LiOH-H\(_2\)O, H\(_2\)O, THF, rt, 3 h; (d) 4,4'-difluorobenzhydryl chloride, Bu\(_3\)N, DMF, 160 °C, 8 h.

<table>
<thead>
<tr>
<th>compd</th>
<th>DA[^3]HWIN 35,428 IC(_{50}) (nM)</th>
<th>[HIDA uptake binding IC(_{50}) (nM)]</th>
<th>5HT[^3]Hiproxetine binding IC(_{50}) (nM)</th>
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<tbody>
<tr>
<td>cocaine</td>
<td>150 ± 20</td>
<td>353.1 ± 36.4</td>
<td>(citalopram) 0.5 ± 0.1 nM</td>
</tr>
<tr>
<td>benzotropine</td>
<td>118 ± 9</td>
<td>403 ± 115</td>
<td></td>
</tr>
<tr>
<td>(±)-5a</td>
<td>4500 ± 600</td>
<td>1083.7 ± 244.3</td>
<td>&gt;10(^4)</td>
</tr>
<tr>
<td>(±)-5b</td>
<td>400 ± 64</td>
<td>193.7 ± 38.2</td>
<td>&gt;10(^4)</td>
</tr>
<tr>
<td>(±)-5c</td>
<td>340 ± 70</td>
<td>79.7 ± 10.8</td>
<td>&gt;10(^4)</td>
</tr>
<tr>
<td>(±)-5d</td>
<td>1300 ± 300</td>
<td>149.4 ± 7.7</td>
<td>&gt;10(^4)</td>
</tr>
<tr>
<td>(±)-5e</td>
<td>600 ± 85</td>
<td>39.1 ± 4.6</td>
<td>&gt;10(^4)</td>
</tr>
<tr>
<td>(±)-5f</td>
<td>12 ± 3</td>
<td>8.1 ± 1.9</td>
<td>11000 ± 1300</td>
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<tr>
<td>(±)-5g</td>
<td>10 ± 2</td>
<td>12.4 ± 1</td>
<td>280 ± 25</td>
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<td>(±)-5h</td>
<td>2500 ± 300</td>
<td>490.9 ± 80.5</td>
<td>3500 ± 400</td>
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<tr>
<td>(±)-5i</td>
<td>140 ± 25</td>
<td>162.3 ± 22.8</td>
<td>&gt;10(^4)</td>
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<tr>
<td>(1S)(+)+6a</td>
<td>975 ± 85</td>
<td>239.7 ± 23.1</td>
<td>&gt;10(^4)</td>
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<tr>
<td>(1R)(-)+6b</td>
<td>276 ± 40</td>
<td>138.8 ± 25.7</td>
<td>&gt;10(^4)</td>
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<td>(1S)(−)+6b</td>
<td>750 ± 70</td>
<td>519 ± 29</td>
<td>&gt;10(^4)</td>
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<td>(1R)(−)+6c</td>
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<td>25 ± 3</td>
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<td>(±)-24</td>
<td>900 ± 100</td>
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<tr>
<td>(±)-25</td>
<td>1125 ± 130</td>
<td>199 ± 28.8</td>
<td>2000 ± 200</td>
</tr>
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</table>

^a Values are expressed as mean ± SE (n = 3–9). \(^b\) Data as reported in ref 22.
produced compounds possessing high DAT activity, and novel profile of activity.

25, high binding affinity. Interpretation of these findings is not easy: as hypothesized for the 2-carboalkoxy-substituted benztropines,19 the disparity in potency in the functional assay compared to binding constants may be related to assay conditions (e.g. 33 °C for uptake experiments vs 0 °C for binding experiments, or different ion concentrations in the buffers) and may be not reflected in vivo.

Thus, the increased potency of the DA uptake inhibition of compounds 5d,e, 24, and 25 suggests that substitution at the bridgehead region of benztropine may provide an interesting opportunity for the construction of new classes of DA-uptake inhibitors with a novel profile of activity.

In summary, bridge-methoxylation of benztropine produced compounds possessing high DAT activity, and the 1R isomers are more potent than the corresponding 1S isomers. Among the results obtained, perhaps the most interesting observation relates to the disparity in DA-uptake potency and binding constants as found for compounds 5d,e, 24, and 25. We observed that, in some cases, manipulation at the two-carbon bridge region of benztropine significantly altered the biological activity of the compounds, and potent DA-uptake inhibitors, possessing a low binding affinity to the DAT, have been discovered. However, some other compounds retained high activity for both DAT binding and inhibition. Our work is currently continuing in the exploration of other 6,7-bridge-substituted benzotropines.

Experimental Section

Different results from literature data23 have been obtained with the following:

**Figure 5.** Correlation of IC50 values for [3H]dopamine uptake vs [3H]WIN35428 binding K; values (n = 5–9 determinations per value). Correlation and probability were determined by using Pearson’s correlation analysis: r = 0.949 (95% confidence interval 0.824–0.986, P < 0.001).

[Graph showing correlation of IC50 values for [3H]dopamine uptake vs [3H]WIN35428 binding K; values (n = 5–9 determinations per value). Correlation and probability were determined by using Pearson’s correlation analysis: r = 0.949 (95% confidence interval 0.824–0.986, P < 0.001).]


[Graph showing correlation of IC50 values for [3H]dopamine uptake vs [3H]WIN35428 binding K; values (n = 5–9 determinations per value). Correlation and probability were determined by using Pearson’s correlation analysis: r = 0.949 (95% confidence interval 0.824–0.986, P < 0.001).]
DH, N.

(±)-3α-(4-(Fluorophenyl)phenylmethoxy)-8-methyl-8-azabicyclo[3.2.1]octane (5c). As described for 5a by means of 4,4-difluorobenzhydryl chloride. White solid (59% yield): mp 194–196 °C; Rf 0.45 (AcOEt:MeOH:NH4OH 5:1:0.1). MALDI-TOF MS: [M+H]+ 342.8. Anal. (C23H27F2NO3): C, H, N.

(±)-3α-(4-(Fluorophenyl)phenylmethoxy)-8-methyl-8-aza-bicyclo[3.2.1]octane (5d). Forty milligrams (0.108 mmol) of 5a was dissolved in MeOH (3 mL), and 100 µL of 37% HCl was added at 0 °C. The reaction was stirred at 60 °C until the starting material completely disappeared (TLC AcOEt:MeOH:NH4OH 3:1:0.1). After 3 h, the reaction mixture was basified with saturated NaHCO3 (5 mL) and extracted with CH2Cl2 (10 mL × 3), and the combined organic layers were washed with brine and dried over anhydrous Na2SO4. The crude product was purified by flash chromatography (AcOEt:MeOH:NH4OH 2:1:0.1) to afford a white solid (5d) as a colorless oil (33 mg, 94%). Rf 0.2 (AcOEt:MeOH:NH4OH 3:1:0.1). MALDI-TOF MS: [M+H]+ 359. Anal. (C21H24ClNO2): C, H, N.

As described for 5b by means of 4,4-difluorobenzhydryl chloride. Colorless oil (yield, 39%). MALDI-TOF MS: [M+H]+ 374. Anal. (C23H27F2NO3): C, H, N.

(±)-3α-(4-(Fluorophenyl)phenylmethoxy)-6α-methoxy-8-methyl-8-aza-bicyclo[3.2.1]octane (5g). As described for 1–7(6c) starting from 20a. Colorless oil (yield, 39%). MALDI-TOF MS: [M+H]+ 360. Anal. (C23H27F2NO3): C, H, N.

References


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