Bioorganic & Medicinal Chemistry Letters 21 (2011) 2962–2965

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and evaluation of novel *N*-fluoropyridyl derivatives of tropane as potential PET imaging agents for the dopamine transporter

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ARTICLE INFO

Article history:
Received 22 January 2011
Revised 14 March 2011
Accepted 15 March 2011
Available online 21 March 2011

Keywords:
Tropanes
Dopamine transporter
Fluorine-18
PET
Parkinson's disease

ABSTRACT

A series of novel *N*-fluoropyridyl-containing tropane derivatives were synthesized and their binding affinities for the dopamine transporter (DAT), serotonin transporter (SERT) and norepinephrine (NET) were determined via competitive radioligand binding assays. Among these derivatives, compound **6d** showed the highest binding affinity to DAT (K_i = 4.1 nM), and selectivity for DAT over SERT (5-fold) and NET (16-fold). Compound **6d** was radiolabeled with Fluorine-18 in two steps. Regional brain distribution and ex vivo autoradiography studies of [^{18}F]**6d** demonstrated that the ligand was selectively localized in the striatum region, where DAT binding sites are highly expressed. [^{18}F]**6d** may be useful as a potential radioligand for imaging DATs with PET.

Dopamine (DA) is one of the key neurotransmitters controlling normal brain function, including movement, emotion and other higher-level cognitive functions. Once it is released into the synaptic cleft as a neurotransmitter, it is readily transported back into presynaptic neurons by the dopamine transporter (DAT). Biochemical and pharmacological studies suggest that changes in the density and function of DAT may play a role in neurodegenerative and neuropsychiatric diseases such as Parkinson's disease (PD), Huntington's chorea, schizophrenia and attention deficit-hyperactivity disorder (ADHD).[1–4]

Positron emission tomography (PET) is a sensitive and specific non-invasive in vivo imaging technology that can provide information about the functional status of neurotransmitter systems in the brain. Since the first PET imaging study of DAT was conducted with [^{11}C]nomifensine in a hemi-parkinson's patient in 1987,[5] there have been many studies demonstrating the utility of DAT PET imaging for diagnostic application. Most PET radiotracers for imaging DAT have been performed with ^{11}C-labeled 2β-carbomethoxy-3β-(4-substituted-phenyl)tropane derivatives. Several ^{18}F-labeled tropane derivatives are also known. These include β-CFT, FECT, FP-CIT, FECNT, FE-PE2I and etc.[6–15] Some of these radioligands have been used in clinical settings for PD diagnosis, but some drawback still existed, such as slow kinetics, a lack of selectivity and the formation of undesired radiometabolites in vivo.[10,16–19] It is necessary to develop new DAT ligands to satisfy the clinical needs for the accurate quantification of DAT.

For PET studies, ^{18}F is a particularly attractive radionuclide. Its 110 min half-life allows imaging studies to be carried out over longer periods of time (2–4 h). Furthermore, ^{18}F has relatively low positron energy (0.635 MeV, 97% abundant) and provides high resolution images. Accordingly, we have focused our recent efforts on developing ^{18}F-labeled tropane derivatives to be used as DAT imaging agents. We report herein the synthesis and biological evaluation of a novel series of *N*-o-halopyridyl substituted phenyltropane derivatives. The results of our study suggest that fluoropyridyl-containing tropane derivatives possess a high affinity and selectivity for DAT and are therefore attractive potential PET radioligands for imaging the DAT in vivo.

The general strategy for the synthesis of tropane derivatives is summarized in Scheme 1. Hydrolysis of **1** in 6 N HCl gave the corresponding acid **2** in excellent yield (95%). The corresponding acid chloride **3**, which was prepared by the action of oxalyl chloride on carboxylic acid **2** at room temperature, were not isolated but reacted with amine **4a–d** to obtain amide compounds **5a–d**. The amide functions of compounds **5a–d** were reduced with BH_3–THF to yield amine compounds **6a–d**.

