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Research Article

Syntheses and radiofluorination of two derivatives of 5-cyano-indole as selective ligands for the dopamine subtype-4 receptor

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Summary

Two fluoroethoxy substituted derivatives, namely 2-[4-(2-(2-fluoroethoxy)phenyl)-piperazin-1-ylmethyl]indole-5-carbonitrile (**5a**) and 2-[4-(4-(2-fluoroethoxy)-phenyl) piperazin-1-ylmethyl]indole-5-carbonitrile (**5b**) were synthesized as analogs of the selective D_4 receptor ligand 2-[4-(4-fluorophenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (FAUC 316). *In vitro* characterization using CHO-cells expressing different dopamine receptor subtypes gave K_i values of 2.1 (**5a**) and 9.9 nM (**5b**) for the dopamine D_4 subtype and displayed a 420-fold D_4-selectivity over D_2 receptors for **5b**. The para-fluoroethoxy substituted candidate **5b** revealed substantially reduced α_1 and serotoninergic binding affinities in comparison to the ortho-fluoroethoxy substituted compound. In order to provide potential positron emission tomography (PET) imaging probes for the dopamine D_4 receptor, ^{18}F-labelling conditions using [^{18}F]fluoroethyl tosylate were optimized and led to radiochemical yields of 81 ± 5% (**[^{18}F]5a**) and 47 ± 4% (**[^{18}F]5b**) ($n = 3$, decay-corrected and referred to labelling agent), respectively. Thus, ^{18}F-fluoroethylation favourably at the para position of the phenylpiperazine moiety of the 5-cyano-indole framework proved to be tolerated by D_4 receptors and could also be applied to alternative scaffolds in order to develop D_4 radioligand candidates for PET with improved D_4 receptor affinity and selectivity. Copyright © 2005 John Wiley & Sons, Ltd.

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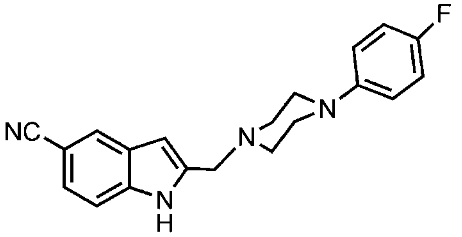
Introduction

The dopamine D_4 receptor subtype belongs to the subfamily of D_2-like distinct dopamine receptors (D_2R, D_3R and D_4R) mediating the action of dopamine in the brain, but differing in their brain distribution and pharmacological profiles. Among them, the D_4 receptor has been cloned[1] and intensively studied *in vitro* using knock-out mice or immunohistochemistry with receptor-specific antibodies.[2–5] The precise function and exact distribution of the dopamine D_4 receptor in the CNS are of great interest, as associations are emerging between D_4 receptors and neuropsychiatric disorders, including schizophrenia, attention-deficit hyperactivity disorder as well as specific personality traits such as novelty seeking.[6–8] Whether the dopamine D_4 receptor fulfills distinct functional roles has not yet been satisfactorily addressed.

Up to now, dopamine D_4 receptor concentrations *in vitro* are usually determined by indirect binding studies, in which [^{3}H]raclopride binding (D_2/D_3 antagonist) is subtracted from total binding measured with [^{3}H]nemonapride.[9,10] However, this method has yielded conflicting results: whereas Seeman *et al.*[9] reported an increased D_4 receptor density in postmortem brain tissue of schizophrenic patients, these results could not be confirmed by others.[11,12] Data on the apparently low D_4 receptor densities in the brain are scarce and were reported in analyses using *in situ* hybridization establishing receptor expression in the prefrontal cortex and hippocampus.[13] Furthermore, *in vitro* autoradiography with [^{3}H]NGD 94-1 reflected a distribution unique among dopamine receptor subtypes and revealed $B_{\max}$-values in the low range from 9 to 30 fmol/mg in distinct human brain regions.[14,15] Thus, the selection criteria for a suitable D_4 receptor radioligand for positron emission tomography (PET) has to consider a high affinity for its target in order to observe a receptor-specific signal *in vivo*. Assuming the maximal concentration of D_4 receptors to be equivalent to approximately 3 nM, the *in vitro* affinity of a radioligand candidate should be significantly less than $B_{\max}$ to achieve a high *in vitro* binding potential ($B_{\max}/K_d$) that could correlate to a suitable target-to-nontarget ratio *in vivo* with good contrast for imaging.[16] However, the *in vivo* distribution of a radioligand at a single time point is likely to be influenced by various factors besides receptor density and affinity, such as blood flow, clearance of the radioligand, metabolism and binding to nonspecific sites.

Up to now, the lack of selective D_4 receptor radioligands suitable for *in vivo* imaging techniques hampers the noninvasive investigation of neurotransmission by single photon emission tomography (SPET) and positron emission tomography (PET) as high-performance tools for understanding the



FAUC 316

Figure 1. Chemical structure of FAUC 316 ($K_i(D_4R) = 1.0$ nM, $K_i(D_2R)/K_i(D_4R) = 19\,000$)[32]

neurochemical basis and pathology of neuropsychiatric diseases.[17] Several efforts were reported on the radiosyntheses of ^{123}I-, ^{18}F- and ^{11}C-labelled ligands for the exploration of D$_4$ receptor density *in vivo* by SPET and PET, including [^{11}C]SDZ GLC 756,[18] methoxybenzamide derivatives,[19,20] SB-235753,[21,22] a ^{123}I-labelled chromeno[3,4-*c*]pyridinone[23] as well as ^{18}F- and ^{123}I-labelled pyrrolo[2,3-*b*]pyridines.[24–26] However, none of these radioligands has been proven suitable, due to lack of specificity *in vivo* or undesirable pharmacological properties.

