Synthesis and Ligand Binding Study of 3β-(4′-Substituted phenyl)-2β-(heterocyclic)tropanes

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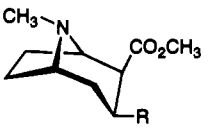
The definition of the pharmacophore for the cocaine (**1a**) binding site on the dopamine transporter has been the subject of considerable scientific interest for the last few years.[1-9] A number of 3β-(substituted phenyl)-tropane-2β-carboxylic acid esters (**1b**) have been synthesized as potential ligands for this binding site and evaluated for their ability to inhibit [^{3}H]WIN 35,428 binding at the dopamine transporter. The results show that substituents on the 3β-aromatic ring greatly influence binding potency at the dopamine transporter and that the nature of the 2β-substituents plays a key role in determining the selectivity for the dopamine transporter relative to the norepinephrine and serotonin transporters. For example, whereas methyl esters show little transporter selectivity, phenyl esters are highly selective for the dopamine transporter.[1]

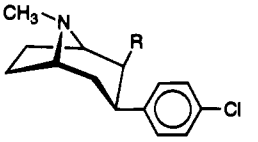
Bioisosterism is an important concept which serves as a valuable aid in structure–activity relationship studies and new drug design.[10,11] In our studies to characterize the pharmacophore for the cocaine binding site on the dopamine transporter, we had found that 3β-(4′-chlorophenyl)-2β-(3-phenyl-1′,2′,4′-oxadiazol-5′-yl)tropane (**3**, RTI-130) had high binding potency at the dopamine transporter, and since the oxadiazole function is stable to chemical and enzymic degradation, this ligand was an excellent, stable bioisostere for 3β-(4′-chlorophenyl)tropane-2β-carboxylic acid phenyl ester (**2**, RTI-113).[12-14] In our studies to further define the requirements of the cocaine binding site, we examined analogues of **3** possessing other 5-membered heterocyclic rings in the 2β-position. We describe in this paper the synthesis of 3β-(4′-chlorophenyl)-2β-(3′-phenylisoxazol-5′-yl)tropane (**4**, RTI-177), 3β-(4′-chlorophenyl)-2β-(5′-phenyl-1′,3′,4′-oxadiazol-2′-yl)tropane (**5**, RTI-188), and 3β-(4′-chlorophenyl)-2β-(5′-phenyloxazol-2′-yl)tropane (**6**, RTI-189) and report that **4** is potent and more selective than **3** for the dopamine transporter.

The syntheses of the 2β-heterocyclic analogues **4**–**6** is outlined in Scheme 1.[15] A solution of 3β-(4′-chlorophenyl)tropane-2β-carboxylic acid methyl ester (**7**)[16] was added to the dilithium salt of acetophenone oxime in THF at 0 °C, and the reaction mixture was warmed to 25 °C. After 18 h at 25 °C, the reaction mixture was added to a THF solution containing sulfuric acid and water and was refluxed for 1 h. Standard workup gave the isoxazole **4** which was characterized as the hydrochloride salt, mp 287 °C dec; $[\alpha]^{24}_D$ −97.5° (*c* 0.28, CH_3OH). Treatment of the acid **8**, obtained by hydrolysis



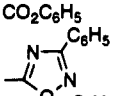
1a, R = OCC_6H_5 (O)

b, R = C_6H_4X

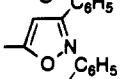


2 (RTI-113), R = $CO_2C_6H_5$

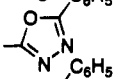
3 (RTI-130), R =



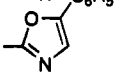
4 (RTI-177), R =



5 (RTI-188), R =



6 (RTI-189), R =



of **7**, with benzoic acid hydrazide in phosphorus oxychloride provided the 1,3,4-oxadiazole **5**. The hydrochloride salt of **5** had mp 160–162 °C; $[\alpha]^{24}_D$ +84.6° (*c* 0.36, CH_3OH). Treatment of **8** with oxalyl chloride, followed by condensation with 2-aminoacetophenone, gave the amide **9**, and cyclization of **9** with phosphorus oxychloride yielded oxazole **6**. The tartrate salt of **6** had mp 126 °C dec; $[\alpha]_D$ +101.4° (*c* 0.26, CH_3OH).

