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Effects of Two-Carbon Bridge Region Methoxylation of Benztropine: Discovery of Novel Chiral Ligands for the Dopamine Transporter

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Abstract—6-Methoxylated and 8-oxygenated benztropines were prepared and evaluated for their DAT and SERT activity (binding and uptake inhibition). Methoxylation at the two-carbon bridge of benztropine produced a novel class of potent and selective DAT ligands. An interesting enantioselectivity was also observed for this new class of chiral benztropines. The inactivity of the 8-oxygenated analogues seems to point out that, unlike cocaine and its analogues, interactions of benztropine ligands with DAT may be strongly governed by the nitrogen atom.

Introduction

A loss of balance in monoaminergic neurotransmission plays a key role in a myriad of neurological and psychiatric diseases (Parkinson's disease, depression, schizophrenia, obsessive compulsive disorder (OCD), panic disorder). Reuptake of released norepinephrine (NE), dopamine (DA), serotonin (5-HT) by Na^+–Cl^--dependent membrane transporters is the primary mechanism for termination of the monoaminergic signal. Although most antidepressants display a low affinity for DA transporter (DAT), drugs like bupropione have been proven to be useful as atypical antidepressants for the management of resistant patients. The DAT system represents the obligatory target for the behavioral and biochemical action of cocaine[1,2] and there is a correlation between craving and OCD; moreover, clinical trials have suggested that a combination of DAT inhibitors with drugs enhancing 5-HT levels may be a successful approach in the treatment of cocaine withdrawal symptoms.[3,4] It is now well accepted that the delineation of transporter domains involved in interaction with blockers, as opposed to those interacting with DA, could lead to medications able to block the effects of cocaine without themselves blocking DA uptake, which may prove useful in the treatment of cocaine addiction.[1,2] Therefore, one approach in the discovery of potential cocaine antagonists is to identify appropriate drugs that bind to a region of the cocaine binding site without inhibiting DA uptake.

In this context, we have recently demonstrated that methoxylation of the cocaine's two-carbon bridge provides compounds of pharmacological interest:[5–9] although the introduction of the methoxy function uniformly resulted in significant reduction in binding affinity to DAT, at least one of these methoxylated analogues was found capable of countering, to a minor extent, the effects of cocaine on DA reuptake.[5]

Among the structural classes of compounds interacting with DAT, which have provided interesting results in the quest of potential cocaine antagonists, the benztropine group has recently received particular attention. After considerable research in this area Newman and co-workers have hypothesized that this class of DA

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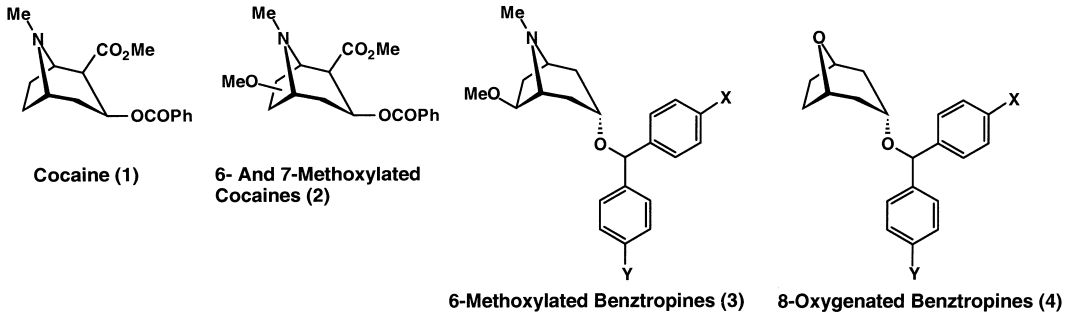


