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Communications to the Editor

3β-(4-Ethyl-3-iodophenyl)nortropane-2β-carboxylic Acid Methyl Ester as a High-Affinity Selective Ligand for the Serotonin Transporter

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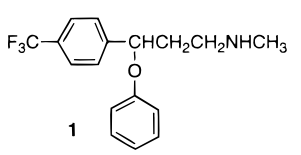
Serotonin-selective reuptake inhibitors (SSRIs) such as fluoxetine (**1**), fluvoxamine (**2**), paroxetine (**3**), and sertraline (**4**) have proved to be important agents in the treatment of depression.[1,2] Even though this class of antidepressants is burdened with fewer side effects than other classes, they still cause gastrointestinal, sleep disturbance, and sexual dysfunction side effects.[3] Thus, the development of new structural classes of clinically useful SSRIs is a subject of continuing interest. Moreover, reports in the literature suggest that inhibition of serotonin (5-HT) uptake modulates the reinforcing properties of cocaine (**5**).[4−6]

In attempts to separate the stimulant (or antidepressant) and local anesthetic actions of cocaine from its toxicity and dependence liability, Clarke and co-workers synthesized several 3β-(substituted phenyl)tropane-2β-carboxylic acid methyl esters (**6**).[7] These compounds are exemplified by WIN 35,065-2 and WIN 35,428, **6a** and



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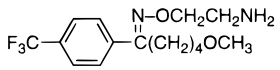

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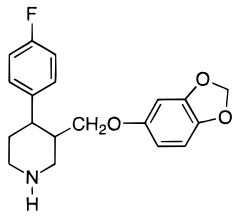

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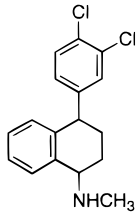

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6b, respectively. Compound **6b** was reported to be about 22 times more active than cocaine in inhibiting the uptake of [^{3}H]norepinephrine (NE) in rat brain.[7] Studies reported in the late 1980s showed that, like cocaine, **6a** and **6b** inhibited the uptake of 5-HT and dopamine (DA) as well as NE. In fact, the potency for inhibition of DA uptake was greater than that for inhibition of 5-HT and NE uptake.[8,9] During the same time frame, [^{3}H]WIN 35,428 ([^{3}H]**6b**) was developed as a radioligand for assaying the inhibition of binding at the dopamine transporter (DAT).[10] We have utilized this ligand for structure activity relationship (SAR) studies to characterize the cocaine binding site on the DAT.[11] Early studies led to the identification of **6c** as a ligand with high affinity for the DAT and low selectivity relative to inhibition of radioligand binding to the serotonin transporter (5-HTT) and norepinephrine transporter (NET). Later studies showed that the phenyl and isopropyl esters **6d** (RTI-113) and **6e** (RTI-114), as well as the 2β-isoxazole analogue **6f** (RTI-177), possess high affinity for the DAT and very low affinity for the 5-HTT and NET.[12,13]

In this study, we report the synthesis of 3β-(4-ethyl-3-iodophenyl)nortropane-2β-carboxylic acid methyl ester (**7**), a cocaine/WIN 35,065-2 (**6a**) analogue which has high affinity for 5-HTT and relatively low affinity for DAT and NET. We also report in vivo competition binding studies in mice which show that the regional distribution and ED_{50} values of **7** are very similar to