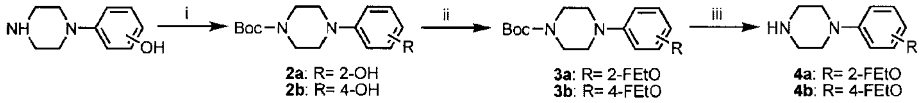
As a part of our drug discovery and SAR investigations on selective dopamine D$_4$ receptor ligands and radiolabelled analogs,[27–31] we identified the indole derivative 2-[4-(4-fluorophenyl)piperazin-1-ylmethyl]indole-5-carbonitrile(FAUC 316; Figure 1) as a dopamine D$_4$ receptor ligand with high affinity ($K_i(D_4R) = 1.0$ nM) and excellent subtype selectivity ($K_i(D_2R)/K_i(D_4R) = 19\,000$).[32] The aims of the present study were the syntheses of fluoroethoxy substituted derivatives of the lead compound FAUC 316, the assessment of their *in vitro* properties with regard to dopamine receptor affinity and subtype selectivity and the radiosyntheses of ^{18}F-labelled analogs. We herein report the effect of fluoroethylation on receptor binding affinities *in vitro* and the ^{18}F-radiosyntheses of the D$_4$ receptor radioligand candidates 2-[4-(2-(2-[^{18}F]fluoroethoxy)phenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (**[^{18}F]5a**) and 2-[4-(4-(2-[^{18}F]fluoroethoxy)phenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (**[^{18}F]5b**).

Results and discussion

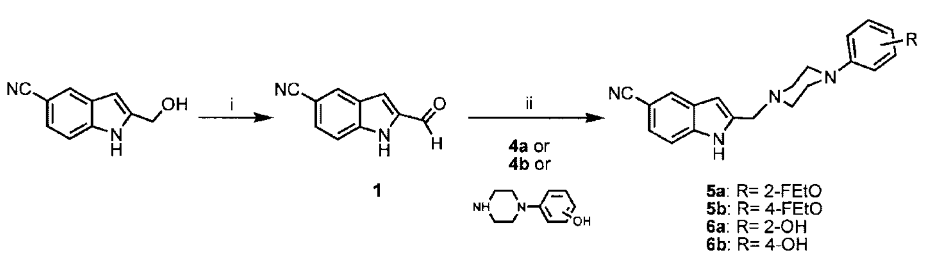
Our previous biological investigation of a representative library of 13 indole derivatives showed highly potent and selective dopamine D$_4$ receptor binding profiles, when positions 2 and 5 proved highly suitable attachment positions for the aminomethyl and cyano groups, respectively.[32] Consequently, this substitution pattern at the indole core unit was maintained for the syntheses of fluoro substituted analogs presented herein. Starting from the commercially available 2- and 4-hydroxyphenylpiperazines, the corresponding fluoroethoxy substituted phenylpiperazines **4a** and **4b** were obtained by a three step procedure including classical N-protection of the piperazine nitrogen as *t*-butyl carbamate (Scheme 1). First, introduction of a *t*-butyloxycarbonyl (BOC) protecting group was accomplished using BOC anhydride in *N*,*N*-dimethyl formamide (DMF) in the presence of triethylamine to obtain compounds **2a,b**. Subsequently, fluoroethylation of the aromatic hydroxyl group was realized by reaction of **2a,b** with l-fluoro-2-tosyloxyethane (fluoroethyl tosylate) in the presence of tetrabutylammonium hydroxide ($N(Bu)_4OH$) to afford **3a** and **3b**, respectively, in good yields (72 and 86%) employing the protocol of Wilson *et al.*[33] Deprotection of the piperazine nitrogen under acidic conditions provided the desired fluoroethoxy substituted phenylpiperazines **4a** and **4b** in yields of 60 and 50%, respectively (Scheme 1).

With the 2- and 4-(2-fluoroethoxy)phenyl substituted piperazines (**4a,b**) in hand, we needed access to the appropriate 5-cyano-indole-2-aldehyde (**1**) in order to allow reductive amination leading to target compounds **5a-6b** (Scheme 2). Starting from 2-(hydroxymethyl)indole-5-carbonitrile,[34] we easily gained the corresponding aldehyde **1** by oxidation using manganese(IV) oxide at ambient temperature. Thus, the fluoroethoxy substituted reference compounds **5a** and **5b** as well as the labelling precursor compounds **6a** and **6b** were obtained by the same experimental procedure using $Na(OAc)_3BH$ as reducing agent in the coupling of **1** with **4a**, **4b**, or 2-/4-hydroxyphenylpiperazine, respectively (Scheme 2).

After purification by flash chromatography on silica gel and confirmation of chemical purity by LC/MS, the novel fluoroethoxyphenyl substituted 5-cyano-



Scheme 1. (i) $(BOC)_2O$, DMF; (ii) 1-fluoro-2-tosyloxyethane, $N(Bu)_4OH$, DMF; (iii) conc. HCl



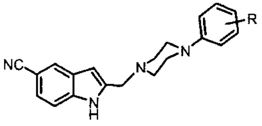
Scheme 2. Syntheses of the target compounds 5a,b as well as the corresponding labelling precursors 6a,b: (i) MnO_2, CH_2Cl_2; (ii) $Na(OAc)_3BH$, CH_2Cl_2

indoles (**5a**, **5b**) and the corresponding hydroxyphenyl substituted derivatives (**6a**, **6b**) were characterized *in vitro* for their ability to displace [^{3}H]spiperone from the cloned human dopamine receptors D_{2long}, D_{2short},[35] D_3[36] and D_4[37] being stably expressed in chinese hamster ovary (CHO) cells.[38] D_1 affinity was determined by employing porcine striatal membrane preparations and the D_1 selective antagonist [^{3}H]SCH 23390. In addition, receptor affinities to the related biogenic amine receptors 5-HT_{1A}, 5-HT_2 and α_1 were evaluated utilizing porcine cortical membranes and the selective radioligands [^{3}H]8-OH-DPAT, [^{3}H]ketanserin and [^{3}H]prazosin, respectively. For comparison of the binding data, the reference compound FAUC 316 was investigated under the same conditions (Table 1).