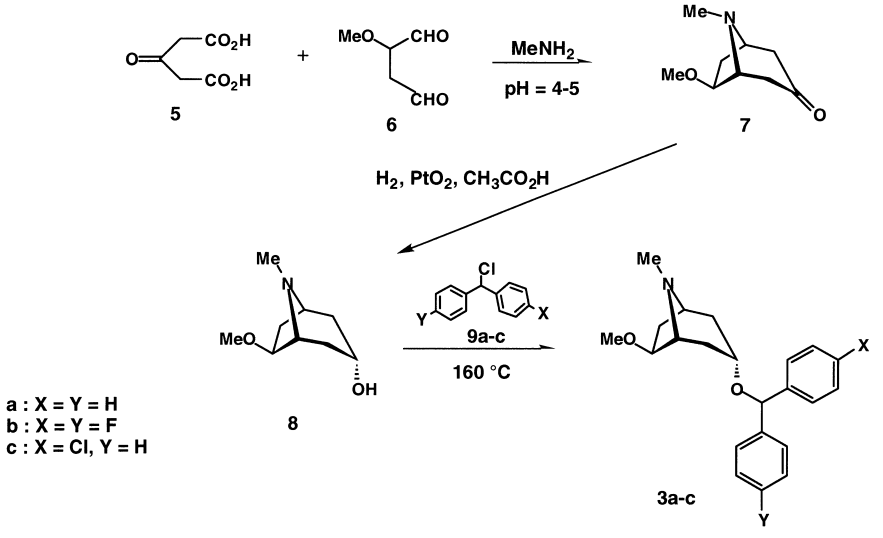
uptake inhibitors may access a DAT binding site distinct from that of cocaine, which would explain their discrepant behavioral profile.[10–12]

Thus, since benztropine contains a tropane ring as cocaine, we were intrigued to verify whether the same structural perturbation at the C6–C7 region as well as at the nitrogen bridge of the tropane skeleton might produce novel ligands for the dopamine transporter. To explore this possibility, we considered it to be of interest to prepare 6-methoxylated analogues **3** of the molecule. Moreover, since 8-oxygenated cocaines and their WIN derivatives proved to be of particular interest for their DAT activity,[13,14] we also evaluated the biological activity of the 8-oxa analogues **4**. These modifications might not only lead to compounds with altered biological activity, but also provide further relevant information upon the question of whether benztropine and cocaine bind to different binding sites.

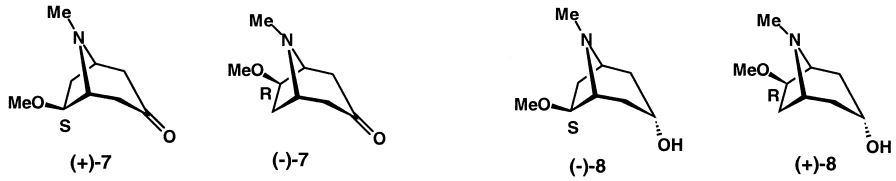
In this communication we describe our findings on a novel class of 6-methoxylated and 8-oxygenated benztropines. We found that the methoxylation of benztropine yields—in some cases—compounds possessing a higher DAT binding activity than benztropine itself, together with interesting discrepancies in the binding versus DA uptake inhibition data. The compounds possess a binding potency comparable to the parent halogenated-diphenylmethoxy benztropines. Moreover, we also describe here a notable enantioselective approach to 6-methoxylated benztropines. We think that the data reported in this communication further expands the understanding of benztropine's SARs, and may provide useful information to aid the discovery of cocaine antagonists or 'partial agonists' potentially useful in abuse treatment.

Chemical Synthesis

For the preparation of the racemic **3a–c** we took, in part, advantage of known procedures.[10] Commercially available acetonedicarboxylic acid was reacted with methylamine hydrochloride and 2-methoxysuccindialdehyde (**6**) at room temperature in a citrate buffer solution at pH 4 to give the key 6-methoxytropinone (**7**) in 60% yield. The starting **6** was obtained by exposure of a mixture of 2,3,5-trimethoxytetrahydrofurane, in turn obtained from 2,5-dimethoxy-2,5-dihydrofurane, by reaction with methanol as reported in the literature, to a 0.2 M aqueous sulfuric acid solution. The stereoselective reduction of **7** by means of hydrogen and PtO_2 produced in 70% yield the 6-methoxytropine (**8**) which was easily reacted with benzhydryl chlorides **9a–c** to afford, in appreciable yields, the desired benztropines **3a–c** (Scheme 1).



Scheme 1.



