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Synthesis and biological evaluation of 2β,3α-(substituted phenyl)nortropanes as potential norepinephrine transporter imaging agents

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Abstract—A series of 2β,3α-(substituted phenyl)nortropanes was synthesized and evaluated in vitro for human monoamine transporters. All compounds studied in this series exhibited nanomolar potency for the norepinephrine transporter (NET). Radiolabeling and nonhuman primate microPET brain imaging studies were performed with the most promising compound, [^{11}C]**1**, to determine its utility as a NET imaging agent. Despite high in vitro affinity for the human NET, the high uptake of [^{11}C]**1** in the caudate and putamen excludes its use as an in vivo PET imaging agent for the NET.

The norepinephrine transporter (NET), a specific marker of noradrenergic neurons, plays a critical role in regulating neurotransmitter concentration at noradrenergic synapses as well as terminating noradrenergic neurotransmission by reclaiming norepinephrine from the extracellular space.[1,2] The NET has been recognized in the involvement of several neurological and psychiatric disorders and is an established molecular target for the treatment of depression, anxiety disorder, and attention-deficit/hyperactivity disorder (ADHD).[3–6] Imaging agents suitable for visualization and quantification of the NET by emission tomography techniques would present unique opportunities to define the function and pharmacology of the NET in the living human brain.

Several potent NET reuptake inhibitors such as desipramine, nisoxetine, and analogues of tomoxetine have been labeled and evaluated as NET imaging agents. However, these radioligands have not shown suitable in vivo properties.[7–9] The recently reported C-11 or F-18-labeled reboxetine derivatives, [*S*,*S*]-MRB,[10–12] [*S*,*S*]-FMeNER-D_2,[13–15] represent by far the most promising candidates based upon preliminary studies in rats and non-human primates.

We became interested in the 3-phenyltropane motif because a variety of analogues have been shown to be substrates for the dopamine transporter (DAT), serotonin transporter (SERT), and NET.[16] However, while numerous efforts have been focused on developing analogues with high affinity as radioligands for the DAT or SERT, their potential as NET radioligands remains largely unexplored. Carroll et al. recently reported structure–activity relationships of tropane analogues related to their binding affinity to the NET. In these studies, they demonstrated that 2β,3α-isomers of 3-(substituted phenyl)nortropanes possessed higher binding affinity at the NET, while their affinity at the DAT and SERT decreased relative to their corresponding 2β,3β-isomers.[17,18] Particularly, 2β,3α-(3′-fluoro-4′-methylphenyl)nortropane (**1**, Fig. 1) is a highly potent and selective compound with a K_i of 0.43 nM at the rat NET and 21- and 55-fold selectivity for the rat NET versus rat DAT and rat SERT, respectively. 4′-Methyl analogue (**2**) also shows high affinity for the rNET (K_i = 5.2 nM) and selectivity over the

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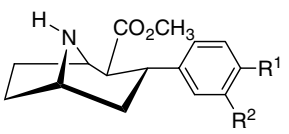


1. $R^1 = CH_3$, $R^2 = F$
2. $R^1 = CH_3$, $R^2 = H$
3. $R^1 = I$, $R^2 = H$
4. $R^1 = -CH{=}CH_2$, $R^2 = H$
5. $R^1 = -C{\equiv}CH$, $R^2 = H$

Figure 1. Structures of 2β,3α-(substituted phenyl)nortropanes.

rDAT (K_i = 33.6 nM) and rSERT (K_i = 46 nM). These findings prompted us to synthesize 2β,3α-(substituted phenyl)nortropanes which may offer promise as potential NET imaging agents. Based upon the observation that 2β,3β-isomers of 3-(4′-iodophenyl)nortropane,[19] 3-(4′-vinylphenyl)nortropane,[20] and 3-(4′-ethynylphenyl)nortropane[20] were reported to have good affinity at the NET with IC_{50} = 7.5, 14.9, and 21.8 nM, respectively, we chose to synthesize their 2β,3α-isomers to see if this conformation change will improve the affinity and selectivity for the NET.