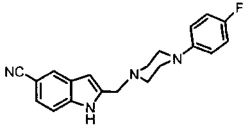
The dopamine receptor binding profiles of the test compounds clearly indicate poor affinities for the D_1, D_2 and D_3 subtypes, with the exception of compound **5a** indicating a distinct influence when introducing a fluoroethoxy substituent at the ortho position of the phenylpiperazin moiety. All test compounds revealed a K_i value in the low nanomolar range for the D_4 receptor (Table 1). Comparison of the D_4 binding data among each other showed significantly higher D_4 receptor affinities for the derivatives with substituents in ortho position of the phenylpiperazinyl group (**5a**: 2.1 nM, **6a**: 1.6 nM). The corresponding derivatives substituted in para position (**5b**: 9.9 nM and **6b**: 3.1 nM) showed decreased D_4 binding affinity, whereas dopamine receptor subtype selectivities were superior in comparison with **5a** and **6a**, respectively. Interestingly, a significant effect on α_1 binding was observed when comparing derivatives substituted at 2-position to those substituted at 4-position. This effect was especially illustrated by a 70-fold loss of α_1 binding of **5b** when comparing to α_1 affinity of **5a**. In general, **5b** was characterized as a high affinity D_4 receptor ligand with good dopamine subtype receptor selectivity, weak receptor affinity for α_1, 5-HT_{1A} and 5-HT_2, and thus illustrating superior *in vitro* properties in comparison with **5a**. When comparing **5b** to the reference lead compound FAUC 316, displacement of the para-fluorine substituent by a para-fluoroethoxy group led to reduced binding properties *in vitro* (Table 1).

It is tempting to speculate whether ^{18}F-labelled indoles **[^{18}F]5a** and **[^{18}F]5b** could be suitable radioligands for the observation of a receptor-specific signal with PET, due to the low D_4 receptor concentration in the brain. However, the *in vitro* binding profile of **5a** demonstrated a K_i value for the D_4 receptor in the low nanomolar range (2.1 nM), which could be acceptable for an adequate binding potential (B_{max}/K_d) when assuming an existing D_4 receptor concentration of roughly 3 nM in the brain. Moreover, **[^{18}F]5b** is characterized by an improved D_4 receptor selectivity including less binding affinity to adrenergic and serotoninergic sites, which could be beneficial when performing *in vitro* autoradiography studies on native tissue. Due to the high D_4 receptor affinity of **5a** and distinct D_4 receptor selectivity of **5b**, we aimed at optimizing

Table 1. Binding affinities of the fluorinated target compounds 5a,b to the human dopamine receptor subtypes D_{2long}, D_{2short}, D_3, D_4 and the porcine D_1 receptor as well as the porcine 5-HT_{1A}, 5-HT_2 and α_1 receptors in comparison with the labelling precursors 6a,b and the reference compound FAUC 316. K_i values are expressed as mean ± SEM of 2–5 experiments each performed in triplicate



5a – 6b



FAUC 316

Compound	K_i values (nM ± SEM)								D_4 selectivity[b] within the D_2 family		
	[^{3}H]spiperone				[^{3}H]SCH 23990	[^{3}H]8-OH-DPAT	[^{3}H]ketanserin	[^{3}H]prazosin			
	D_{2long}	D_{2short}	D_3	$D_{4.4}$	D_1	5-HT_{1A}	5-HT_2	α_1	$K_i(D_{2long})/K_i(D_{4.4})$	$K_i(D_{2short})/K_i(D_{4.4})$	$K_i(D_3)/K_i(D_{4.4})$
5a (R = 2-OEtF)	11 ± 1.3	16 ± 1.5	67 ± 14	2.1 ± 0.5	2600 ± 190	190[c]	3400 ± 1300	27 ± 3.4	5.2	7.6	32
5b (R = 4-OEtF)	4100 ± 830	4200 ± 330	2500 ± 690	9.9 ± 0.4	38 000 ± 5000	5300 ± 300	24 000 ± 5500	1900 ± 290	410	420	250
6a (R = 2-OH)	730 ± 50	450 ± 50	280[c]	1.6 ± 0	3700 ± 300	nd	nd	77 ± 14	460	280	180
6b (R = 4-OH)	3500 ± 350	2000 ± 300	4700 ± 1800	3.1 ± 0.9	12 000 ± 1000	nd	nd	690 ± 95	1100	650	1500
FAUC 316	28 000 ± 6500	19 000 ± 4000	15 000 ± 2000	1.0 ± 0.1	8600 ± 2400[a]	nd	nd	nd	28 000	19 000	15 000

nd: not determined.

[a] Determined with bovine receptors.

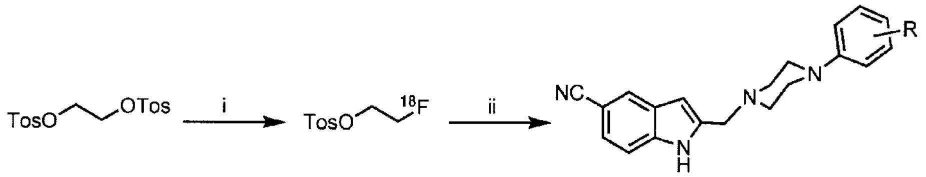
[b] D_4 receptor selectivity was expressed as the dimensionless ratio between K_i (nM) for each subtype and K_i for the D_4 receptor (nM).