The IC_{50} values for the inhibition of [^{3}H]WIN 35,428, [^{3}H]nisoxetine, and [^{3}H]paroxetine binding at the dopamine, norepinephrine, and serotonin transporters, respectively, for the previously reported analogues **2** and **3** and the new analogues **4**–**6** are listed in Table 1. Binding affinities were determined as described previously.[12] The phenyl ester **2**, the 1,2,4-oxadiazole **3**, and the isoxazole **4** possess almost identical IC_{50} values for the dopamine transporter. In contrast, the IC_{50} values for inhibition of binding at the dopamine transporter for the 1,3,4-oxadiazole **5** and the oxazole **6** are 1 order of magnitude higher. The isoxazole **4**, with NE/DA and 5-HT/DA ratios of 394 and 1890, respectively, is highly selective for the dopamine transporter relative to the norepinephrine and serotonin transporters. Comparison of **4** to the parent phenyl ester **2** shows that it possesses slightly higher affinity for the dopamine transporter and greater selectivity relative to the serotonin transporter while retaining a 394-fold selectivity, relative to the norepinephrine transporter.

We, and others, have reported binding affinities for various 2-substituted analogues of cocaine and of 3β-(4′-substituted phenyl)tropanes.[1,3,5,12-14] The results have shown that a 2β-substituent is required for high potency binding at the dopamine transporter. Results from some of the analogues suggest an electrostatic interaction between the 2β-substituent and the binding site, whereas results from other analogues are more consistent with a hydrophobic or steric interaction of the 2β-substituent with the binding site. One explanation for this apparent contradiction is that the binding of analogues possessing 2β-substituents which are

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Scheme 1

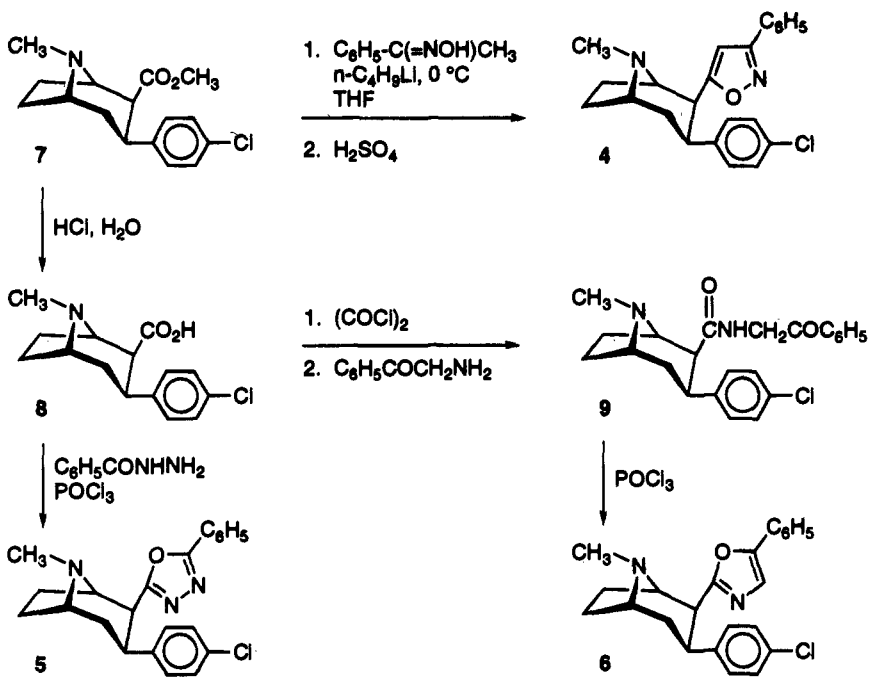
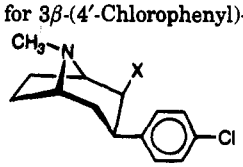
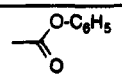
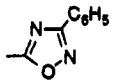
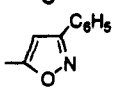
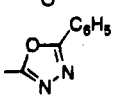
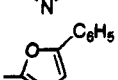


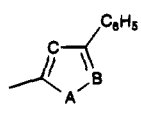
Table 1. Comparison of Transporter Binding Potencies for 3β-(4′-Chlorophenyl)-2β-(heterocyclic)tropanes



compd	X	IC_{50} (nM)[a] DA[^{3}H]WIN 35,428	NE[^{3}H]Nisoxetine	5-HT[^{3}H]Paroxetine	NE/DA ratio[b]	5-HT/DA ratio[b]
2[c]		1.98 ± 0.05	2960 ± 223	2340 ± 176	1490	1180
3[d]		1.62 ± 0.02	245 ± 13	195 ± 4.8	151	120
4		1.28 ± 0.18	504 ± 29	2420 ± 136	394	1890
5		12.6 ± 1.03	929 ± 88	3300 ± 196	74	262
6		19.7 ± 1.98	496 ± 42	1120 ± 107	25	57