In this paper, we report the synthesis of 2β,3α-isomers of 3-(4′-iodophenyl)nortropane (**3**), 3-(4′-vinylphenyl)nortropane (**4**), and 3-(4′-ethynylphenyl)nortropane (**5**). The K_i values for these compounds along with the reference compounds **1** and **2** were measured through in vitro competition assays utilizing cells expressing human NET, DAT, and SERT. A radiolabeling method was developed for the most promising compound **1**, and [^{11}C]**1** was evaluated through microPET imaging studies in nonhuman primates to assess in vivo binding to the NET in the brain.

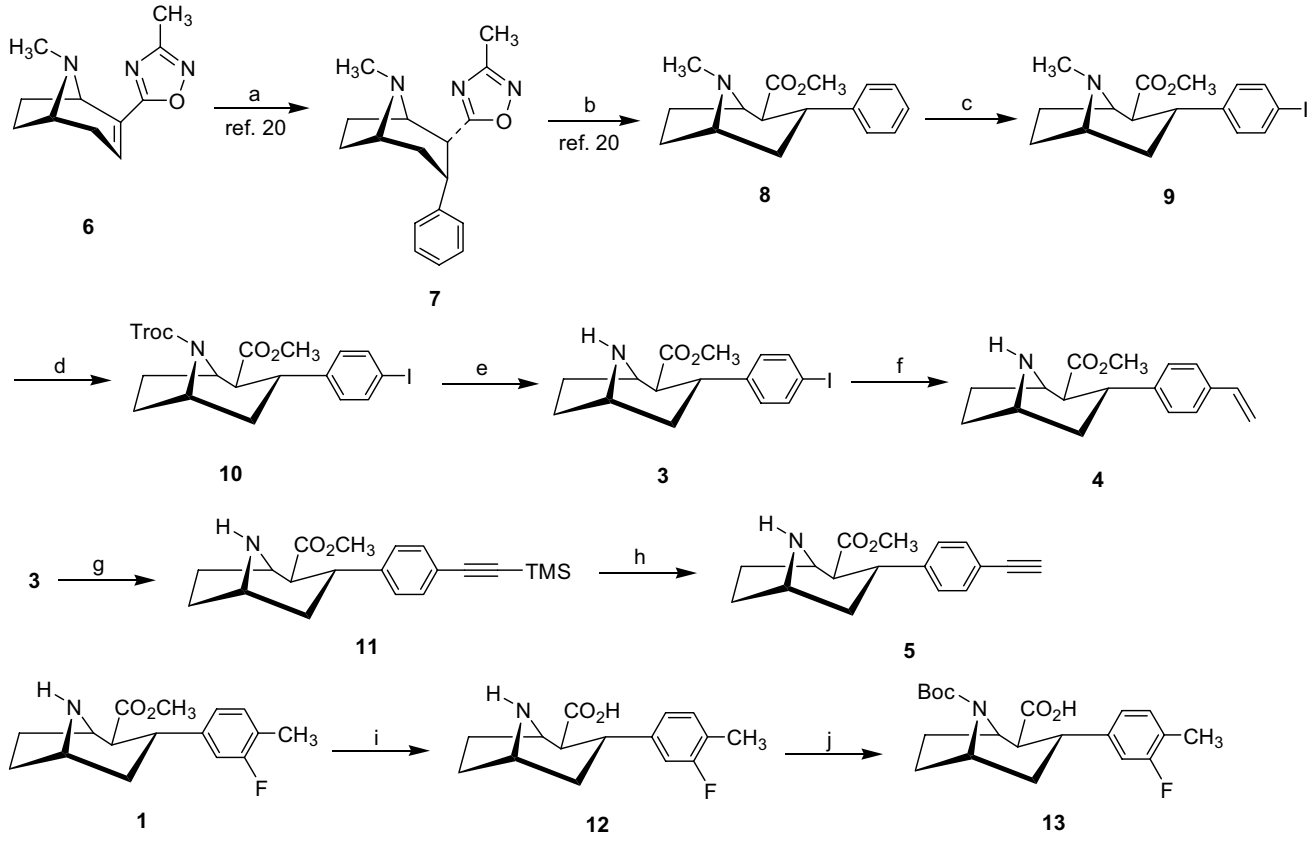
Compounds **1** and **2** were prepared as previously described.[18] The synthesis of **3**, **4**, and **5** is outlined in Scheme 1. Attempts to prepare **9** by addition of 4-iodophenyl lithium to oxadiazole (**6**) followed by reduction with nickel boride as reported in the literature[21] were unsuccessful in our hands. In this case, the addition reaction was very messy and gave us the desired isomer in less than 10% yield. Alternatively, **9** was prepared in a high yield by a three-step procedure: addition of phenyllithium to oxadiazole (**6**);[21] conversion of the oxadiazole (**7**) to the methyl ester (**8**) by reduction with nickel boride and hydrochloric acid in refluxing methanol;[21] and direct iodination of **8** using I_2 and silver trifluoromethanesulfonate in CH_2Cl_2.[22] The conversion of **9** to its corresponding carbamate using trichloroethylchloroformate followed by Zn–acetic acid reduction gave 3-(4′-iodophenyl)nortropane (**3**). Palladium-catalyzed coupling of **3** with tributyl(vinyl)tin produced **4** in 87% yield. Reaction of **3** with trimethylsilylacetylene in the presence of a catalytic amount of copper(I) iodide and bis(triphenylphosphine)palladium dichloride afforded **11** in 84% yield, and subsequent removal of the silyl-protecting group with tetrabutylammonium fluoride provided **5** in 90% yield. Hydrolysis of **1** in refluxing 1,4-dioxane/H_2O afforded nortropane acid **12**, which was *N*-Boc protected to give the radiolabeling precursor **13**.