[c] Data of a single experiment.

the radiosyntheses of the corresponding ^{18}F-labelled analogs, in order to provide putative D$_4$ receptor radioligands that could be further investigated by *in vitro* assays or used to assess the pharmacokinetics of these 5-cyano-indole derivatives *in vivo*.

6a and **6b** were used for the following ^{18}F-radiolabelling studies with [^{18}F]fluoroethyl tosylate under varied reaction parameters. Compounds **5a** and **5b** served as authentic reference compounds in analytical radio-HPLC to confirm chemical identity of **[^{18}F]5a** and **[^{18}F]5b**. The radiosyntheses of **[^{18}F]5a** and **[^{18}F]5b** are depicted in Scheme 3. Based on the nucleophilic ^{18}F-for-OTos substitution on bistosyloxyethane as described by Block and coworkers,[39] we isolated [^{18}F]fluoroethyl tosylate by semipreparative reversed-phase HPLC followed by solid phase extraction. Slight modifications of the procedure described by Block *et al.*[39] were introduced concerning reaction time and reaction temperature. In order to improve the radiochemical yield (RCY) of the prosthetic group [^{18}F]fluoroethyl tosylate, the reaction temperature was increased to 90°C, while the reaction time was shortened to 3 min. These reaction conditions provided limited amounts of an ^{18}F-labelled hydrophilic by-product (<5%; probably 2-[^{18}F]fluoroethanol occurring under basic reaction conditions) and an improved radiochemical yield of 84% as determined by radio-HPLC (Scheme 3, step 1). The reaction parameters for the ^{18}F-fluoroethylation key step were investigated with respect to the choice of reaction solvent, base and reaction time as summarized in Table 2.

Using DMF and sodium methoxide (NaOMe) as a base to generate the phenoxide from precursor **6a** did not lead to a rapid progression of the ^{18}F-fluoroethylation in ortho position. By increasing the reaction temperature from 100 to 140°C, the RCY of **[^{18}F]5a** decreased to 13%. At 100°C the remaining radioactivity at the end of reaction was present as [^{18}F]fluoroethyl tosylate (50 ± 3%, $t = 5$ min), while at 140°C an accelerated degradation of [^{18}F]fluoroethyl tosylate was observed probably due to the formation of



[^{18}F]5a: R= 2-[^{18}F]FEtO
[^{18}F]5b: R= 4-[^{18}F]FEtO

Scheme 3. Two-step procedure for the radiosynthesis of [^{18}F]5a and [^{18}F]5b: (i) [$K \subset 222]^+$[^{18}F]F$^-$, bistosyloxyethane, CH$_3$CN, T = 90°C, 3 min; (ii) for [^{18}F]5a: 6a, NaOMe, DMSO, T = 120°C, 5 min; for [^{18}F]5b: 6b, N(Bu)$_4$OH, DMF, T = 140°C, 5 min

Table 2. Radiochemical yields (RCY) for the ^{18}F-fluoroethylation of 6a and 6b using 1-[^{18}F]fluoro-2-tosyloxyethane

Product	Solvent/base	T (°C)	t (min)	RCY (%)[a]
[^{18}F]5a	DMF/NaOMe	100	1	12; 13 ($n = 2$)
			5	37; 39 ($n = 2$)
			15	67 ± 5
		120	1	14 ± 5
			5	34 ± 3
			10	47 ± 1
		140	1	7; 11 ($n = 2$)
			3	8; 16 ($n = 2$)
			10	7; 20 ($n = 2$)
	DMSO/NaOMe	120	1	31; 45 ($n = 2$)
			2	57; 67 ($n = 2$)
			3	70 ± 5
			5	80 ± 2
			10	81 ± 5
			15	70 ± 5
[^{18}F]5b	DMF/NaOMe	120	1–15	0[b]
	DMSO/NaOMe	140	1–15	0[b]
	DMF/NaH	120	1–15	0[b]
	DMF/N(Bu)$_4$OH	100	1	9[c]
			3	10[c]
			5	7[c]
		120	1	24 ± 5
			5	37 ± 8
			10	31 ± 4
			15	34 ± 5
		140	1	41 ± 10
			5	47 ± 4
			15	36 ± 5
	DMSO/N(Bu)$_4$OH	140	1–15	0[b]

[a] RCY were determined by radio-HPLC and related to 1-[^{18}F]fluoro-2-tosyloxyethane. Values are expressed as mean ± standard deviation (SD) of three independent experiments ($n = 3$). Independent experimental values are expressed for $n = 2$ (10.8 μmol precursor, $V = 350$ μl).

[b] Determined by radio-TLC.

[c] Data of a single experiment.

2-[^{18}F]fluoroethanol (~70%, $t = 10$ min). The low RCY of **[^{18}F]5a** using the solvent system DMF/NaOMe could be due to limited solubility of the sodium phenoxide in DMF and thus decreased nucleophilicity. Thus, we examined the influence of the solvent on the RCY under identical experimental conditions. We used dimethyl sulfoxide (DMSO) instead of DMF, since this solvent provides excellent properties for dissolving sodium salts as already has been exploited in numerous ^{18}F-fluoroethylation procedures, such as the radiosynthesis of O-(2-[^{18}F]fluoroethyl)-L-tyrosine and others.[40–42] As shown in Table 1, the use of DMSO revealed an accelerated formation of the desired product **[^{18}F]5a**, obtaining a RCY of about 80% (decay-corrected and related to [^{18}F]fluoroethyl tosylate) within 5–10 min at 120°C.