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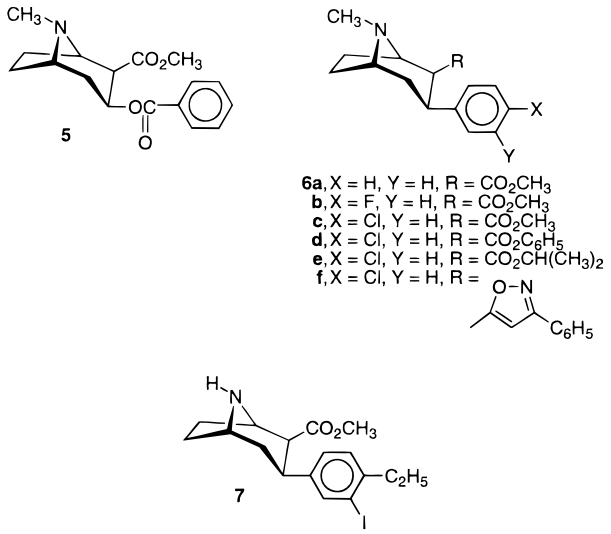
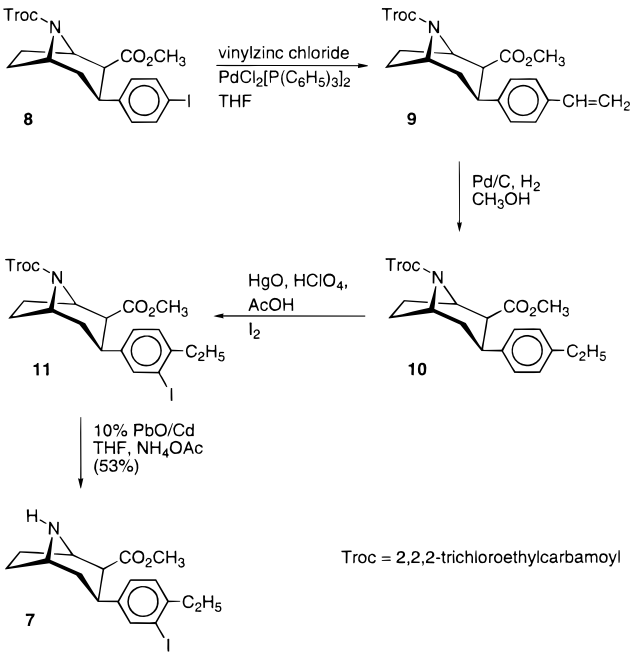


those of **1** and **4**. These results suggest that **7** may be useful as an antidepressant and that an iodine-123 analogue would have potential as a single photon emission computed tomography (SPECT) ligand for imaging 5-HT neurons in living brain.

Chemistry. The route used to synthesize **7** is shown in Scheme 1. Subjection of *N*-[(2,2,2-trichloroethyl)-carbamoyl]-3β-(4-iodophenyl)nortropane-2β-carboxylic acid methyl ester (**8**)[14] to a Stille-type coupling using vinylzinc and bis(triphenylphosphine)palladium(II) chloride in tetrahydrofuran gave 73% of the 4-vinyl compound **9**. The ^{1}H NMR spectrum of **9** was complicated by the existence of rotamers caused by the *N*-(2,2,2-trichloroethyl)carbamoyl group; however, the expected vinyl resonances at 6.65 (1H, dd, J = 10.9 and 17.6 Hz), 5.70 (1H, d, J = 17.6 Hz), and 5.20 ppm (1H, d, J = 10.9 Hz) were clearly delineated. Catalytic reduction of **9** using 10% palladium on carbon catalyst in methanol afforded 4-ethyl compound **10** in 84% yield. The ^{1}H NMR spectrum showed the expected resonances for the ethyl group and the absence of the vinyl resonances. Iodination of **10** using iodine in a mixture of acetic acid and perchloric acid containing mercuric oxide provided *N*-[(2,2,2-trichloroethyl)carbamoyl]-3β-(4-ethyl-3-iodophenyl)nortropane-2β-carboxylic acid methyl ester (**11**) in 68% yield. The ^{1}H NMR spectrum of **11** showed broad resonances at 7.58 (1H) and 7.08 ppm (2H) for the three aromatic protons. The *N*-[(2,2,2-trichloroethyl)carbamoyl group was removed under the experimentally mild conditions of 10% lead oxide−cadmium[15] in a mixture of tetrahydrofuran and 1 M aqueous ammonium acetate at 25 °C to give **7** in 53% yield. The substitution pattern of the phenyl ring was confirmed by heteronuclear multiple bond correlation which showed a three-bond interaction between the ethyl methylene protons (quartet at 2.68 ppm) and the carbon bearing the iodine (unique at 100 ppm). The chemical shift and coupling pattern of the C-2 and C-3 protons are consistent with previously reported compounds that possess the 2β,3β-stereochemistry.[14] The compound was further characterized as the tartrate salt: mp 160−161 °C; $[\alpha]^{25}_D$ −56.9° (c 0.26, CH_3OH).

In Vitro Binding. The binding affinities of the compounds for DAT, 5-HTT, and NET were determined using competitive binding assays following previously reported procedures.[16] The radioligands used were 0.5 nM [^{3}H]WIN 35,428 for the DAT, 0.2 nM [^{3}H]paroxetine

Scheme 1



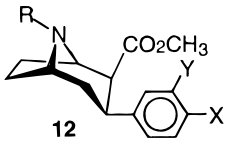
for the 5-HTT, and 0.5 nM [^{3}H]nisoxetine for the NET. The results are listed in Table 1.