The radiolabeling of [^{11}C]**1** was accomplished through O-methylation of *N*-Boc acid precursor **13** with [^{11}C]CH_3I in the presence of Bu_4NOH followed by deprotection with 6 M HCl and HPLC purification (Scheme 2). The entire procedure required approximately 40 min after the delivery of [^{11}C]CH_3I to the reaction vessel. [^{11}C]**1** was prepared in an average 45% decay-corrected yield. Analytical HPLC demonstrated that the radiolabeled product was over 98% radiochemically pure, and the specific activity of the product was 1.2–2 Ci/μmol at time of injection. The distribution coefficient of [^{11}C]**1** was determined to be $\log P_{7.4}$ = 1.17 between 1-octanol and phosphate buffer at pH 7.4 according to a known procedure.[23]

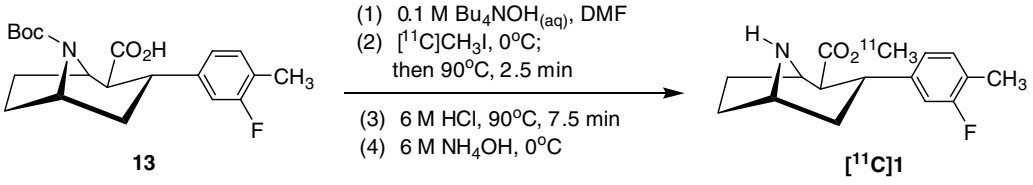
The affinities of 2β,3α-nortropanes **1**–**5** for the human NET, SERT, and DAT were determined through in vitro competition assays in transfected HEK-293 cells according to a previously reported procedure.[24] The data shown in Table 1 indicate that all of the compounds tested in this series exhibit great potency for the hNET with K_i of 1.78–4.88 nM. Compared to its 2β,3β-isomer, **3**, **4**, or **5** showed increased affinity at the NET, while they are also approximately equipotent for the hSERT and hDAT. Compound **1** had the highest affinity for the hNET (K_i = 1.78 nM) with 24-fold selectivity over the hSERT and 3.6-fold selectivity over the hDAT. The lack of selectivity of **1** for the hNET versus hDAT differs from the literature reports[18] using rat brain homogenates and this discrepancy may be due to the different methods used and due to species differences between rodent and human monoamine transporters.