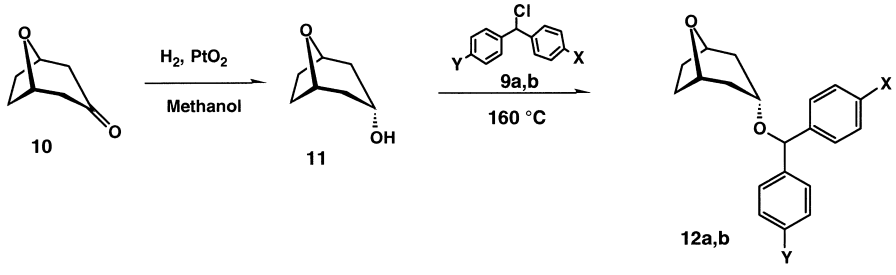
Since the synthesized compounds **3a**–**c**, different from the parent benztropine and analogues, bear a chiral center, we were intrigued to verify whether stereochemistry could play a role in their binding to the recognition site on DAT and, consequently, we needed access to the enantiomers of our racemic methoxylated benztropines. Indeed, it has been well documented by Carroll and others that stereochemistry plays an important role in the binding of cocaine and its analogues to the recognition site on DAT and, for example, the seven stereoisomers of cocaine are less potent than the natural product.[2] Moreover, a profound enantioselectivity has previously been demonstrated in the 2-carbomethoxy-substituted 3α-[bis(4′-fluorophenyl)-methoxy]tropane series of DAT ligands.[15] Therefore, we have investigated methods aimed at obtaining our methoxybenztropine analogues in non-racemic form. We considered that methoxytropinones (+)-**7** and (−)-**7** possessing the known configuration at the carbon atom bearing the methoxy functionality, would constitute appropriate starting materials for our purposes. Accordingly, the stereoselective reduction of the ketones (+)-**7** and (−)-**7** produced the corresponding alcohol derivatives (−)-**8** and (+)-**8** in optically pure form, which were easily transformed, as described above for the racemic **8**, into the desired enantiomeric (+)-**3b** and (−)-**3b** and diastereomeric (+)-**3c** and (−)-**3c** which retain the same absolute configuration at the tropane skeleton as the starting compounds.

Finally, the synthetic route depicted in Scheme 2 allowed facile preparation of the 8-oxygenated analogues **12a**,**b**. The ketone **10** was easily reduced by means of PtO_2 in methanol solution to provide the alcohol derivative **11** in 80% yield. Reaction with the appropriate benzhydryl chlorides **9a**,**b** affords in appreciable yields the desired **12a**,**b**.

Biological Results and Discussion

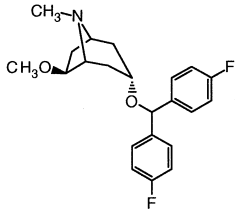
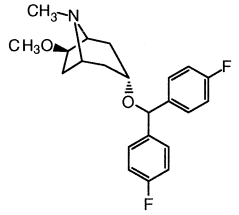
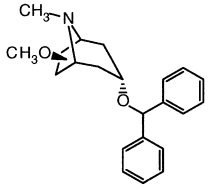
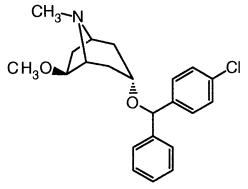
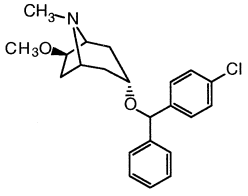
Enantiomers (+)-**3b** and (−)-**3b**, diastereoisomers (+)-**3c** and (−)-**3c**, and the racemic mixtures **3a** and **12a**,**b** were examined for their ability to displace [^{3}H]WIN 35,428 and [^{3}H]paroxetine from DA and 5-HT transporters in the rat caudate putamen. Additionally, compounds possessing an appreciable binding affinity to DAT were tested for their ability to inhibit high-affinity uptake of [^{3}H]DA into striatal nerve endings (synaptosomes). In general, compounds with the tropane skeleton retain activity relative to their parent structures, namely benztropine and cocaine, in both binding and functional assays (Table 1). In the case of the enantiomeric pairs (+)-**3b** and (−)-**3b** as well as of the diastereoisomers (+)-**3c** and (−)-**3c** the levo 6*R* isomers were the most potent. Interestingly, compound (−)-**3c** showed a binding activity comparable to the corresponding 4′-chlorobenztropine whereas (−)-**3b** demonstrated only a 2-fold decrease in binding affinity as compared to the parent ligand 4′,4″-difluorobenztropine.[10] On the contrary, the 8-oxabenztropine analogues **12a**,**b** were devoid of any activity. Additionally, none of the newly synthesized benztropines displayed affinity to SERT.

Among the results obtained, perhaps the most interesting observation relates to the differences in binding versus DA reuptake inhibition for compounds (−)-**3b** and (−)-**3c**; in the case of the 6*R* isomers the IC_{50} for binding is about 4 to 5 times smaller than the IC_{50} for inhibition of DA uptake. Vice versa, a different behavior has been observed with the *S* isomers: the IC_{50} for binding is only slightly lower or higher than the IC_{50} for inhibition of DA uptake. Consequently, notwithstanding that only a relatively small number of chiral compounds have been described here, the current findings



Scheme 2.