Surprisingly, these reaction conditions were not transferable to the ^{18}F-fluoroethylation of the para substituted precursor **6b** to give **[^{18}F]5b** (Table 2). More than 90% of the remaining radioactivity at end of reaction was detected as unreacted [^{18}F]fluoroethyl tosylate when using the solvent/base systems DMF/NaOMe or DMSO/NaOMe. Changing the base from NaOMe to equimolar amounts of sodium hydride also did not reveal any reaction or degradation of [^{18}F]fluoroethyl tosylate. However, following the protocol of Wilson *et al.*[33] we used tetrabutylammonium hydroxide as base promotor to generate the para phenoxide of **6b** in DMF. Under these conditions, addition of [^{18}F]fluoroethyl tosylate lead to satisfactory radiochemical yields of **[^{18}F]5b** at 140°C (40–50%, decay-corrected and related to [^{18}F]fluoroethyl tosylate; Table 2). In comparison with the radiosynthesis of the [^{18}F]fluoroethoxy substituted derivative **[^{18}F]5a**, ^{18}F-fluoroethylation in the para position of the phenylpiperazine moiety using the solvent system DMF/$N(Bu)_4OH$ allowed the use of a higher reaction temperature (140°C) and proceeded more rapidly reaching a maximum RCY of **[^{18}F]5b** within a shorter reaction time of 1–5 min. At later time points, the RCY of **[^{18}F]5b** slightly decreased, suggesting degradation of the final product under basic reaction conditions. As the major by-product we detected an unknown hydrophilic ^{18}F-labelled compound ($R_t = 2.9$ min, radio-HPLC), probably due to the formation of 2-[^{18}F]fluoroethanol as also observed for the reaction of **6a** with [^{18}F]fluoroethyl tosylate at 140°C (see above). Efforts to improve the RCY of the para substituted product **[^{18}F]5b** by using DMSO/$N(Bu)_4OH$ failed (Table 2), so that the solvent system DMF/$N(Bu)_4OH$ at 140°C turned out to be the optimal reaction medium for the radiosynthesis of **[^{18}F]5b**.

This optimization study for the syntheses of the ^{18}F-labelled radioligands **[^{18}F]5a** (DMSO/NaOMe, 120°C, 5 min) and **[^{18}F]5b** (DMF/$N(Bu)_4OH$, 140°C, 5 min) led to adequate radiochemical yields of 80 and 47%, respectively, permitting further *in vitro* and *in vivo* studies.

Experimental

General

All chemicals and reagents were of analytical grade and obtained from commercial sources. [^{18}F]Fluoride was produced by the $^{18}O(p, n)^{18}F$ reaction on ^{18}O-enriched (95%) water using a 11 MeV proton beam generated by a RDS 111 cyclotron (PET Net GmbH, Erlangen, Germany). Solid phase cartridges (Sep-Pak®Plus C18 cartridges) were purchased from Waters (Eschborn, Germany). Thin layer chromatography (TLC) was carried out on silica gel-coated aluminium plates (silica gel/TLC-cards, with fluorescent indicator 254 nm, layer thickness 0.2 mm, Fluka); for radio-TLC plastic sheets (Polygram®, Sil G/UV_{254}, Macherey-Nagel) were used. Compounds were visualized by UV light (254 nm). Analytical radio-HPLC was performed on the following system: HPLC Hewlett Packard (HP 1100) with a quarternary pump and variable wavelength detector (HP 1100) and radio-HPLC-detector D505TR (Canberra Packard). Computer analysis of the HPLC data was performed using FLO-One software (Canberra Packard). NMR spectra were recorded on a Bruker Avance 360 or Bruker Avance 600 using TMS as internal standard (all data were expressed in parts per million (ppm)). LC-MS analyses were performed on an Agilent 1100 Series analytic HPLC system with a VWL detector, coupled to a Bruker esquire 2000 mass spectrometer with atmospheric pressure chemical ionization (APCI). Melting points (m.p.) were uncorrected and obtained with a Büchi apparatus. 1-fluoro-2-tosyloxyethane was synthesized as previously described by Block *et al.*[39] and identity was confirmed by TLC (ethyl acetate/hexane 7:3, $R_f = 0.89$), ^{1}H-NMR ($CDCl_3$: δ 2.46 (s, 3H), 4.24–4.32 (dt, 2H, OCH_2), 4.45–4.63 (dt, 2H, FCH_2), 7.34–7.38 (m, 2H) 7.79–7.83 (m, 2H)) and HPLC (Lichrosorb RP18, 250 × 4.6 mm 1 ml/min, 30–80% CH_3CN in water (0.1% TFA) in 25 min: $R_t = 13.40$ min). 2-(Hydroxymethyl)indole-5-carbonitrile (LC-MS (APCI): *m*/*z* 173.0 $[M + H]^+$) was available as a product of a former research study (Department of Medicinal Chemistry).[34] Each ^{18}F-labelled compound was identified by retention time (R_t) on the radio-HPLC system and co-injection of the corresponding reference compound.

Chemistry

*5-Cyano-indole-2-aldehyde (***1***).* **1** was synthesized by oxidation of 2-(hydroxymethyl)indole-5-carbonitrile (1.4 g, 8.14 mmol) using manganese(IV) oxide (7.08 g, 80.28 mmol) in 30 ml CH_2Cl_2. The suspension was stirred at room temperature for 24 h. The reaction mixture was filtered through cellulose and the precipitate was washed with ethyl acetate. The organic layer was removed under reduced pressure to give the product as a pale yellow solid (1.09 g, 6.41 mmol, 79%), which was used for subsequent reactions without further purification. TLC (CH_2Cl_2/MeOH 95:5): $R_f = 0.43$. ^{1}H-NMR (DMSO): δ 7.53 (s, 1H), 7.64 (m, 2H), 8.36 (s, 1H), 9.96 (s, 1H, CHO), 12.48 (s, 1H, NH).

