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New 2α-tropane amides as potential PET ligands for the dopamine transporter

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Abstract

Positron emission tomography (PET) imaging of dopamine transporter (DAT) density in the brain is a potentially valuable tool for studying the etiopathology of degenerative brain disorders. The present study evaluated five new potential competitive inhibitors of DAT as ligands for PET. The evaluation of the new compounds measured their ability to compete with the binding of the reference ligand 2β-carbomethoxy-3β-(4-[^{131}I]iodophenyl)tropane [^{131}I]β-CIT to striatal and cortical membranes from rat and pig brain. Four of the new compounds structurally related to cocaine were synthesized in their 2α,3β configuration; the most potent one, 3β-(4-iodo-phenyl)-8-methyl-8-aza-bicyclo[3.2.1]octane-2α-carboxylic acid (2-fluoro-ethyl)-amide, was synthesized also in the 2β,3β configuration. For comparative studies in rat brain and new evaluation in pig brain homogenate, the established compounds β-CIT, FP-CIT, PE2I and FETT were also synthesized and evaluated. Contrary to expectation, the 2α,3β and 2β,3β isomers of 3-(4-iodo-phenyl)-8-methyl-8-aza-bicyclo[3.2.1]octane-2-carboxylic acid (2-fluoro-ethyl)-amide showed the same affinity constant for rat striatum (K_i=200 nM±34), but in pig striatum and rat and pig cortex the 2α,3β form even had a higher affinity than the 2β,3β form.

Keywords: DAT ligands; PET; Cocaine derivatives; 2α,3β Tropanes

1. Introduction

A suitable noninvasive in vivo imaging tool for quantifying the dopamine transporter (DAT) density in human brain is a focus of radiopharmaceutical investigation. Such a tool could serve the early recognition of degenerative brain disorders especially Parkinson's disease (PD). The DAT is a presynaptic, transmembrane spanning protein that controls the transport of the neurotransmitter dopamine (DA) across the neuronal membrane [1]. In the central nervous system, DAT mediates the re-uptake of released dopamine from the synaptic cleft back into the nerve terminals for subsequent storage and release [2]. This DAT-mediated re-uptake process is the main mechanism for the termination of dopaminergic neurotransmission [3] in the striatum. The function of DAT and its pharmacology have been studied since the mid-1960s [4,5]. Rising interest in the etiology of PD [6] and drugs of abuse [7] has generated new information about the DAT. PD is a degenerative brain disorder with reduced DAT density and impaired striatal uptake of suitable ligands [8,9]. For commonly abused drugs such as cocaine and amphetamine, DAT represents the main target site.

When combined with positron emission tomography (PET), suitable radiolabeled ligands binding specifically to the DAT may be useful as noninvasive in vivo imaging tools for studying the DAT density. The result could allow a conclusion of the stadium of degenerative brain disorders like PD or monitor changes in DAT density after treatment of PD patients with new promising pharmaceuticals. Several chemical classes [10] have been tested as DAT ligands, especially derivatives of cocaine **1**, aryl 1,4-dialkyl piperazines e.g., GBR 12783 **2**, methylphenidates **3** and

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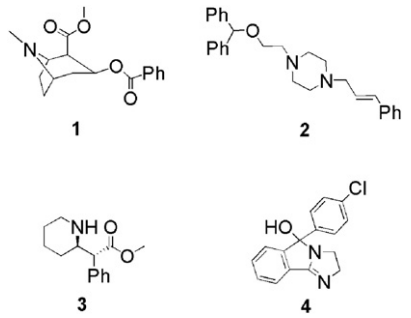


Fig. 1. Cocaine **1**, GBR 12783 **2**, methylphenidate **3**, mazindole **4**.

mazindoles **4** (Fig. 1), cocaine derivatives being the most promising candidates.

Besides satisfying criteria such as target affinity, selectivity, brain uptake and feasibility of labeling with radionuclides suitable for PET, a useful radioligand must possess sufficient in vivo stability. Compounds with known DAT affinity based on cocaine, e.g., 2β-carbomethoxy-3β-(4-iodophenyl)tropane **5** (β-CIT) [11], N-(3-fluoropropyl)-2β-carbomethoxy-3β-(4-iodo-phenyl)nortropane **6** (FP-CIT) [12], N-(3-iodoprop-2-(*E*)-enyl)-2β-carbomethoxy-3β-(4-methylphenyl)-nortropane **7** (PE2I) [13], 2β-carboflouroethoxy-3β-(4-methylphenyl)tropane **8** (FETT) [14], N-(3-fluoroethyl)-2β-carbomethoxy-3β-(4-chloro-phenyl) nortropane **9** (FECNT) [15] and N-(3-fluoroprop-2-(*E*)-enyl)-2β-carbomethoxy-3β-(4-methyl-phenyl)-nortropane **10** (LBT-999) [16–18], have a carboxylic acid ester function (Fig. 2). The hydrolysis of these esters to carboxylic acids is a major reason for their instability in vivo [19,20]. Because amides are less susceptible to in vivo hydrolysis, the corresponding amides of known DAT ligands were selected for the present study.