[a] Data are mean ± standard error of three or four experiments performed in triplicate. [b] Ratios of IC_{50} values. [c] The IC_{50} values are from ref 1. Cocaine has IC_{50} values of 89, 3298, and 1045 nM at the DA, NE, and 5-HT transporters (ref 1). [d] The IC_{50} values are from ref 12.

capable of participating in electrostatic interactions may be dominated by factors different from those involved in the binding of substituents incapable of such interactions. The set of ligands **3**–**6** possesses 2β-substituents with near equal molecular shapes and volumes but with variations in the location and, in some cases, the number of heteroatoms, providing an ideal set for evaluation of the effects of interactions of ester bioisosteres at the 2β-position on the tropane ring with the binding site. The oxadiazoles **3** and **5** both have the same number and type of heteroatoms in the 2β-substituent; however, the binding potency at the dopamine transporter is 8 times greater for **3** than for **5**. Similarly, **4** and **6** both have one nitrogen and one oxygen in the 2β-substituent, but **4** is 15 times more potent at the dopamine transporter than **6**. Since these data are not accommodated by steric and/or hydrophobic factors, it appears that for this set of analogues there are additional interactions between the 2β-substituent in cocaine-like compounds and the cocaine binding site at the dopamine transporter which contribute significantly to the binding potency. In order to explore the possibility that this is an electrostatic interaction, molecular electrostatic potentials (MEP) in the vicinity of the atoms at positions A–C in the model compound **10** were examined. Using the appropriate phenylhet-



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erocycles in which the phenyltropane moiety was replaced by methyl (**10**) as simplified models of compounds **3**–**6**, and superimposing the heterocyclic and phenyl rings so as to minimize steric and conformational effects, differences in the MEPs were obtained from semiempirical (AM1) quantum mechanics calculations.[17-19] A strong correlation between differences in the electrostatic potential minima near position A, ΔV_{min}(A), and affinity at the dopamine transporter is apparent for the four structure models of compounds **3**–**6**. Relative to the lowest V_{min}(A) (compound **3**), the 2β-substituent models of the higher affinity compounds **3** and **4** have ΔV_{min}(A) values of 0 and −4 kcal/mol, while those of the lower affinity compounds **5** and **6** have ΔV_{min}(A) values of −50 and −63 kcal/mol, respectively. In general, increasingly negative V_{min} in the vicinity of hydrogen bond acceptor atoms is correlated with increases in the strength of associated hydrogen bonds.[20,21] Such correlations have been incorporated into quantitative structure–activity relationships (QSAR) of muscarinic ligands where increasingly negative V_{min} has also been correlated to higher binding affinities.[22,23] In contrast to these reports of relationships between MEP and efficacy at muscarinic receptors for compounds bearing similar heterocyclic substituents, higher affinity at the dopamine transporter appears to be associated with relatively less negative V_{min} values. Therefore, although these calulations support a role for electrostatic interactions in binding at the dopamine transporter for this group of substituents, the observed correlation argues against the interaction being associated with hydrogen bond acceptor sites in the 2β-substituents of these analogues.

At the NE transporter, binding potency is higher in the bioisosteres **3**–**6** than in the parent ester **2** and does not vary substantially between them, while at the 5-HT transporter, **3** is 1 order of magnitude more potent than the parent ester **2** and those of the bioisosteres **4**–**6**. Thus it appears that the 2β-substituent in **3** possesses unique electronic features favorable to binding at the 5-HT transporter. The extreme sensitivity of binding affinity at the 5-HT transporter to changes in the 2β-substituent had been noted previously.[1]

In summary, the biochemical results from this study show that the 2β-isoxazole **4** is a potent, selective, and stable ligand for the dopamine transporter. Since pharmacological effects associated with the inhibition of the NE and 5-HT uptake would be absent for **4** and its in vivo half-life should be greatly extended over that of an ester analogue, this ligand possesses properties needed for medications for treatment of cocaine abuse. In addition, this study has provided evidence for an electrostatic interaction of some 2β-substituents of some phenyltropane analogues with the cocaine binding site.

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(15) Compounds **4**–**6** were analyzed for C, H, and N. The results agreed to within ±0.4% of the theoretical values. Since compounds **4**–**6** were prepared from **7** (ref 12), they possess the same absolute stereochemistry of natural (−)-cocaine. The 2β,3β-stereochemical assignment was based on an analysis of ^{1}H NMR which showed chemical shift values of the C-1, C-2, C-3, C-4$_{ax}$, C-4$_{eq}$, and C-5 protons almost identical with those found for **3**.

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