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Synthesis, Cocaine Receptor Affinity, and Dopamine Uptake Inhibition of Several New 2β-Substituted 3β-Phenyltropanes

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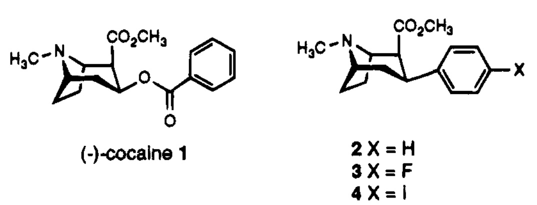
Cocaine (**1**) is believed to mediate its pharmacologic effects through the occupation of high-affinity saturable stereoselective binding sites associated with biogenic amine uptake mechanisms.[1-5] The reinforcing effects of cocaine have been shown to be associated with the inhibition of dopamine uptake; however, the mechanistic details of dopamine uptake inhibition are unclear at this time.[6-8] It is conceivable that occupation of the cocaine receptor results in the physical blockade of dopamine at the dopamine reuptake site or that allosteric modulation of the dopamine transporter occurs such that dopamine uptake is not possible.[3,9,10] In order to obtain a greater understanding of the mechanism by which cocaine affects dopamine uptake inhibition and elicits its reinforcing effects, a study of the structure–activity relationships (SAR) of cocaine and cocaine-related derivatives has been initiated in these laboratories.



The 2β-carbomethoxy-3β-phenyltropanes **2** (WIN 35,065-2),[11,12] **3** (WIN 35,428),[11,12] and **4** (RTI-55)[12,13] are among the most potent tropane derivatives for cocaine receptors. From the SAR of these compounds and 2β-substituted cocaine derivatives it is clear that the substitution at C2 of the tropane nucleus has a profound effect on the affinity of the cocaine receptor ligands.[8] Recent reports by Carroll *et al.*,[14,15] Davies *et al.*,[16,17] and Kozikowski *et al.*[18] have demonstrated that a variety of 2β-carbonyl- or 2β-unsaturated 3β-phenyltropane derivatives exhibit high affinity for cocaine receptors. However, the nature of the interaction between the 2β-substituents and the binding site has not been clearly identified. To explore the nature of the interaction between the cocaine receptor and high-affinity ligands as well as attempt to identify the proximal effect of electron-rich/unsaturated 2β-substituents, a series of 2β-substituted 3β-phenyltropanes were synthesized and tested *in vitro* for cocaine receptor affinity and for dopamine uptake inhibition.

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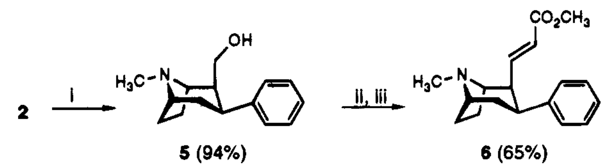
Scheme 1[a]



[a] Reagents: (i) $LiAlH_4$, Et_2O, 0 °C; (ii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C; (iii) $(CH_3O)_2POCH_2CO_2CH_3$, LiCl, *i*Pr_2NEt, CH_3CN, 25 °C.

The 2β-substituted 3β-phenyltropane derivatives **5–10** were prepared from **2**. *N*-Methyl-2β-carbomethoxy-3β-phenyltropane **2** was prepared from cocaine according to the modified procedure of Carroll *et al.*[13] As illustrated in Scheme 1, $LiAlH_4$ reduction of the 2β-ester group of **2** afforded the 2β-(hydroxymethyl)-3β-phenyltropane **5**.[11] Swern oxidation of **5** to the corresponding aldehyde followed by subsequent olefination, furnished the α,β-unsaturated ester **6** in 65% overall yield.[19] The mild conditions of the Masamune–Rousch olefination procedure afforded the ester **6** as a single crystalline isomer resulting from no epimerization of the labile intermediate 2β-aldehyde.[20] The structure of **6** was determined by NMR and the 2β-stereochemistry was unequivocally confirmed by X-ray crystallographic analysis (Figure 1).

With stereochemistry at the 2β-position no longer susceptible to epimerization, the 2β-derivatives **7–10** were prepared in a straightforward fashion from the unsaturated ester **6** (Scheme 2). Hydrogenation of **6** afforded saturated ester **7** in quantitative yield, while reduction of **6** gave the allyl alcohol **8** (95% yield). Conversion of ester **7** into **9** was achieved by reduction with $LiAlH_4$. Finally, Swern oxidation/Masamune-Rousch olefination of **9** furnished the unsaturated ester **10** (89% yield).