In Vivo Binding. Male CD-1 mice (Charles-River, Wilmington, MA), weighing 28−32 g, were pretreated with increasing doses of **7** (0.1−10 mg/kg, i.p., 0.2 mL of saline) 60 min before intravenous injection of 2 μCi (in 0.2 mL of saline) of [^{125}I]RTI-55 ([^{125}I]-3β-(4-iodophenyl)tropane-2β-carboxylic acid methyl ester) into the tail vein. The animals were sacrificed 2 h later as previously described.[17] The brains were immediately dissected on ice, the individual tissues were weighed and placed in plastic tubes, and the radioactivity was assayed in an automatic gamma counter. The counting error was kept below 3%. Aliquots of the injectate were prepared as standards and counted along with the tissue samples. The percent injected dose per gram tissue (%ID/g) was calculated.

Results and Discussion. In our SAR studies, we had noticed that removing the *N*-methyl group from **6c** to give 3β-(4-chlorophenyl)nortropane-2β-carboxylic acid methyl ester **12a** increased affinity at both the DAT and 5-HTT; however, the effect on the 5-HTT was much larger[18] (see Table 1). Since our SAR studies also had shown that the addition of a 4-ethyl group to the phenyl moiety of WIN 35,065-2 to give 3β-(4-ethylphenyl)-tropane-2β-carboxylic acid methyl ester **12b** reduced affinity at the DAT and increased affinity at the 5-HTT, we expected that 3β-(4-ethylphenyl)nortropane-2β-carboxylic acid methyl ester **12c** would have selectivity for 5-HTT.[18] This compound, **12c**, with IC_{50} values of 8.13 and 49.9 nM for the 5-HTT and DAT, was the first WIN 35,065-2 analogue whose affinity at the 5-HTT was greater than at the DAT.[18]

We also had reported that adding a 3-iodo group to WIN 35,065-2 to give 3β-(3-iodophenyl)tropane-2β-carboxylic acid methyl ester **12d** had little effect on affinity at the DAT.[19] Since present data demonstrated substantial enhancement of affinity for **12d** at 5-HTT relative to WIN 35,065-2 (Table 1), we reasoned that the addition of a 3-iodo group to **12c** might lead to a compound with increased affinity and selectivity for the

Table 1. Comparison of Inhibitors of Radioligand Binding to Monoamine Transporters



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				IC_{50}(nM)[a]			ratio[b]	
compd	X	Y	R	DA [^{3}H]WIN 35,428	NE [^{3}H]nisoxetine	5-HT [^{3}H]paroxetine	NE/DA	5-HT/DA
cocaine[c]				89.1	3300	1050	37	12
6a, WIN 35, 065-2[c]	H	H	CH_3	23	920	2000	40	87
6b, WIN 35,428[c]	F	H	CH_3	13.9 ± 1.7	835 ± 45	692 ± 71	60	50
6c (RTI-31)[c]	Cl	H	CH_3	1.12 ± 0.1	37 ± ± 2.1	44.5 ± 1.3	33	40
7 (EINT)	C_2H_5	I	H	329 ± ± 13	148 ± ± 9.2	0.69 ± 0.07	0.5	0.002
12a (RTI-110)[c]	Cl	H	H	0.62 ± 0.09	4.13 ± 0.62	5.45 ± 0.21	7	9
12b (RTI-83)[c]	C_2H_5	H	CH_3	55.0 ± 2.1	39.0 ± 381	28.4 ± 3.8	71	0.52
12c (RTI-173)[c]	C_2H_5	H	H	49.9 ± 7.3	122 ± ± 12	8.13 ± 0.3	2.5	0.2
12d (RTI-92)[d]	H	I	CH_3	26 ± ± 1.7	1590 ± ± 120	38.8 ± 3.8	61	1.5

[a] Data are mean standard error of three or four experiments performed in tripliate. [b] Ratios of IC_{50}values. [c] IC_{50} values taken from ref 18. [d] IC_{50} value for DA taken from ref 19.