The in vitro binding affinities (K_i) of the novel derivatives to three different monoamine transporters were evaluated by competition binding assays with radioligands that are highly selective for DAT, SERT and NET. Membrane homogenates of transfected LLC-PK$_1$ cell lines which overexpress a single monoamine transporter were obtained for the binding assays as detailed

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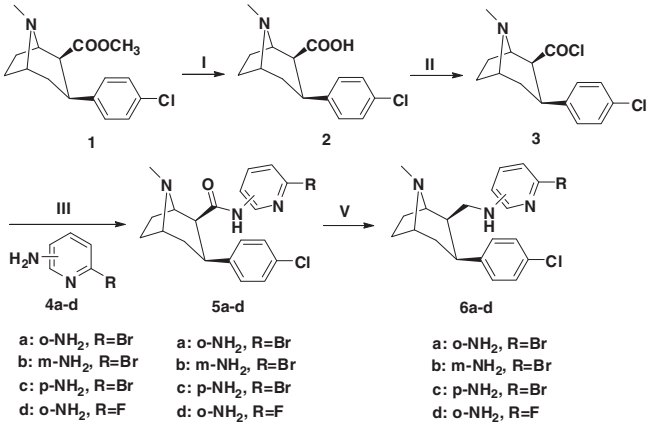
doi:10.1016/j.bmcl.2011.03.051



Scheme 1. Reagents and conditions: (I) 6 N HCl, reflux, 12 h; (II) $(COCl)_2$, CH_2Cl_2, 0 °C to rt, 1.5 h; (III) Et_3N, CH_2Cl_2, 6 h; (V) BH_3–THF, reflux, 8 h.

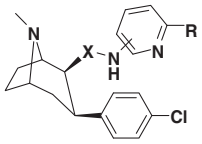
previously.[20] Two other DAT ligands, 2β-carbomethoxy-3β-(4-chlorophenyl)tropane (β-CCT) and 2β-carbomethoxy-3β-(4-bromophenyl)tropane (β-CBT) were also tested for comparison (Table 1). Selectivities for specific transporter-types are expressed as K_i ratios (Table 1).

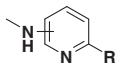
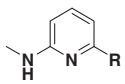
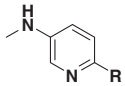
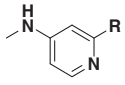
The goal of this preliminary study was 2-fold. First, we wished to explore whether the C2-ester of the parent 2-carbomethoxy analogs could be replaced by 2-formamide or 2-methyleneamine while maintaining binding affinity to DAT. Secondly, we wished to evaluate whether a halopyridyl substituent with an amino group at a different position (*o*-, *m*- or *p*-substituted) could affect the DAT binding affinity. Data in Table 1 show that all of the novel amide and amine derivatives of tropane showed a high binding affinity to DAT in contrast to that observed for β-CCT and β-CBT. The amine derivatives displayed higher affinity to DAT (K_i from 4.2 to 17.9 nM) than the amide derivatives did. Yet, the amide derivatives exhibited a higher selectivity for DAT over both SERT and NET binding sites.

For the new amine derivatives, it's interesting to note that in the halopyridyl substituent the position of the amino group (*o*-, *m*- or *p*-substituted) affected the DAT binding affinity and selectivity. The 2-bromo-5-aminopyridine derivative of compound **6b** displayed the lowest binding affinity to DAT (K_i = 17 nM) as compared to those for other amino derivatives of tropane. Although compound **6b** showed a high selectivity for the DAT over the NET, it displayed a lower selectivity for the DAT over the SERT and higher binding affinity to SERT (K_i = 10.5 nM). The 2-amino-6-halopyridine derivatives showed the higher binding affinities to the DAT. Compound **6a**, the 2-amino-6-bromopyrine derivative showed an excellent binding affinity to DAT with a K_i of 5.6 nM. The fluoro compound, **6d**, showed the highest binding affinity to DAT (K_i = 4.1 nM) amongst all of the compounds tested. Compound **6d** also showed a higher selectivity for the DAT over SERT (5-fold) and NET (16-fold) amongst all of the amine derivatives tested. Therefore, compound **6d** was chosen for further ^{18}F-radiolabeling and biological evaluation.

Initial attempts to label the 2-pyridine ring of **6a** with ^{18}F were not successful. This failure may be due to the fact that in an alkaline labeling condition a negative charge is introduced on the N-atom at the position 6. As a result, the amino substituent no longer acts as an electron-withdrawing substituent and the nucleophilic displacement of the o-bromo group by [^{18}F]fluoride is no longer feasible. To overcome this problem, we introduced a N-BOC protecting group on the amine at position 6. The new precursor, **7**, was synthesized from **6a** in the reported method.[21] The ^{18}F-radioligand was prepared via aromatic nucleophilic substitution of a [^{18}F]fluoride for displacing the bromo group.[22,23] The precursor **7** and the K[^{18}F]F–$TBAHCO_3$ complex were heated in DMSO at 150 °C for 15 min, followed by deprotection with trifluoroacetic acid (Fig. 1). The crude mixtures were purified using semi-preparative reversed phase HPLC (RP-HPLC). The overall radiochemical yield of [^{18}F]**6d** from [^{18}F]fluoride was 5%

