

Synthesis and Monoamine Transporter Affinity of 2 β -Carbomethoxy-3 β -(2''-, 3''- or 4''-substituted) Biphenyltropanes

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Abstract—A series of 11 novel 3 β -substituted biphenyltropanes was synthesized and evaluated by selective radioligand binding assays for affinity to monoamine transporters. Both 5-HTT potency and selectivity for 5-HTT over DAT was greatest with electron withdrawing group at the 3''-position. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Tropane (8-azabicyclo[3.2.1]octane) derivatives have been studied extensively in recent years, particularly in relation to the pharmacology of the benzoyloxytropane stimulant cocaine (**1**), encouraged by strong interest in developing treatments for cocaine addiction. Several 3 β -phenyltropanes have been used as radiotracers for quantification of DAT in brain tissue, with clinical applications in SPECT or PET brain scanning.^{1–4} In addition, radiolabeled derivatives of 2 β -carbomethoxy-3- β -(4-iodophenyl) tropane (β -CIT)⁵ and its precursor norCIT have been used to quantify the serotonin (5-hydroxytryptamine) transporter (5-HTT), with limited success because of inadequate selectivity for 5-HTT over DAT.

Information is accumulating on structure–activity relationships of tropanes that bind at specific membrane transporter proteins for DA, 5-HT and norepinephrine (NE). Transporters are critical for the physiological inactivation of monoamine neurotransmitters following their extraneuronal release at synapses, and they are blocked by cocaine and other stimulants. Assessment of substitution at the 2 β , 3 β , and 8 positions of the tropane ring indicates that high affinity for DAT or 5-HTT requires an aromatic group at the 3 β position.⁶ Observations by Davies^{7,8} and Carroll⁹ suggest that having unsaturated substituents on

the phenyl ring and removing the *N*-Me group enhance selectivity for 5-HTT over DAT. We therefore hypothesized that adding a second substituted phenyl moiety at position 4' of the 3 β -phenyl ring would increase selectivity for the 5-HTT over DAT.

The proposed biphenyltropanes have the effect of adding unsaturation to the 3 β -phenyl ring, which is believed to interact with specific amino acid residues in the binding region of the transporter peptide chains. For example, in the 5-HTT protein, tyrosine residue Tyr-95 in the putative first hydrophobic transmembrane domain and Tyr-176 in the third transmembrane domain have been proposed as critical sites of molecular interaction with 5-HT and aromatic tropanes.^{10,11} For DAT, phenylalanine residues play the same role.¹² Interactions of the aromatic rings of these amino acid residues with the phenyl ring of the phenyltropane would explain the absolute requirement for the 3 β -phenyl substitution for DAT and 5-HTT potency. In addition, the presence of an OH group in tyrosine residues in 5-HTT and its absence in the phenylalanine residues of DAT suggests that appropriate substituents on a 4'-phenyl substituted-tropane might yield differential affinity for 5-HTT over DAT. These aromatic residues in transporter polypeptides may interact with 4'-substituted phenyltropanes by both π -stacking and hydrogen bonding to increase potency and selectivity for 5-HTT. In this study, we evaluated the effect of aromatic substitution on the 3 β -phenyl ring of 2 β -carbomethoxy-3 β -(4'-iodophenyl)tropane (β -CIT; RTI-55) on affinity and selectivity for 5-HTT over DAT.

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Synthesis

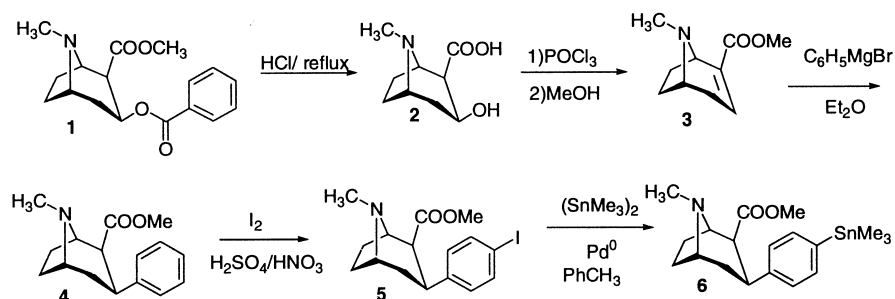
Cocaine (**1**) was treated with 6 N HCl at reflux to give ecgonine (**2**; Scheme 1). Treatment with POCl₃ and MeOH converted **2** to ecgonidine methyl ester (**3**). Grignard addition of phenyl-magnesium bromide to **3** led to compound **4**. Treatment of **4** with iodine gave β-CIT (**5**). The tin derivative **6** was obtained by treatment of β-CIT with hexamethylditin in the presence of tetrakis(triphenylphosphine) palladium (Scheme 1).

