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BICYCLO[3.2.1]OCTANES: SYNTHESIS AND INHIBITION OF BINDING AT THE DOPAMINE AND SEROTONIN TRANSPORTERS

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Abstract: Herein we report the synthesis of a series of bicyclo[3.2.1]octanes and their binding characteristics at the dopamine and serotonin transporters. The data confirm that a heteroatom at position 8 of the tropane nucleus is not a prerequisite for binding since the bicyclo[3.2.1]octanes prove potent inhibitors of both transporters. Therefore the three-dimensional topology of the ligand may be more important than specific functionality with respect to stereospecific binding at the acceptor site.

Cocaine is a potent stimulant. Its abuse potential likely stems from inhibition of dopamine reuptake as a result of the binding, which it exhibits on the dopamine transporter (DAT).[1–6] In the search for potential medications for cocaine abuse, there has been considerable research on the structure–activity relationships of ligands that bind to the DAT. Our exploration of the interaction of cocaine with the DAT has focused on congeners of the 3-aryl-8-azabicyclo[3.2.1]octanes (3-aryltropanes)[7](Fig. 1).[5,8–16]

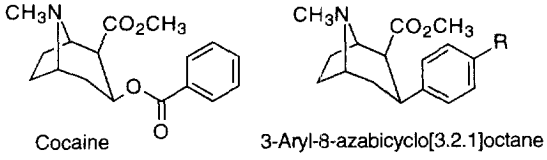

Figure 1. Cocaine and 3-aryltropanes

Recently, we have reported that the nitrogen in the classical 3-aryltropanes can be exchanged for oxygen and substantial binding at the DAT maintained. We presented a model of the 12 transmembrane domain DAT. We also suggested that the specific acceptor site for the many disparate DAT ligands may in fact be different, albeit within the channel of the membrane-bound DAT itself. We proposed that blockage of the channel by ligand binding should inhibit binding of competing ligands whether they bind at the same molecular acceptor sites or not.[12,14]

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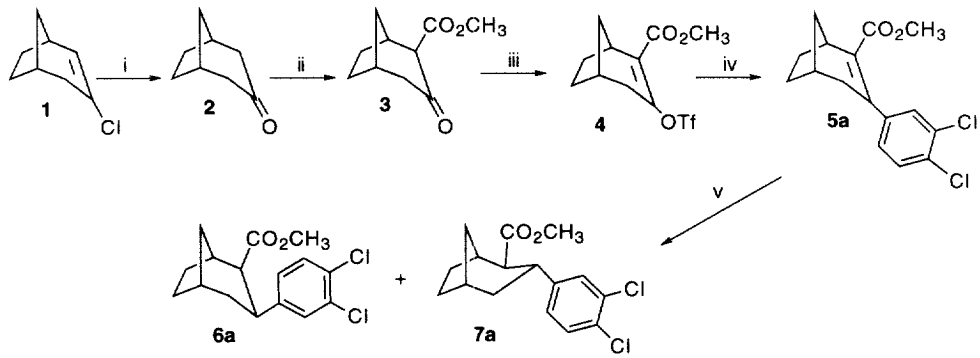
While it had been proposed that an 8-nitrogen was necessary for ionic bonding of a ligand to the DAT, the tight binding exhibited by the 8-oxabicyclo[3.2.1]octane analogs suggested that an ionic bond between the ligand and the macromolecule was not a prerequisite for binding. Indeed, these results implied that a hydrogen bond might suffice.[14] In order to explore this further, we have now prepared bicyclo[3.2.1]octanes devoid of 8-heteroatoms. These surprisingly potent DAT inhibitors indicate that neither ionic nor hydrogen bonding is a prerequisite for inhibition of the DAT. We report that the 2,3-unsaturated aza, oxa, and carba bicyclo[3.2.1]octanes are quite potent, and that the 3α-aryl and 2,3-unsaturated compounds are strikingly selective.

Chemistry

The general syntheses of the 8-aza compounds[9] and 8-oxa compounds have been published previously.[14] The new analogs presented here were synthesized as shown in Scheme 1.

Scheme 1. Synthesis of 3-aryl-bicyclo[3.2.1]octanes



Reagents: (i) H_2SO_4, 77%; (ii) $CNCOOCH_3$, LDA, 83%; (iii) $Na(TMS)_2N$, $Ph(Tf)_2N$, THF, -78 °C, 75%; (iv) $ArB(OH)_2$, Pd_2dba_3, Na_2CO_3, 69%; (v) SmI_2, Methanol, -78 °C, 72%: after column chromatography **6a**: 7%; **7a**: 6%