The five 2β-substituted 3β-phenyltropane derivatives **6–10** were tested for their ability to displace bound [^{3}H]-**3** from rat caudate–putamen tissue.[21] In addition, the compounds **6–10** were tested for their ability to inhibit high affinity uptake of [^{3}H]dopamine into rat caudate–putamen tissue.[21] The K_i values reported in Table 1, are the dissociation constants derived for the unlabeled ligands. The linear portion of the [^{3}H]dopamine uptake inhibition curves were analyzed using standard analysis of variance and linear regression techniques.

Both cocaine and **2** modeled better for two binding sites than for one, and therefore two K_i values are given in the table.[22,23] In contrast, compounds **6–10** did not model for two sites better than for one. The affinities of compounds **6–10** are generally similar to the high affinity values obtained for **1** and **2**. The saturated alcohol **9** exhibited a 2-fold increase in potency in both *in vitro* paradigms compared to the esters **6**, **7**, and **10** and the unsaturated alcohol **8**. This result was somewhat surprising since most potent 2β-substituted 3β-aryltropane derivatives derived from either cocaine or 3β-phenyltropanes reported to date possess an unsaturated moiety such as an ester,[11-14] a ketone,[16,17] an oxadiazole,[15] or a vinyl group[18] directly bound to the tropane ring system. Moreover, the 2β-hydroxymethyl analog **11**, homoesters, and C2-unsubstituted 3β-aryltropanes have been reported to have diminished activity at cocaine receptors.[11,14]

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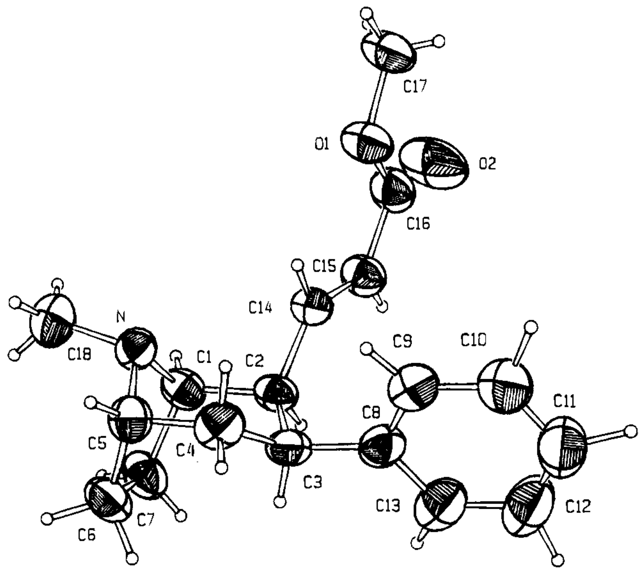
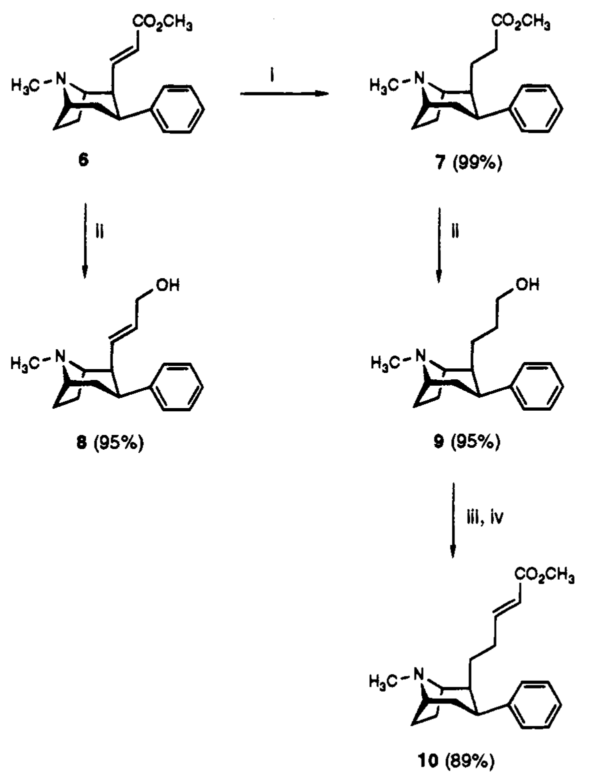


Figure 1. X-ray crystal structure of compound **6** (free base).

Scheme 2[a]



[a] Reagents: (i) 10% Pd/C, MeOH; (ii) $LiAlH_4$, Et_2O, 0 °C; (iii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C; (iv) $(CH_3O)_2POCH_2CO_2CH_3$, LiCl, *i*Pr_2NEt, CH_3CN, 25 °C.