Although **1** was potent for both the hNET and the hDAT, microPET studies were performed with [^{11}C]**1** to determine the imaging properties of this series of compounds. A baseline study was initially performed in an anesthetized rhesus monkey according to our previously reported procedure[24] in order to assess the brain regional distribution. The time–activity curves (TACs) obtained after administration of [^{11}C]**1** indicate a high uptake of radioactivity in the caudate, putamen, thalamus, midbrain, medulla, and cerebellum, regions rich in DAT and NET, with peak uptake achieved between 9.5 and 12.5 min (Fig. 2). In vivo microPET studies of [^{11}C]**1** with pharmacologic interventions were performed in the same rhesus monkey to further determine whether the observed uptake in the baseline study reflected [^{11}C]**1** binding to the DAT or NET. Administration of the DAT-selective ligand RTI-113 (0.3 mg/kg) at 60 min post-injection of radiotracer produced a decrease of [^{11}C]**1** binding to the caudate and putamen, which are known to have a high DAT density, indicating that the uptake of [^{11}C]**1** in these regions was due to binding to the DAT (Fig. 3). This is consistent with the binding data shown in Table 1. A blocking study was performed with the NET ligand desipramine (0.25 mg/kg) administered intravenously at 30 min prior to the injection of [^{11}C]**1**. Uptake in the thalamus and midbrain was not observed, indicating that the uptake in these regions was due to binding to the NET.

Arterial plasma samples from the rhesus monkey following antecubital vein injection of [^{11}C]**1** were analyzed for nonpolar, potentially brain permeable metabolites by a HPLC method. The fraction of plasma radioactivity



Scheme 1. Reagents and conditions: (a) C_6H_5Li, THF, −78 °C; TFA, −78 °C, 66%; (b) Ni_2B, HCl, CH_3OH, reflux, 80%; (c) I_2, HOAc, AgOTf, CH_2Cl_2, rt, 83%; (d) Troc–Cl, toluene, reflux, 93%; (e) Zn, AcOH, rt, 79%; (f) $Bu_3SnCH{=}CH_2$, toluene, $(PPh_3)_4Pd$, 130 °C, 87%; (g) ≡-TMS, CuI, $(PPh_3)_2PdCl_2$, $[(CH_3)_2CH]_2NH$, rt, 84%; (h) TBAF, THF, 90%; (i) 1,4-dioxane, H_2O, reflux, 82%; (j) $(Boc)_2O$, Et_3N, CH_2Cl_2, rt, 74%.



Scheme 2. Radiosynthesis of [^{11}C]**1**.

Table 1. In vitro evaluation of 2β,3α-(substituted phenyl)nortropanes in competition assays with human monoamine transporters[a]

Compound	K_i for hNET	K_i for hSERT	K_i for hDAT	hSERT/hNET	hDAT/hNET
1	1.78 ± 0.19	42.80 ± 3.07	6.42 ± 1.12	24	3.6
1(literature)[b]	0.43±0.02	23.8 ± 4.4	9.0 ± 4.5	55	21
2	4.88 ± 0.64	136.29 ± 14.82	11.47 ± 1.01	28	2.3
3	1.98 ± 0.10	4.16 ± 0.93	2.25 ± 0.55	2.1	1.1
4	4.67 ± 0.94	9.04 ± 1.29	10.09 ± 0.42	1.9	2.2
5	1.86 ± 0.37	3.45 ± 1.72	2.26 ± 0.07	1.8	1.2