Commercially available 3-chlorobicyclo[3.2.1]oct-2-ene **1** was treated with sulfuric acid to obtain the ketone **2** in 77% yield.[17] Introduction of the 2-carbomethoxy group was achieved analogously to the route previously reported for synthesis of the 8-oxa compounds.[14] Thus, the ketone **2** was treated with methyl cyanoformate in the presence of lithium diisopropyl amide to provide the racemic keto ester **3** in 83% yield. The ester **3** exists in equilibrium in three isomeric forms, namely the 2α-carboxy ester, the enol ester, and the 2β-carboxy ester. Purification and isolation of each of these isomers was not readily achievable nor was it necessary, since conversion to the enol triflate **4** could be achieved by treatment of a mixture of the three

compounds with sodium bis(trimethylsilyl)amide and phenylbis(trifluoromethanesulfonyl)amine to obtain **4** in 75% yield. Suzuki coupling[18,19] of the enol triflate with 3,4-dichlorophenylboronic acid then provided the 2,3-unsaturated analog **5a** in 69% yield. Samarium iodide reduction of **5a** gave a mixture of all four isomers in 72% yield. These compounds presented as a single spot in all TLC systems evaluated and consequently proved extremely difficult to separate. Notwithstanding, careful gravity column chromatography with a compound to silica ratio of 1:100 provided the pure 3β- (**6a**) and 3α-(3,4-dichlorophenyl)-2-carbomethoxybicyclo[3.2.1]octanes (**7a**) in 7% and 6% yield, respectively.

Structural assignment of **6a** and **7a** was complicated by the fact that there is no heteroatom at position 8 and therefore interpretation of ^{1}H NMR data is difficult. However, the structure of **6a** was proved conclusively by X-ray crystallographic analysis to be the 2β,3β-substituted compound. The ^{1}H NMR spectra (NOE, COSY and HETCOR) of **6a** compared with the spectra of **7a** then allowed the unambiguous structural assignment of **7a** as the 2β,3α-substituted isomer. As expected for 3α-aryl compounds and evidenced by the NMR experiments, **7a** adopts the boat conformation.

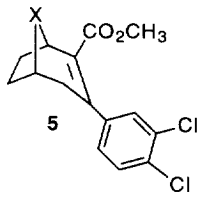
Biology

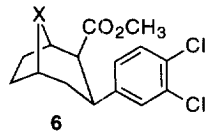
The affinities (IC_{50}) for the dopamine and serotonin transporters were determined in competition studies using [^{3}H]3β-(4-fluorophenyl)tropane-2β-carboxylic acid methyl ester ([^{3}H]WIN 35,428 or [^{3}H]CFT) to label the dopamine transporter and [^{3}H]citalopram to label the serotonin transporter.[5] Competition studies were conducted with a fixed concentration of radioligand and a range of concentrations of the test drug. All drugs inhibited [^{3}H]WIN 35,428 and [^{3}H]citalopram binding in a concentration-dependent manner. Binding data are presented in the Table. The 8-aza analogs are pure (1*R*)-enantiomers. The 8-carba analogs are racemic. For comparative purposes, data for both the racemic and pure (1*R*)-enantiomers of the 8-oxa series are provided.

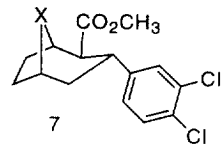
Discussion

The role of the 8-substituent on the tropane skeleton has been questioned. It was initially assumed that an 8-nitrogen provides an ionic anchor at the DAT acceptor site at physiological pH.[20] Subsequently it was demonstrated that ionic bonding was not a prerequisite for binding to the receptor[21] and 8-oxa analogs were found to be potent inhibitors of the DAT.[14] Herein we show that in fact an 8-functional anchor is not a prerequisite for binding to the DAT at all, and **5a**, **6a**, and **7a** bind potently at the DAT (IC_{50} range = 7–14 nM). This preliminary study focuses on the 3,4-dichlorophenyl analogs in particular, since our earlier work[9] had clearly demonstrated that this substitution provides compounds that are more potent than those with alternate functionality (e.g., 4-F or 4-H) and they therefore provide an excellent basis for comparison of 8-substitution within this bicyclo[3.2.1]octane series.

Table. Inhibition of [^{3}H]WIN35,428 binding to the dopamine transporter (DAT) and [^{3}H]citalopram binding to the serotonin transporter (SERT) in cynomolgus monkey caudate-putamen.[24]







		IC_{50} (nM)			IC_{50} (nM)			IC_{50} (nM)	
X	Compd 5	DAT	SERT	Compd 6	DAT	SERT	Compd 7	DAT	SERT
CH_2 (*R/S*)	**a**. O-1231	7.1 ± 1.7	5160 ± 580	**a**. O-1414	9.6 ± 1.8	33.4 ± 0.6	**a**. O-1442	14.3 ± 1.1	180 ± 65
O (*R/S*)	**b**. O-1014	12.8 ± 1.4	1960 ± 840	**b**. O-914	3.4 ± 0.5	6.5 ± 2.9	**b**. O-913	3.1 ± 0.1	64.5 ± 14.5
O (*R*)	**b**. O-1059	4.2 ± 0.6	2120 ± 732	**b**. O-1072	3.9 ± 0.5	4.7 ± 1.2	**b**. O-1066	2.3 ± 0.6	30.3 ± 9.8
CH_3N (*R*)	**c**. O-1109	1.2 ± 0.2	867 ± 75	**c**. O-401	1.1	2.5 ± 0.1	**c**. O-1157	0.38 ± 0.1	27.6 ± 0.8
HN (*R*)				**d**. O-456	0.7 ± 0.2	1.24 (n=1)			