A series of 4'-phenyl-substituted aromatic phenyltropanes was synthesized by Stille coupling between **6** and a desired bromo or iodo phenyl derivative (Scheme 2).¹³

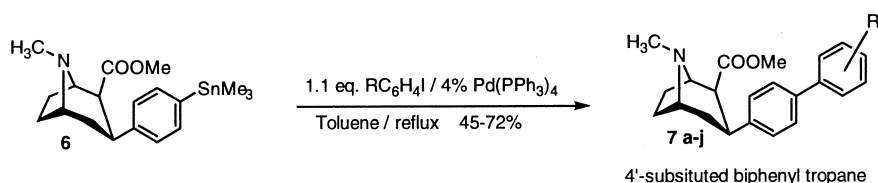
Phenol **7j** was obtained as the major product of reaction of 3-bromophenol trimethylsilyl ether with **6** after work up and purification on silica gel. Compound **7k** was obtained by catalytic reduction of **7e** under hydrogen with 5% Pd/C.

Binding and Discussion

The potency (K_i , nM) of the substituted biphenyltropane compounds synthesized was evaluated by competition against radiolabeled ligands selective for 5-HTT, DAT, and NET in rat forebrain tissue evaluated by competition against radiolabeled ligands selective for 5-HTT, DAT, and NET in rat forebrain tissue (Table 1).^{14–17} In general, 4'-substitution on the primary phenyl ring with phenyl (i.e., *p*-biphenyl derivatives) led to much lower affinity at NET than at either 5-HTT or DAT. To retain high potency for 5-HTT, *meta* (3'')-substitution was preferred over *ortho* (2'') and *para* (4''), particularly with small electronegative substituents (NO₂, OH, and OCH₃), which yielded K_i values < 2 nM, (Table 1). Moreover, when the *meta* position was substituted with a bulkier electronegative group (3''-COCH₃ compound **7h**), 5-HTT potency fell by more than 22-fold to K_i = 42 nM compared to **7e** (Table 1). In contrast, substitution to the *para* (4'') position on the secondary phenyl ring favored binding to DAT over 5-HTT.



Scheme 1.



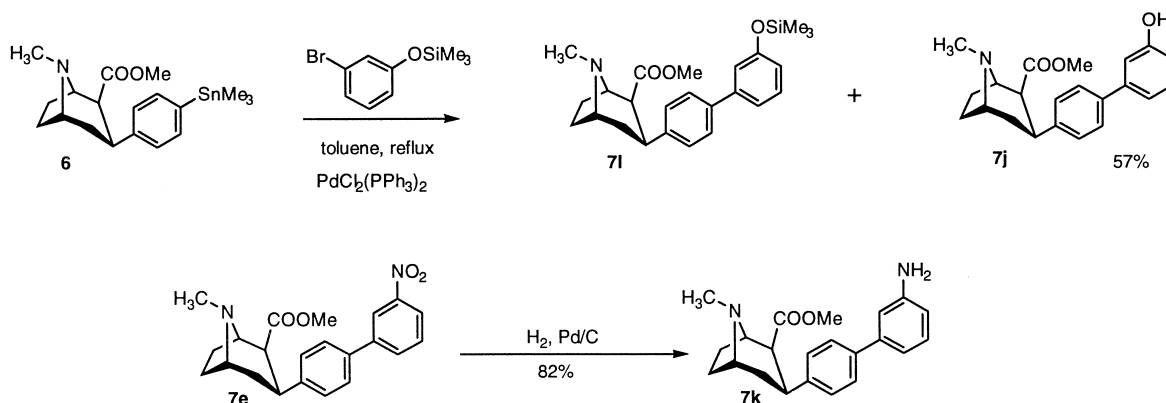
Scheme 2.