Table 1
Comparison of transporter binding potencies for novel tropane analogues[a]



Compd		X	R	Affinity (K_i ± SE, nM)[b]			Selectivity	
				DAT	SERT	NET	SERT/DAT	NET/DAT
β-CCT	––	––	––	5.1 ± 0.4	––	––	––	––
β-CBT	––	––	––	3.5 ± 0.6	––	––	––	––
β-CFT[c]	––	––	––	14.7 ± 2.9	181 ± 21	635 ± 110	12.3	43.2
5a		C=O	Br	86.7 ± 33.7	3211 ± 334	3855 ± 23	37	44
5d		C=O	F	127 ± 16	29104 ± 457	16877 ± 2314	229	133
6a		CH_2	Br	5.6 ± 0.7	7.4 ± 0.7	29.1 ± 2.7	1.3	5.2
6d		CH_2	F	4.1 ± 0.6	20.6 ± 3.7	66.2 ± 12.7	5.0	16
5b		C=O	Br	106 ± 14	15315 ± 1649	16211 ± 2334	144	153
6b		CH_2	Br	17.9 ± 2.1	10.5 ± 0.6	49.1 ± 4.6	0.6	2.7
5c		C=O	Br	82.2 ± 10.8	10298 ± 4767	12090 ± 4543	125	147
6c		CH_2	Br	7.9 ± 0.9	11.0 ± 4.5	27.7 ± 1.5	1.4	3.5

[a] Each K_i value represents data from at least three independent experiments, each performed in duplicate.
[b] Transporter binding affinity assays are conducted as reported previously. For DAT, LLC-DAT membrane homogenates incubated with 0.08 nM [^{125}I]IPT (K_d = 0.25 nM); for SERT, LLC-SERT membrane homogenates incubated with 0.1 nM [^{125}I]IDAM (K_d = 0.2 nM); for NET, LLC-NET membrane homogenates incubated with 0.07 nM [^{125}I]2-INXT (K_d = 0.06 nM).
[c] Standard reference compounds reported previously.[24]

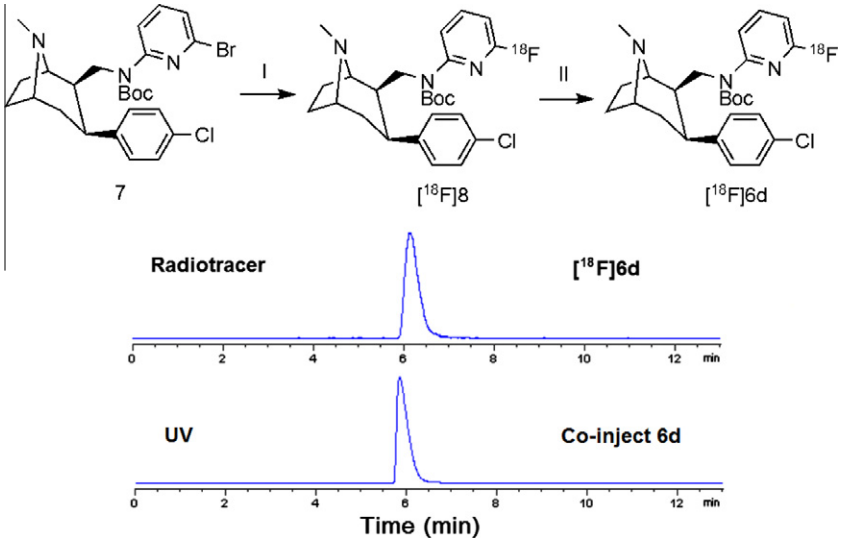


Figure 1. Radiosynthesis and HPLC chromatogram of [^{18}F]**6d**. The upper trace showed the radio-profile, and the lower tracer showed the UV profile at 254 nM.

(uncorrected for decay), and the total time for radiolabeling and purification was 100 min. The radiochemical purity was found to be more than 99% and the specific radioactivity was 100–220 GBq/μmol. The identity of the tracers was confirmed by co-elution with the authentic non-radioactive compounds after co-injection on the same analytical HPLC system showing the same retention time (Fig. 1).