[a] All K_i values are reported with nanomolar (nM) units. The data are expressed as means ± standard deviation of at least three separate experiments performed in triplicate. The following radiotracers were used: [^{3}H]nisoxetine for hNET, [^{3}H]citalopram for hSERT, and [^{125}I]RTI-55 for hDAT.
[b] Literature values performed in rat brain homogenates.[18]

corresponding to unmetabolized [^{11}C]**1** rapidly decreased from 25% at 3 min to 7.5% at 10 min, and 1.6% at 30 min. The major radioactive metabolite found in arterial plasma using HPLC separation and gamma-counter detection was a more polar component which was eluted immediately after the void volume. The abundance of the polar metabolite as a percent of total plasma activity increased from 75% at 3 min to 98% at 45 min. The polar metabolite is probably the corresponding free acid which was not expected to cross the blood–brain barrier. This hypothesis is based on our experience with other tropanes and a plasma metabolite study reported in the literature[25] showing that the major metabolite obtained from [*N*-methyl-^{11}C]β-CIT was the

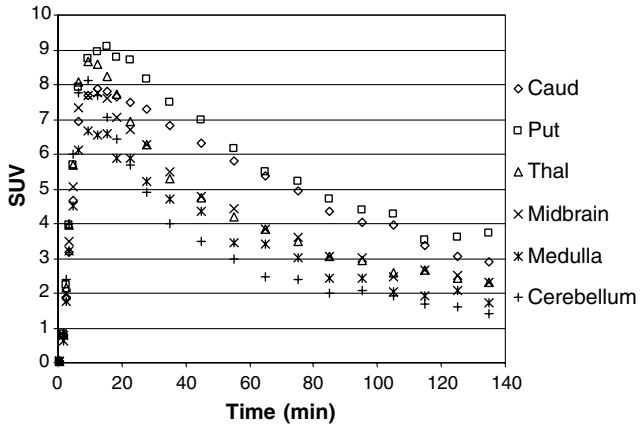


Figure 2. MicroPET baseline study TACs for the brain regions of a rhesus monkey after intravenous injection of 6.2 mCi of [^{11}C]**1**.

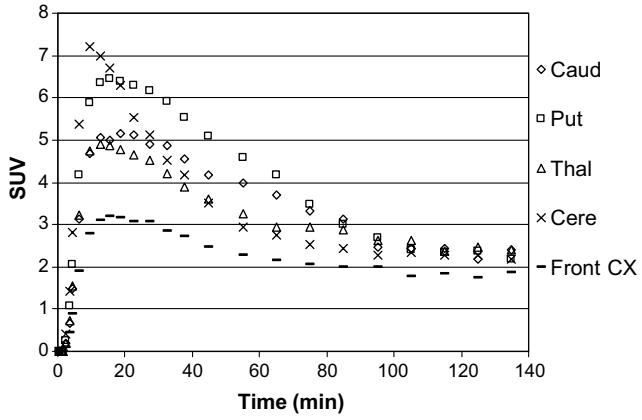


Figure 3. Time–activity curves for [^{11}C]**1** in an anesthetized rhesus monkey with RTI-113 (0.3 mg/kg) administered at 60 min postinjection of [^{11}C]**1**.

[*N*-methyl-^{11}C]β-CIT acid which did not enter the brain. Thus, there was no detectable formation of lipophilic radiolabeled metabolites capable of entering the brain and contributing to brain radioactivity.

In summary, we have prepared and determined the monoamine transporter binding affinity of several 2β,3α-(substituted phenyl)nortropanes. The binding results showed that all of the compounds tested in this series exhibit great potency for the hNET. Despite the lack of selectivity of this series of compounds for the hNET vs hDAT, the most promising compound, [^{11}C]**1**, was prepared in 45% dcy and evaluated in microPET studies in a rhesus monkey to assess the imaging properties of this class of compounds. The regional distribution of radioactivity after injection of [^{11}C]**1** showed potential binding to the NET and DAT in vivo, and further chase and blocking studies verified this binding. Although **1** has a high affinity for the hNET in vitro with K_i of 1.78 nM, the high uptake of [^{11}C]**1** in caudate and putamen excludes its utility as a PET imaging agent for the NET.

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