Table 1. IC_{50} values for the 6-methoxylated and 8-oxygenated benztropine analogues in DA[^{3}H]WIN 35,428 and 5HT[^{3}H]paroxetine binding and dopamine uptake experiments

Compd		DA[^{3}H]WIN 35,428 Binding, IC_{50} (nM)	[^{3}H]DA Uptake IC_{50} (nM)	5HT[^{3}H]Paroxetine Binding, IC_{50} (μM)
Cocaine		89±8	208±12	
Benztropine		118±9	403±115	
(+)-**3b**		95±5	165±10	>100
(−)-**3b**		25±3	104±11	>100
(±)-**3a**		650±50	2280±73	>100
(+)-**3c**		750±70	519±29	>100
(−)-**3c**		32±2	179±9	>100
12a		>10,000	—	—
12b		2000±200	—	—

clearly demonstrate that also in the benztropine class of DAT inhibitors an interesting enantioselectivity may exist when chiral carbon atoms are present in the tropane carbon skeleton. In particular, a marked enantioselectivity is seen for (−)-**3c**, which is 23 times more active in binding affinity than (+)-**3c**. It is also worth noting that, although a large body of literature exists concerning the different biological activity of cocaine's stereoisomers, very few observations have hitherto been reported regarding chiral benztropines, probably because of the absence of chiral carbon atoms in the starting benztropine. However, Newman et al. have recently demonstrated that chiral benztropines could be of particular interest in the quest for analogues that retain high affinity for DAT but less for muscarinic receptors.[16] Therefore, the study of chiral benztropines could be of particular interest in research dealing with selective DAT ligands.

Since in the last decade there has been considerable effort to determine the SARs of cocaine and congeneres, it has become evident that DAT inhibitors do not all interact with DAT in the same manner. It has been found that neither nitrogen nor oxygen[14,17,18] are

prerequisites for binding of cocaine-based ligands to monoamine transporters and Meltzer et al.[17] had postulated that the three-dimensional topology of the ligand may be more important for binding to the biological macromolecule (DAT or SERT) than the specific functionality present. However, the inactivity of the 8-oxygenated benztropines **12a**,**b** seems to point out that, unlike cocaine and its analogues, interaction of this class of tropane-based DAT inhibitors may be governed to a large extent by the nitrogen atom. To this regard it is worth noting that *N*-acyl- and *N*-sulfonyl-benztropines were also reported to be inactive.[19]

Additionally, we wish to draw attention to the biological results obtained from this class of methoxylated benztropines with respect to the two-carbon bridge methoxylated cocaines. In fact, as the introduction of a methoxyl function at 6 and 7 positions of cocaine strongly compromises mazindol binding at DAT, the methoxylated benztropines still retain potent activity and, in some cases, the compounds are more active than the parent benztropine. Therefore, in our opinion, these findings may add further support to the notion that the domains associated with the cocaine and the benztropine recognition sites are not identical.

In summary, the results obtained with the stereoisomers (+)-**3b**, (−)-**3b**, (+)-**3c** and (−)-**3c** demonstrate that when a chiral center is present in the benztropine molecule its activity is closely related to the stereochemistry of the compounds and the 6*R* isomers are the most interesting. Considering that methoxylation of benztropine produces compounds possessing high DAT activity, whereas the 8-oxygenated benztropines are devoid of any activity, and that these data are in sharp contrast with those obtained in the cocaine series of compounds, these results further substantiate the hypothesis that benztropine may be interacting with a different binding domain at DAT as compared to cocaine and its analogues. Moreover, since the 8-oxygenated benztropines are not active, it is likely that, at least for this class of compounds, the nitrogen atom present in the tropane system plays an important role in modulating the DAT activity of the compounds.

In conclusion, methoxylation at the benztropine's two-carbon bridge provides important leads toward mapping the topology of DAT. Furthermore, these novel molecular probes, and the structural information they provide, will very likely contribute to the development of novel ligands for DAT and possible novel pharmacotherapeutic tools for cocaine abuse. The study of other two-carbon bridge modified analogues of benztropine would therefore be strongly warranted, and as evidenced by the foregoing work, particular attention must be given to the stereochemistry of the substituents attached to the tropane ring system.

Acknowledgements

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