*4-(2-Hydroxyphenyl)piperazine-1-carboxylic acid t-butyl ester (***2a***).* Anhydrous di-*t*-butyldicarbonate (720 mg, 3.3 mmol) was dissolved in 10 ml DMF and slowly added to a solution of 1-(2-hydroxyphenyl)piperazine (534 mg, 3.0 mmol) and triethylamine (334 mg, 3.3 mmol) in 20 ml DMF. After stirring at room temperature for 1 h, the reaction was stopped by adding 30 ml of a saturated $NaHCO_3$ solution. The solution was extracted with ethyl acetate (3 × 20 ml) and the organic solvent was removed under reduced pressure. The crude product was purified by silica gel chromatography using ethyl acetate/hexane (3:7) as eluant. **2a** was isolated in 58% yield (511 mg, 1.74 mmol) as a yellow oil. TLC (ethyl acetate/hexane 3:7): $R_f = 0.60$. ^{1}H-NMR (CDCl$_3$): δ 1.48 (s, 9H, *t*-butyl), 2.85 (t, 4H), 3.59 (t, 4H), 6.87–7.15 (m, 4H).

*4-(4-Hydroxyphenyl)piperazine-1-carboxylic acid t-butyl ester (***2b***).* **2b** was synthesized as described for **2a**. Starting from 178 mg (1.0 mmol) 1-(4-hydroxyphenyl)piperazine, **2b** was obtained as a yellow oil in a yield of 64% (188 mg, 0.64 mmol). TLC (ethyl acetate/hexane 3:7): $R_f = 0.30$. ^{1}H-NMR (CDCl$_3$): δ 1.50 (s, 9H, *t*-butyl), 2.91 (t, 4H), 3.56 (t, 4H), 6.76 (m, 2H), 6.90 (m, 2H).

*4-(2-(2-Fluoroethoxy)phenyl)piperazine-1-carboxylic acid t-butyl ester (***3a***).* Following a protocol reported by Wilson *et al.*,[33] a mixture of **2a** (149 mg, 0.51 mmol), 1-fluoro-2-tosyloxyethane (171 mg, 0.79 mmol) and a solution of tetrabutylammonium hydroxide (0.51 ml, 1.4 N in dry MeOH) were dissolved in 8 ml dry DMF and stirred at room temperature for 24 h. The reaction was quenched by adding 50 ml 0.05 N NaOH. After extraction with ethyl acetate (3 × 25 ml) and drying over Na$_2$SO$_4$ the organic layer was evaporated and the residue was purified by silica gel chromatography using CH$_2$Cl$_2$/ethyl acetate (90:10) to give **3a** (126 mg, 0.37 mmol, 72%) as a yellow oil. TLC (ethyl acetate/hexane 3:7): $R_f = 0.60$. ^{1}H-NMR (CDCl$_3$): δ 1.49 (m, 9H, *t*-butyl), 3.02 (t, 4H), 3.58 (t, 4H), 4.21–4.30 (dt, 2H, FCH_2), 4.71–4.83 (dt, 2H, FCH_2), 6.82–7.02 (m, 4H).

*4-(4-(2-Fluoroethoxy)phenyl)piperazine-1-carboxylic acid t-butyl ester (***3b***).* **3b** was synthesized as described for **3a**. Starting from 225 mg (0.77 mmol) **2b**, chromatographic isolation on silica gel yielded 86% of **3b** as a dark yellow oil after evaporation of the organic solvent (225 mg, 0.66 mmol). TLC (ethyl acetate/hexane 3:7): $R_f = 0.75$. ^{1}H-NMR (CDCl$_3$): δ 1.49 (m, 9H, *t*-butyl) 3.02 (t, 4H), 3.61 (t, 4H), 4.21–4.31 (dt, 2H, FCH_2), 4.69–4.85 (dt, 2H, FCH_2), 6.81–7.01 (m, 4H).

*1-(2-(2-Fluoroethoxy)phenyl)piperazine (***4a***).* A solution of **3a** (595 mg, 1.75 mmol) in 10 ml of concentrated hydrochloric acid was stirred at room temperature for 15 min. Cleavage of the *t*-butyl ester was analysed by TLC. The solution was carefully diluted with water and slowly adjusted to pH 8–10 with sodium hydroxide pellets. Extraction with ethyl acetate, drying over Na$_2$SO$_4$ and removal of the solvent under reduced pressure yielded 60% of **4a** (237 mg, 1.06 mmol) as a yellow oil. TLC (MeOH/CH$_2$Cl$_2$ 3:1, 0.5% triethylamine): $R_f = 0.20$. ^{1}H-NMR (CDCl$_3$): δ 3.09 (m, 8H), 4.18–4.31 (dt, 2H, FCH_2), 4.68–4.86 (dt, 2H, FCH_2), 6.82–7.02 (m, 4H).

*1-(4-(2-Fluoroethoxy)phenyl)piperazine (***4b***).* Ester cleavage of **3b** to obtain **4b** was performed as described above for the synthesis of **4a**. Chromatography on silica gel yielded **4b** as a yellow oil (121 mg, 0.53 mmol, 50%). TLC ($MeOH/CH_2Cl_2$ 3:1, 0.5% triethylamine): $R_f = 0.20$. ^{1}H-NMR ($CDCl_3$): δ 3.08 (m, 8H), 4.19–4.32 (dt, 2H, FCH_2), 4.68–4.88 (dt, 2H, FCH_2), 6.80–6.89 (m, 2H) 6.90–7.02 (m, 2H).

