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Synthesis and monoamine transporter affinity of 3′-analogs of 2-β-carbomethoxy-3-β-(4′-iodophenyl)tropane (β-CIT)

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Abstract—The 3′-iodo positional isomer of 2-β-carbomethoxy-3-β-(4′-iodophenyl)tropane (β-CIT) and other 3′-substituted analogs were synthesized and evaluated for binding to monoamine transporters in rat forebrain and membranes of cell lines selectively expressing human transporter genes. All 3′-substituted compounds displayed affinity for both serotonin (SERT) and dopamine (DAT), but much less for norepinephrine transporters (NET), with selectivity for rat (r) or human (h) SERT over NET, but only 3′-iodo-substituted phenyltropanes showed selectivity for SERT versus DAT. The 3′-iodo, *N*-methyl analog of β-CIT (**7**) displayed 29-fold selectivity and high affinity for hSERT ($K_i = 9.6$ nM) over hDAT ($K_i = 279$ nM), and its nor-congener (**8**) showed even higher hSERT potency ($K_i = 1.2$ nM) and selectivity over DAT (415-fold).

1. Introduction

Cocaine (**1**) and other psycho-stimulants exert multiple physiological and behavioral effects by inhibiting the presynaptic neuronal transport of dopamine (DA), serotonin (5-HT), and norepinephrine (NE) in mammalian central nervous systems. Altered abundance or functioning of these monoaminergic neurons is implicated in the pathophysiology or treatment of several neuropsychiatric disorders, including Parkinson's disease, and schizophrenia, major mood disorders, and attention deficit-hyperactivity disorder, as well as various forms of substance abuse.[1–4]

Cocaine binds to a stereoselective recognition site of the membrane DA transporter protein (DAT), a specific molecular marker of DA neurons in mid- and forebrain tissues. Neuronal reuptake by the DAT is the principal physiological means of regulating synaptic availability of DA. Inhibition of DA transport by stimulants increases and prolongs extracellular concentrations of neuronally released DA, and so potentiates dopaminergic neurotransmission to result in the behaviorally reinforcing, stimulating, and euphoriant effects associated with abuse of these drugs.[5–9] Although effects of cocaine on the DAT have received a great deal of research attention, cocaine is an even more potent inhibitor of the transporters selective for 5-HT (SERT) and norepinephrine (NET) on central neurons that produce these monoamine neurotransmitters. Moreover, potentiation of 5-HT probably contributes to the behaviorally reinforcing effects of cocaine, indicating that effects at both SERT and DAT may be important for the neuropharmacodynamics and potential treatment of cocaine addiction.[10–12]

Studies of the design of novel antagonists or high-affinity partial-agonists for the DAT have focused mainly on modifications of the nonhydrolyzable phenyltropane 2β-carbomethoxy-3β-phenyltropane (WIN 35,065-2; **2**, Fig. 1).

This and similar stable analogs replace cocaine's metabolically labile benzoate ester with a substituted aromatic ring at the 3-position of the tropane moiety.[13] Substituents in position-2 (**3**, **3b**, **3c**), as well as replacement of the bridge heteroatom with oxygen or

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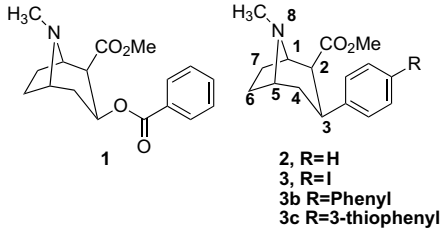


Figure 1.