5-HTT. This compound, 3β-(4-ethyl-3-iodophenyl)nortropane-2β-carboxylic acid methyl ester (**7**), was prepared and was found to have an IC_{50} value of 0.69 nM at the 5-HTT and IC_{50} values of 329 and 148 nM at the DAT and NET, respectively. To our knowledge, **7** is the most potent and selective WIN 35,065-2 analogue thus far prepared for the 5-HTT.

The efficacy of the antidepressant drugs **1**−**4** as well as the modulation of cocaine self-administration has been associated with serotonin transporters. Since **7** is a potent and selective compound for the 5-HTT and also contains an iodine that could be replaced by an iodine-123, this compound could be useful as an SPECT ligand to measure serotonin transporters in living humans if it provided high levels of specific binding. To examine the potential of **7** as a SPECT imaging agent, and to compare its brain levels to those of the antidepressant drugs, sertraline and fluoxetine, the inhibition of the binding of [^{125}I]RTI-55, which binds to both DATs (especially in striatum) and 5-HTTs, in mouse brain in vivo was investigated; the results are shown in Figure 1. The regional distribution of [^{125}I]RTI-55 binding in mouse brain varied, with the striatum having the highest, and the cerebellum having the lowest, radioactivity. Consistent with the observed in vitro selectivity of **7** for 5-HTT relative to DAT, the analogue **7** inhibited [^{125}I]RTI-55 binding in the hypothalamus and thalamus, regions with high concentrations of 5-HTTs. This effect was dose-dependent with [^{125}I]RTI-55 binding decreasing with increasing doses of **7**. In contrast, in the striatum and olfactory tubercle, regions with high concentrations of DATs relative to 5-HTTs, binding of [^{125}I]RTI-55 increased, in a dose-dependent manner, with administration of **7** (Figure 1). The effectiveness of **7** in decreasing the binding of [^{125}I]RTI-55 in 5-HTT-rich regions (hypothalamus and thalamus) supports the notion that **7** is selective for 5-HTT. The increase of [^{125}I]RTI-55 binding in DAT-rich regions is likely due to increased availability of [^{125}I]RTI-55 following its displacement from 5-HTTs by **7**. The calculated ED_{50} of **7** in hypothalamus, 3.5 μmol/kg, is in the range of ED_{50} values reported for sertraline (2.6 μmol/kg) and fluoxetine (6.8 μmol/kg).[17,20]

In summary, the removal of the *N*-methyl group combined with the addition of 4-ethyl and 3-iodo substituents to the 3β-phenyl group of WIN 35,065-2 affords

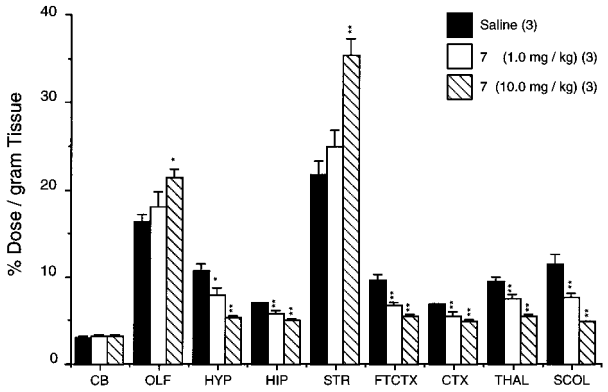

Figure 1. Inhibition of [^{125}I]RTI-55 binding by **7** in mouse brain. Compound **7** (1.0 or 10 mg/kg in 0.2 mL of saline) or saline (0.2 mL) (controls) was injected intraperitoneally 60 min before intravenous injection of the tracer via tail vein. All animals were sacrificed 120 min after tracer injection. The number of animals is given in parentheses. CB = cerebellum; OLF = olfactory tubercle; HYP = hypothalamus; HIP = hippocampus; STR = striatum; FTCTX = prefrontal cortex; CTX = parietal cortex; THAL = thalamus; SCOL = superior colliculus. Data are means ± SEM, $n = 3$, $^*P < 0.05$; $^{**}P < 0.01$.

7, a compound possessing high affinity and selectivity for the 5-HTT. In vivo competition binding studies in mouse suggest that the iodine-123 analogue of **7** may be a useful SPECT ligand for studying the 5-HTT in living brain and that **7** may also be useful as an antidepressant.

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Supporting Information Available: Experimental data for the syntheses of **7** (4 pages). Ordering information is given on any current masthead page.

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