*2-[4-(2-(2-Fluoroethoxy)phenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (***5a***).* A mixture of **4a** (237 mg, 1.05 mmol) and **1** (270 mg, 1.59 mmol) was dissolved in 20 ml dry CH_2Cl_2, $Na(OAc)_3BH$ (335 mg, 1.59 mmol) was added to the suspension in one portion. After stirring at room temperature for 24 h the reaction was stopped by adding 30 ml saturated $NaHCO_3$. The product was extracted with ethyl acetate and the organic layer was dried over Na_2SO_4. The solvent was evaporated and the residue was purified by silica gel chromatography in a two step procedure (a: CH_2Cl_2/ethyl acetate 90:10; b: CH_2Cl_2/MeOH 95:5) yielding **5** as a pale yellow solid (98 mg, 0.26 mmol, 24%). M.p.: 186°C. TLC (CH_2Cl_2/MeOH 95:5): $R_f = 0.50$. ^{1}H-NMR ($CDCl_3$): δ 2.71 (t, 4H), 3.17 (t, 4H), 3.83 (s, 2H, CH_2), 4.21–4.28 (dt, 2H, OCH_2), 4.71–4.82 (dt, 2H, FCH_2), 6.47–7.90 (m, 8H), 8.91 (s, 1H, NH). HPLC (Lichrosorb RP18, 250 × 4.6, 1 ml/min, 30–80% CH_3CN in water (0.1% TFA) in 25 min): $R_t = 10.60$ min. LC-MS (APCI): m/z 379.1 $[M+H]^+$, 225.1 (fluoroethoxyphenyl piperazinyl fragment $[C_{12}H_{17}FN_2O+H]^+$).

*2-[4-(4-(2-Fluoroethoxy)phenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (***5b***).* Compound **5b** was synthesized as described for **5a** starting from **4b** (125 mg, 0.56 mmol) and **1** (140 mg, 0.83 mmol). The pale yellow solid **5b** was obtained in a yield of 27% (56 mg, 0.15 mmol). M.p.: 205°C. TLC (CH_2Cl_2/MeOH 95:5): $R_f = 0.55$. ^{1}H-NMR ($CDCl_3$): δ 2.68 (t, 4H), 3.22 (t, 4H), 3.74 (s, 2H, CH_2) 4.09–4.20 (dt, 2H, OCH_2), 4.34–4.77 (dt, 2H, FCH_2), 6.46 (s, 1H), 6.88 (m, 4H), 7.42 (s, 2H), 7.91 (s, 1H), 8.90 (s, 1H, NH). HPLC (Lichrosorb RP18, 250 × 4.6, 1 ml/min, 30–80% CH_3CN in water (0.1% TFA) in 25 min): $R_t = 10.13$ min. LC-MS (APCI): m/z 379.1 $[M+H]^+$, 225.0 (fluoroethoxyphenyl piperazinyl fragment $[C_{12}H_{17}FN_2O+H]^+$).

*2-[4-(2-Hydroxyphenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (***6a***).* 1-(2-Hydroxyphenyl)piperazine (397 mg, 2.23 mmol) and **1** (253 mg, 1.49 mmol) were dissolved in 10 ml dry CH_2Cl_2 and $Na(OAc)_3BH$ (1263 mg, 5.96 mmol) was added to the suspension. After stirring at room temperature for 1 h, the reaction was terminated by adding 30 ml saturated $NaHCO_3$. The crude product was purified by silica gel chromatography using CH_2Cl_2/MeO H (95:5) as eluant yielding 32% (159 mg, 0.48 mmol) of **6a** as a pale yellow solid. M.p.: 148°C. TLC (CH_2Cl_2/MeOH 95:5): $R_f = 0.30$. ^{1}H-NMR ($CDCl_3$): δ 2.44 (t, 4H), 3.33 (t, 4H), 3.74 (s, 2H, CH_2), 6.43–8.05 (m, 8H), 8.87 (s, 1H, N*H*), 11.69 (s, 1H, O*H*). LC-MS (APCI): *m*/*z* 333.1 $[M+H]^+$, 179.0 (hydroxyphenyl piperazinyl fragment $[C_{10}H_{14}N_2O+H]^+$).

*2-[4-(4-Hydroxyphenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (***6b***).* **6b** was synthesized as described above for compound **6a**. The reaction yielded **6b** as a pale yellow solid (183 mg, 0.55 mmol, 36%). M.p.: 149°C. TLC (CH_2Cl_2/MeOH 95:5): $R_f = 0.50$. ^{1}H-NMR ($CDCl_3$): δ 2.53 (t, 4H), 3.31 (t, 4H), 3.73 (s, 2H, CH_2), 6.51 (s, 1H), 6.59 (d, 2H), 6.82 (d, 2H), 7.39–7.98 (m, 3H), 8.79 (s, 1H, N*H*), 11.70 (s, 1H, O*H*). LC-MS (APCI): *m*/*z* 333.1 $[M+H]^+$, 179.0 (hydroxyphenyl piperazinyl fragment $[C_{10}H_{14}N_2O+H]^+$).