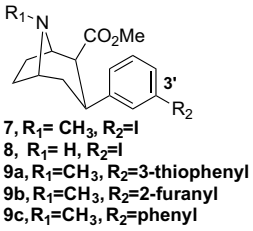


Figure 2.

carbon, also can be tolerated without significant loss of binding affinity for the DAT (Fig. 1).[14,15]

Radiolabeled phenyltropane derivatives have been used successfully for SPECT and PET radionuclide imaging to visualize and quantify DAT in human brain tissue.[16–20] Derivatives of β-CIT (RTI-55; **3**) and its *N*-desmethyl analog nor-β-CIT[21] have also been considered as potential radiotracers to quantify SERT, but with limited success owing to their poor selectivity for SERT versus DAT. To seek more SERT-selective ligands, we built on observations reported by Davies and colleagues[22,23] and Blough et al.[24] indicating that selectivity for SERT over DAT was enhanced by unsaturated substituents on the phenyl ring and by N-demethylation of phenyltropanes. Our previous findings confirmed these trends with a novel series of 2″-, 3″-, or 4″-substituted biphenyl tropanes.[25] We now report on the synthesis of novel 3'-substituted analogs (**7**–**9**; Fig. 2) and their binding affinity to the three major monoamine transporters, SERT, DAT, and NET, in rat forebrain and in membranes of cells selectively expressing the human genes for these membrane proteins.

2. Chemistry

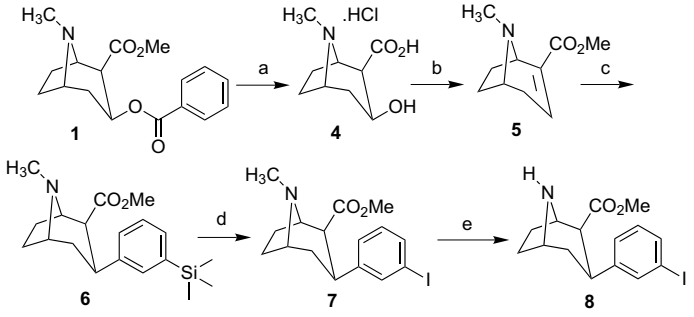
As summarized in Scheme 1, cocaine (**1**) was hydrolyzed by refluxing in HCl to give ecgonine **4** (98% yield), which was transformed into the corresponding ecgonidine methyl ester (**5**, 54% yield) with $POCl_3$ and MeOH. Grignard addition to **5** with 3-trimethylsilylphenylmagnesium bromide in anhydrous ether at −78 °C gave silane **6** (43%). Treatment of **6** with I_2 and $AgBF_4$ in MeOH at rt yielded the 3′-isomer of β-CIT **7** (60%). Demethylation of the nitrogen was achieved with 1-chloroethyl chloroformate in 1,2-dichloroethane at reflux followed by refluxing in MeOH to produce the nortropane derivative **8** (62%).

Additional five- and six-membered ring substituents (Scheme 2) were introduced by means of Rieke coupling between **7** and 3-thiophenylzinc iodide in THF at rt (**9a**, 97%). Stille coupling of **7** with tributylfuran-2-ylstannane in DMF at rt gave **9b** (67%) and with trimethylphenylstannane in refluxing toluene gave **9c** (30% yield).

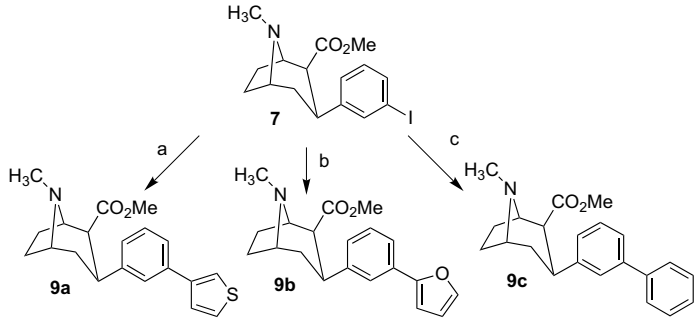
3. Transporter affinities

Transporter potency (K_i, nM) of the novel 3′-substituted phenyltropanes was evaluated by their competition against radioligands selective for SERT, DAT, and NET, using rat (r) forebrain tissue and membranes of cell lines transfected to express human (h) genes for these transporter proteins selectively (Table 1). Selectivity for SERT versus DAT or NET is expressed as K_i ratios, with larger values indicating greater selectivity (Table 2).