Table 1. Affinity of biphenyltropane derivatives to transporters in rat forebrain tissue for dopamine, serotonin and norepinephrine transporter ($K_i \pm$ SE, nM)

Compound	Substitution	5-HTT ^a [³ H]Paroxetine	DAT ^a [³ H]GBR-12935	NET [³ H]Nisoxetine	Selectivity 5-HTT/DAT
β-CIT ^b	I	0.46 ± 0.06	0.96 ± 0.15	2.80 ± 0.40	2.08
7e	3''-NO ₂	0.80 ± 0.08	9.54 ± 2.32	963 ± 110	11.9
7j	3''-OH	1.39 ± 1.10	15.2 ± 3.2	—	11.0
7b	3''-OCH ₃	1.89 ± 0.20	58.7 ± 10.0	>3000	31.1
7f	4''-NO ₂	29.7 ± 0.9	5.10 ± 0.17	370 ± 13	0.17
7c	4''-OCH ₃	32.0 ± 4.5	13.2 ± 0.9	352 ± 35	0.41
7h	3''-COCH ₃	42.0 ± 3.0	12.4 ± 0.8	>50,000	0.30
7k	3''-NH ₂	67.3 ± 2.9	24.2 ± 3.9	—	0.36
7a	2''-OCH ₃	79.5 ± 4.1	357 ± 49	= 10,000	4.49
7i	4''-COCH ₃	179 ± 20	19.1 ± 1.8	754 ± 80	0.11
7g	2''-COCH ₃	5000	1016 ± 431	>20000	0.20
7d	2''-NO ₂	≥ 3000	≥ 3000	>3000	1.00

^aSelectivity 5-HTT/DAT: higher numbers are more selective for 5-HTT.

^bβ-CIT is 4-iodophenyltropane.⁵



Scheme 3.

The most 5-HTT-selective compounds were also those with the highest 5-HTT potency (Table 1). They ranked: 3''-OCH₃ > 3''-NO₂ > 3''-OH, with lesser selectivity found with substitution at the 2'' or 4'' positions (Table 1). The most 5-HTT-over-DAT selective compound, the 3'-OCH₃ derivative (**7b**), had a *K_i* ratio >30, representing the greatest selectivity reported to date for an *N*-methyl tropane derivative.

The present results, combined with previous findings,^{7,10} suggest a possible packing effect between a transporter amino acid such as Tyr-95 or Tyr-176 and the aromatic ring of the phenyltropane. The high affinity and selectivity of 3''-electronegative-substituted (NO₂, OH, OCH₃) compounds (**7e**, **7j**, and **7b**) may also suggest hydrogen bonding of this compound may also occur at the transporter, again perhaps Tyr-95 or Tyr. The possibility of hydrogen bonding between a 3''-methoxy substituent on the secondary phenyl ring of phenyltropanes and Tyr-176 or 95 of the transporter protein also seems consistent with the rank order of selectivity of the phenyltropanes tested: 3''-OCH₃ > 3''-NO₂ = 3''-OH > 2''-OCH₃; other compounds were not 5-HTT-selective (Table 1). A 3''-methoxy group yielded higher 5-HTT-over-DAT selectivity than a 3''-nitro or acyl substitution. To further test the hydrogen bonding hypothesis, we evaluated the 3''-hydroxy and 3''-amino derivatives **7j** and **7k** (Scheme 3). High 5-HTT affinity and selectivity were still observed with the 3''-hydroxy compound. While hydrogen bonding may exist other factors also must play a role in binding.

In general, the present results indicate that secondary 4'-phenyl substitution was tolerated on the phenyl ring attached at position 3β of the tropane ring with retention of high 5-HTT affinity. Moreover, *meta*- (3'')-substitution on the secondary phenyl ring appears favored over *ortho*-(2'') and *para*-(4'') to retain 5-HTT affinity.

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