The 2β,3β tropanes synthesized from (−)-cocaine have the highest affinity for the DAT, and tropanes having a 2α,3β or other configuration had little or no biological activity. Several analogues of (−)-cocaine, namely, the 2β,3β, 2α,3β, 2β,3α and 2α,3α isomers, have been examined by different groups [21–26], as well as the respective isomers of cocaine and pseudococaine itself [27]. The 2α,3β isomers had by far the lowest affinity to the DAT with affinity values up to 120-fold higher than the 2β,3β isomers, e.g., IC_{50} (2β,3β CIT)= 1.6±0.15 nM and IC_{50} (2α,3β CIT)=87.6±2.9 nM.

The 2β,3β configuration is the structure of natural (−)-cocaine, the main alkaloid from *Erythroxylon (Erythroxylum) coca*, the coca bush. All known ligands with DAT affinity based on cocaine such as **5**−**10** have this structure. Thus, the 2β,3β tropanes are in the focus of research although the 2α,3β isomers are thermodynamically more stable and simpler to obtain.

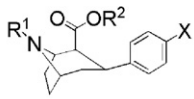
This study evaluated five new potential competitive DAT inhibitors as ligands for PET. Four of the new compounds were synthesized in their 2α,3β configuration; the most potent one also in its 2β,3β configuration. The established compounds β-CIT, FP-CIT, PE2I and FETT were synthesized for the purpose of comparison and for their new evaluation on pig brain membranes. Among five new cocaine derivatives, this study presents amide **16a** with 2α,3β configuration that showed the same affinity constant K_i as did its 2β,3β isomer **17a** in rat striatum homogenate, whereas in pig striatum as well as in rat and pig cortex the 2α,3β form was even more affine than the 2β,3β form. A parallel evaluation from our group measured the ability of the same compounds to antagonize the binding of [^{131}I]β-CIT and [^{18}F]FP-CIT in rat and pig brain by autoradiography. The affinity values that arose from the different studies are comparable to each other [28].

2. Materials and methods

2.1. Chemistry

Melting points were measured on an electrothermal apparatus and are uncorrected. Mass spectra ESI (positive) were obtained on a Finnigan Automass III mass spectrometer (Thermo Quest, Dreieich, Germany). ESI-HR-MS spectra were recorded using a Finnigan MAT 900 ST mass spectrometer with the peak-matching method. Thin-layer chromatography employed precoated silica sheets (4×8 cm, Polygram, Machery-Nagel, Düren, Germany). Column chromatography (CC) was performed using silica gel (Sigma-Aldrich, Taufkirchen, Germany). ^{1}H, ^{13}C and ^{19}F NMR spectra were obtained on either a Bruker DPX-200 spectrometer (Avance 200) or Varian INOVA 400 MHz or Varian INOVA 600 MHz in ≈5% solution at 25°C. Chemical shifts are given in δ ppm using the residual proton signals of the appropriate deuterated solvents as reference ($\delta_{H(CDCl_3)}$=7.24, $\delta_{C(CDCl_3)}$=77.0). The multiplicity symbols s, d, t and m refer to singlet, doublet, triplet and multiplet, respectively.

All solvents and reagents in the highest state of purity were obtained commercially (Sigma-Aldrich, Taufkirchen, Germany). Reference tropanes β-CIT **5** [11], FP-CIT **6** [29], PE2I **7** [13] and FETT **8** [14] were prepared as described. The carboxylic acids **14a**–**c** were obtained from esters **13a**–**c** suspended in a 12 mol/L solution of KOH in 95% ethyl alcohol and stirred at 50°C overnight.



R^1	R^2	X	No
CH_3	CH_3	I	**5**
F-C_3H_6	CH_3	I	**6**
(E)-I-CH=CH-CH_2	CH_3	CH_3	**7**
CH_3	C_2H_4-F	CH_3	**8**
F-C_2H_4	CH_3	Cl	**9**
(E)-F- CH_2-CH=CH-CH_2	CH_3	CH_3	**10**

Fig. 2. β-CIT **5**, FP-CIT **6**, PE2I **7**, FETT **8**, FECNT **9**, LBT-999 **10**.