The 3′-iodo or *meta*-substituted positional isomer **7** was similarly less potent than the 4′-iodo or *para*-congener β-CIT (**3**) at both rSERT (21-fold) and hSERT (14-fold) proteins, but was >3571 times less potent at NET, and 104–581 times less potent at rDAT and hDAT sites (Table 1), with substantial selectivity for SERT over DAT (by 10- to 29-fold) or NET proteins (108- to >1018-fold). Similar to our previous findings,[25] selectivity for SERT was increased even further by N-demethylation, such that compound **8** exhibited



Scheme 1. Reagents and conditions: (a) HCl aq, Δ; (b) $POCl_3$, MeOH, Δ; (c) 3-$SiMe_3$–C_6H_5MgBr, Et_2O; (d) $I_2/AgBF_4$, MeOH; (e) (1) ACE-Cl, $C_2H_4Cl_2$, Δ, (2) MeOH, Δ.



Scheme 2. Reagents and conditions: (a) 3-thiophenylzinc iodide, $PdCl_2(PPh_3)_2$, THF, rt; (b) tributylfuran-2-stannane, $PdCl_2(PPh_3)_2$, DMF, rt; (c) trimethylphenylstannane, $Pd(PPh_3)_4$, toluene, Δ.

Table 1. Binding affinity to monoamine transporters [$K_i \pm$ SEM (nM)]

Compound	rDAT	hDAT	rSERT	hSERT	rNET	hNET
7* (YP 241)	100 ± 11	182 ± 8	9.82 ± 0.80	9.63 ± 0.4	>10,000	1039 ± 53
8 (YP 256)	21.2 ± 4.6	438 ± 52	1.35 ± 0.13	1.23 ± 0.04	282 ± 12	118 ± 3
9a* (YP-242)	23.7 ± 1.3	29.8 ± 2.6	82.5 ± 4.8	74.1 ± 5.1	>10,000	>20,000
9b* (YP 243)	30.8 ± 4.9	54.0 ± 1.3	41.7 ± 2.2	55.1 ± 2.2	>10,000	3024 ± 438
9c* (YP 244)	9.59 ± 0.34	8.83 ± 0.98	35.2 ± 1.1	38.6 ± 2.0	>10,000	1360 ± 183
β-CIT[26]	1.33 ± 0.15	0.48 (2)	0.41 ± 0.03	N/D	2.80 ± 0.40	11.2 ± 0.7
Nor-β-CIT[26]	0.64 ± 0.097		0.062 ± 0.001		1.85 ± 0.21	

*Tested as HCl salt;[26] (2) K_d value from Scatchard plot.

Table 2. Binding selectivity ratios

Compound	rSERT/ rDAT	hSERT/ hDAT	rSERT/ rNET	hSERT/ hNET
7	10.2	18.9	>1020	108
8	15.7	356	241	95.9
9a	0.280	0.402	>121	>270
9b	0.740	0.980	>240	54.9
9c	0.270	0.229	>283	35.2

16-fold (r) or 415-fold (h) selectivity toward SERT over DAT, and 96 (h) to 241-fold (r) selectivity toward SERT over NET (Table 2).

Substitution at position 3′ with larger, aromatic groups, including the 3′-phenyl, thiophenyl, and furanyl derivatives (compounds **9a**–**9c**) resulted in 4- to 8-fold lower SERT potency and 3- to 10-fold higher DAT affinity, with loss of selectivity for SERT over DAT, all compared to the corresponding *N*-methyl, 3′-iodo congener **7**, and in the descending rank order of rSERT potency: **9c** (3′-furanyl) ⩾ **9b** (3′-thiophenyl) > **9a** (3′-phenyl; Tables 1 and 2).

4. Conclusions

These results indicate an unexpected effect of iodo *meta*-substitution of phenyltropanes at position 3′ of the accessory phenyl ring to increase selectivity for SERT over DAT with moderately high ($K_i = 1$–10 nM) affinity for SERT. Given its particularly high SERT potency and high selectivity for SERT over both DAT and NET, radiolabeling and in vivo assessment of the N-demethylated compound **8** (nor-**7**) as a potentially useful SERT radioligand are now required.

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