Tissue (4 mg/mL original wet tissue weight) was incubated with each radioligand and 7–14 concentrations of a cocaine congener. Nonspecific binding of [^{3}H]WIN 35,428, was measured with 30 μM (-)-cocaine and of [^{3}H]citalopram with 1 μM fluoxetine. IC_{50} values were computed by the EBDA computer program and are the means (± S.D. or S.E.M.) of 2–4 independent experiments, each conducted in triplicate.[5]

The data presented in the table confirm that, irrespective of whether the 8-position is occupied by a nitrogen, oxygen or carbon, these bicyclo[3.2.1]octane analogs are potent inhibitors of the DAT. Inhibition of WIN 35,428 binding at the DAT occurs between 0.4 and 14 nM for all these compounds. For comparison, the IC_{50} for (-)-cocaine itself at the DAT is 95.6 ± 14.4 nM while at the SERT it is 270 ± 120 nM.[9] The data show the surprising fact that not only is the 8-nitrogen of the classical tropanes not a prerequisite for ligand binding to the DAT, but replacement with an 8-oxygen[12] or even with an 8-carbon does not effect inhibition of this transporter too markedly. This latter finding calls into question the notion of an assumed three-point binding requirement[22,23] for stereospecific inhibition of a biological macromolecule (e.g., DAT) by a ligand. Indeed, the fact that a bicyclo[3.2.1]octane, devoid of a heteroatom, binds potently to the DAT supports the notion that the overall three dimensional topology of the ligand, at least in the case of the DAT, is more important than specific functionality with respect to stereospecific binding of the ligand at its acceptor site.[16]

A comparison of the selectivity for DAT inhibition vs SERT inhibition is extremely interesting. Irrespective of the presence of a N, O or C at the 8-position, the 2,3-unsaturated compounds show a particularly striking selectivity (between 460- to 720-fold) with only micromolar inhibition of the SERT (IC_{50} range = 867–5160 nM). The 3α-aryl (boat conformation) compounds are substantially more selective for the DAT vs SERT (between 10- to 70-fold) than are their 3β (chair conformation) counterparts (between 2- to 4-

fold). It is clear that conformation of the molecule plays a substantial role in control of binding to the SERT. While diastereomers that have the 3-aryl group in the 3β-position (chair) bind most potently to the SERT (IC_{50} = 2.5 to 33.4 nM), the 3α oriented (boat) compounds bind less well (IC_{50} = 27.6 to 180 nM) and the "flattened" 2,3-unsaturated compounds bind least well of all (IC_{50} = 867 to 5,160 nM). This difference in binding to the SERT accounts for the increased DAT selectivity manifested, since potency for DAT inhibition remains approximately similar for all three 3α, 3β and 2,3-unsaturated compounds (IC_{50} = 0.38 to 14.3 nM)

In a comparison of the three-dimensional structures of these three classes, namely the 3α, 3β and 2,3-unsaturated compounds, two features in particular could be envisioned to provide an explanation for the observed selectivity. First, the orientation of the 3-aryl ring with respect to the molecular skeleton differs in each of these classes. Second, the relative orientation of the 2-carbomethoxy group with respect to the aromatic ring is different in each class. One or other, or perhaps both, of these aspects must control placement of the 3,4-dichlorophenyl ring and 2-carbomethoxy substituent in the acceptor site[14] of the DAT and SERT. This placement must then affect relative affinity. Thus, while the 3β-aryl diastereomers bind quite potently at the acceptor sites of both the DAT and the SERT, the 3α-aryl diastereomers and 2,3-unsaturated compounds do not fit the SERT acceptor site as well. This suggests that the selectivity of these 3-arylbicyclo[3.2.1]octanes can be controlled by the conformation of the 3-aryl ring as well as the conformation of the 2-carbomethoxy group.

Conclusion

In summary, bicyclo[3.2.1]octanes show a surprising potency at monoamine transporters thus suggesting that a functional anchor at this site is unnecessary for binding at the DAT acceptor site. The striking selectivity for the DAT manifested by the 3α-aryl compounds and the 2,3-enes in particular suggests that the DAT is considerably more flexible with respect to binding than is the SERT. Could it be that there are multiple tropane acceptor sites[14] on the DAT with perhaps very few, or even only a single, tropane acceptor site on the SERT?

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24. All new compounds exhibited ^{1}H NMR, ^{13}C NMR and combustion analyses consistent with reported structures. X-ray crystallographic data, refined atomic coordinates, tables of bond angles and distances have been deposited with the Cambridge Crystallographic Data Centre.