The high affinity of the saturated alcohol **9** suggests that the flexible nature of the saturated hydroxypropyl unit allows the ligand to better conform to the structural constraints imposed by this region of the cocaine binding site. The other analogs **6−8** and **10** possess some degree of unsaturation which increases the rigidity of the side chain and perhaps decreases the ability of the ligand to adopt a higher affinity conformation at the cocaine receptor. Despite recent studies by Kozikowski *et al.*[18] which have shown that 2β-vinyl-3β-aryltropane analogs **12−15** possess high affinity for cocaine receptors, clearly unsaturation does not facilitate binding in this system. Moreover, since the unsaturated alcohol **8** demonstrates no greater affinity than esters **6**, **7**, and **10**, the hydroxyl group of **9** probably has little effect or at most a nonspecific effect on the affinity of the ligand.

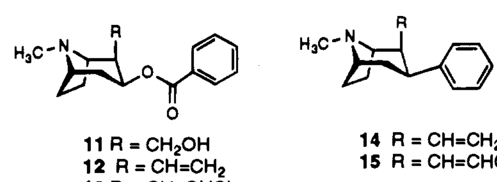
Table 1. K_i Values for Displacement of Receptor Bound [^{3}H]-**3** and IC_{50} Values for Inhibition of [^{3}H]Dopamine Uptake[a]

analog	K_i (nM)	IC_{50} (nM)
1[b]	32 ± 5 388 ± 221	405 ± 91
2[b]	33 ± 17 314 ± 222	373 ± 100
6	22 ± 2	123 ± 65
7	23 ± 2	166 ± 68
8	26 ± 3	159 ± 43
9	11 ± 1	64 ± 32
10	20 ± 2	203 ± 77

[a] All values are the mean ± SEM of three experiments performed in triplicate. [b] The binding data for these drugs are reproduced from ref 23 and were collected under conditions identical to the present ones.

The compounds **6**, **7**, **8**, and **10** were synthesized in order to evaluate the effect of the proximity of an unsaturated group and or an electron-rich group relative to the tropane ring on cocaine receptor affinity. Interpretation of the *in vitro* affinity data indicates that the proximity of an unsaturated group or an electron-rich group to the tropane nucleus has little effect on the ligand affinity. Despite the fact that the functional groups were displaced by as many as four carbon atoms from the tropane nucleus, the K_i values of the analogs **6**, **7**, **8**, and **10** were all equivalent to that of **2**. It is equally noteworthy that the cocaine binding site was able to accommodate the large ester side chain of **10** with no effect on the affinity of the ligand. This result is consistent with data obtained by Carroll *et al.* in which large 2β-substituents of 3β-phenyltropane and cocaine derivatives demonstrated high affinity for cocaine binding sites.[14,15]

From the results of this study and from known SAR of 2β-substituted 3β-phenyltropane and cocaine derivatives,[14−18] it can be concluded that the region of the cocaine binding site which is occupied by the 2β-substituent must either be a large cleft in the dopamine transporter protein or is exterior to the binding site where the 2β-substituent lies above the surface of the dopamine transporter protein. Either case would explain the ability of the binding site to accommodate the large side chains of potent ligands. Secondly, this region appears to be relatively insensitve to electrostatic and lipophilic interactions. This is consistent with only the slight difference in affinity observed for compounds **2**, **6−10**. The proposed nature of this region of the cocaine binding is in agreement with the models recently proposed by Kozikowski *et al.* [18] and Srivastava and Crippen.[25]



11 R = CH_2OH
12 R = $CH=CH_2$
13 R = CH=CHCl
14 R = $CH=CH_2$
15 R = CH=CHCl

In summary, it is the stereochemical orientation of substituents at C2 which is the primary requirement for high-affinity binding at cocaine receptors, while the steric bulk and the lipophilic character of the 2βsubstituents exhibit minimum effects on the affinity of the ligand. The results of this study are in complete agreement with these observations. Moreover, the SAR reported herein supports the hypothesis that electrostatic interactions (including hydrogen bonds) between 2β-substituents and the cocaine binding site are of minimal importance while hydrophobic interactions may contribute significantly to the free energy of binding and lead to the enhanced potency of ligands.[15] Further studies aimed at the elucidation of the substituent effects of high-affinity 2β-substituted 3β-phenyltropane derivatives in both *in vitro* and *in vivo* systems is warranted and are the subject of current investigations.

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Supplementary Material Available: Experimental procedures, physical data for compounds **6–10**, and X-ray crystallographic data for compound **6** (12 pages). Ordering information is given on any current masthead page.

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