A biodistribution study of [^{18}F]**6d** was performed in normal male Sprague–Dawley rats. [^{18}F]**6d** (25 μCi) was injected through the femoral vein. The rats (n = 5 for each time point) were sacrificed post-injection at indicated time points. The organs of interest were removed and weighed, and the radioactivity was counted with an automatic gamma counter. The percent dose (%ID) per organ was calculated by a comparison of the tissue counts to suitably diluted aliquots of the injected dose. Different regions of rat brain corresponding to striatum (ST), hippocampus (HP), cerebellum (CB) and cortex (CX) were dissected and counted to obtain the regional distribution of the tracer (Table 2)

[^{18}F]**6d** showed a high initial accumulation in the heart, lung and kidney (2.85, 9.85 and 3.70%ID/g, respectively) and then cleared rapidly from these organs at later time points. The results demonstrated that [^{18}F]**6d** can cross the BBB into the brain. The initial brain uptake was 0.40%ID/g at 2 min. The molecule's lipophilicity plays an important role in BBB penetration. The log P (1.88) of [^{18}F]**6d** was slightly lower than the optimal value (2.0–3.5) for crossing the BBB.[25] Therefore, there is still room for improvement in the initial brain uptake. The results of regional brain distribution

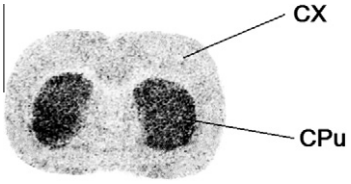


Figure 2. Ex vivo autoradiographic localization of [^{18}F]**6d** binding sites in rats at 60 min postinjection. High levels of radioactivity were observed in the caudate putamen (CPu). CX, cortex.

study suggested that [^{18}F]**6d** will selectively localize in the ST region, where the dopamine neurons are highly concentrated. Using the CB uptake as the background (a non-target region containing a minimal concentration of DAT), the ST/CB ratio exhibited a moderate increase over the time course, reaching 2.02 at 60 min and 3.14 at 180 min.

The anatomical distribution of [^{18}F]**6d** in the rat brain was confirmed by ex vivo autoradiography (Fig. 2). For ex vivo studies, the normal Sprague–Dawley rats were injected intravenously with 2.5 mCi [^{18}F]**6d** and sacrificed at 60 min post injection. The brain was removed immediately and frozen for section preparation. The dried tissue sections were then exposed overnight to Kodak Biomax MR films in an autoradiographic cassette. The highest radioactivity observed was in the striatum (ST), which is the target region for DAT. The low background and high signal-to-noise ratio

Table 2
Rat brain uptake and regional distribution of [^{18}F]**6d** in normal Sprague–Dawley rats (% ID/g, average of five rats ± S.D)

	2 min	60 min	120 min	180 min
Brain	0.40 ± 0.06	0.26 ± 0.04	0.07 ± 0.00	0.04 ± 0.01
Region				
Cerebellum	0.49 ± 0.04	0.23 ± 0.03	0.05 ± 0.00	0.03 ± 0.00
Striatum	0.40 ± 0.10	0.47 ± 0.13	0.12 ± 0.01	0.09 ± 0.02
Hippocampus	0.36 ± 0.06	0.28 ± 0.04	0.08 ± 0.01	0.05 ± 0.01
Cortex	0.45 ± 0.10	0.28 ± 0.03	0.07 ± 0.01	0.03 ± 0.01
Hypothalamus	0.40 ± 0.07	0.29 ± 0.04	0.06 ± 0.01	0.04 ± 0.01
Ratio (vs CB)				
Striatum	**0.82**	**2.02**	**2.54**	**3.14**
Hippocampus	0.72	1.21	1.61	1.81
Cortex	0.91	1.21	1.40	0.96
Hypothalamus	0.81	1.24	1.31	1.51

observed by autoradiography were consistent with the data obtained by the regional brain tissue dissection method (Fig. 2).

In conclusion, we report a series of novel *N*-fluoropyridyl-containing tropane derivatives with binding affinity and selectivity to DAT over SERT and NET. Within this series, compound **6d** showed the highest binding affinity to DAT (K_i = 4.1 nM) and selectivity over SERT and NET. Radiolabeling of [^{18}F]**6d** was successful. Regional brain distribution and ex vivo autoradiography studies of [^{18}F]**6d** demonstrated high specific binding in the striatum. These results suggest that further exploration of its derivatives as new imaging agents for the DAT in the brain is warranted.

Acknowledgments

This work was supported by grant from the National 973 Program (2006CB500705 and 2011CB504100) from Ministry of Science and Technology and the National 863 Program (2006AA02A408), China.

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