Radiochemistry

1-[^{18}F]Fluoro-2-tosyloxyethane[39]. No-carrier-added [^{18}F]fluoride (200–650 MBq) was delivered on a QMA-cartridge and eluted with a solution of 15 mg Kryptofix® 2.2.2/15 μl 1 M K_2CO_3 in 1 ml acetonitrile/water (8:2). The solution was evaporated using a stream of nitrogen at 85°C and co-evaporated to dryness with CH_3CN (2 × 200 μl). Following the procedure described by Block *et al.*,[39] 4.5 mg (12 μmol) bistosyloxyethane in 500 μl anhydrous acetonitrile were added to the reaction vessel and the mixture was stirred for 3 min at 90°C. The reaction was quenched by dilution with 500 μl water. The prosthetic group 1-[^{18}F]fluoro-2-tosyloxyethane was isolated by gradient reversed-phase HPLC (Lichrosorb RP18, 125 × 8 mm, 4 ml/min, CH_3CN/H_2O (40/60) (0.1% TFA)). The product fraction was diluted 1:10 with water and fixed on a C18-cartridge (Waters Sep-Pak®Plus), dried in a nitrogen stream and eluted with 1 ml DMF in a reaction vessel. 1-[^{18}F]Fluoro-2-tosyloxyethane was obtained in a radiochemical yield (RCY) of 84% as determined by radio-HPLC from a sample withdrawn from the reaction mixture. Radio-TLC (ethyl acetate/hexane 7:3): $R_f = 0.87$. Radio-HPLC (Lichrosorb RP18, 250 × 4.6 mm, 1 ml/min, 30–80% CH_3CN in water (0.1% TFA) in 25 min): $R_t = 14.34$ min.

*2-[4-(2-(2-[^{18}F]Fluoroethoxy)phenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (***[^{18}F]5a***).* 3.6 mg (10.8 μmol) 2-[4-(2-hydroxyphenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (**6a**) were dissolved in 250 μl dry DMSO and 50 μl sodium methoxide in dry methanol (8 mg/ml) were added to the reaction vial. The reaction mixture was stirred for 3 min at 120°C under a nitrogen atmosphere. Subsequently, 1-[^{18}F]fluoro-2-tosyloxyethane in 50 μl dry DMF (5–20 MBq) were added to the reaction mixture. The progression of the reaction was analysed by radio-HPLC. The radiochemical yield of **[^{18}F]5a** was 81 ± 5% after 5–10 min at 120°C. Radio-HPLC (Lichrosorb RP18, 250 × 4.6 mm, 1 ml/min, 30–80% CH_3CN in water (0.1% TFA) in 25 min): $R_t = 10.66$ min.

2-[4-(4-(2-[^{18}F]Fluoroethoxy)phenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (**[^{18}F]5b**). 3.6 mg (10.8 μmol) 2-[4-(4-hydroxyphenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (**6b**) were dissolved in 250 μl dry DMF. 113 μl 1.4 N tetrabutylammonium hydroxide in dry MeOH were added to the reaction vial. The reaction mixture was stirred for 3 min at 120°C under a nitrogen atmosphere. Subsequently, 50 μl of the 1-[^{18}F]fluoro-2-tosyloxyethane solution in dry DMF (5–20 MBq) were added to the reaction mixture at 140°C. The progression of the reaction was analysed by radio-HPLC. The radiochemical yield of **[^{18}F]5b** was $47 \pm 4\%$ after 5 min at a reaction temperature of 140°C. Radio-HPLC (Lichrosorb RP18, 250 × 4.6, 1 ml/min, 30–80% CH_3CN in water (0.1% TFA) in 25 min): $R_t = 10.24$ min.

Optimization of the ^{18}F-fluoroethylation procedure. The ^{18}F-fluoroethylation of **6a** and **6b** was optimized by repeating the reaction with varying parameters as indicated in Table 1.

Receptor binding experiments and data analysis

Receptor binding studies were performed as described previously.[38] In brief, the dopamine D_1 receptor assay was done with porcine striatal membranes at a final protein concentration of 40 μg/assay tube and the radioligand [^{3}H]SCH23390 at 0.3 nM ($K_d = 0.5$ nM). Competition experiments with the human D_{2long}, D_{2short}, D_3 and $D_{4.4}$ receptors were run with preparations of membranes from CHO cells expressing the corresponding receptor and [^{3}H]spiperone at a final concentration of 0.5 nM. The assays were carried out at a protein concentration of 6–30 μg/assay tube and K_d values of 0.10 nM for D_{2long}, D_{2short} and D_3 and 0.10–0.13 nM for $D_{4.4}$. Serotonin 5-HT_{1A}, 5-HT_2 and adrenergic α_1 binding were measured utilizing porcine cortical membranes and the selective radioligands [^{3}H]8-OH-DPAT, [^{3}H]ketanserin and [^{3}H]prazosin, respectively, each at a final concentration of 0.5 nM. The resulting competition curves were analysed by nonlinear regression using the algorithms in PRISM (GraphPad Software, San Diego, USA). The data were fitted using a sigmoid model to provide an IC_{50} value, representing the concentration corresponding to 50% of maximal inhibition. IC_{50} values were transformed to K_i values according to the equation of Cheng and Prusoff.[43]

Conclusion

In conclusion, two isomeric fluoroethoxy substituted derivatives of 5-cyano-indole were synthesized and characterized as high-affinity dopamine D_4 receptor ligands *in vitro*. A para-fluoroethoxy substituent at the phenylpiperazine moiety was tolerated by D_4 receptors and more advantageous than an ortho substituent with respect to dopamine D_4 receptor selectivity. For both ^{18}F-labelled derivatives of 5-cyano-indole the reaction conditions for

^{18}F-fluoroethylation using [^{18}F]fluoroethyl tosylate were successfully optimized providing potential PET imaging probes for the dopamine D$_4$ receptor. The methodology of ^{18}F-fluoroethylation could also be applied to alternative lead compounds of the FAUC series, such as derivatives of pyrazolo[1,5-*a*]pyridine, in order to provide D$_4$ radioligand candidates for PET with improved D$_4$ receptor affinity and selectivity.

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