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^{18}F-Labeled FAUC 346 and BP 897 Derivatives as Subtype-Selective Potential PET Radioligands for the Dopamine D3 Receptor

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*Disturbances of neutrotransmission at the dopamine D3 receptor are related to several neuropsychiatric diseases and in particular to drug addiction. Herein, we report the computer-assisted prediction of D3 selectivities of new fluoroalkoxy-substituted receptor ligands by means of 3D-QSAR analysis. As close analogues of the D3-selective lead compound FAUC 346 and BP 879, the ^{19}F-substituted test compounds **4a–d** were synthesized and evaluated. In vitro investigation of their binding characteristics in transfected Chinese Hamster Ovary (CHO) cells led to excellent K_i values between 0.12 and 0.69 nM at the dopamine D3 subtype. The benzothiophene-substituted carboxamide **4a** (K_i = 0.12 nM) displayed 133 and 283-fold selectivity over the structurally related $D2_{Long}$ and D4 subtypes, respectively. Mitogenesis assays showed the behavior of partial agonists. Based on these data, we synthesized the [^{18}F]fluoroethoxy-substituted radioligands [^{18}F]**4a–d**. The N-[4-[4-(2-hydroxyphenyl)piperazin-1-yl]butyl]-2-carboxamides **3a–d** were prepared and labeled with 2-[^{18}F]fluoroethyltosylate in a two-step procedure. Optimization of the ^{18}F-labeling conditions led to radiochemical yields between 24 and 65%.*

Introduction

Dopamine receptors are classified into the G_s-coupled D1-like (D1 and D5) and $G_{i/o}$-coupled D2-like (D2, D3, and D4) receptor subtypes[1–2] although D3 has attracted special attention, in recent years.[3] Similar to the neurotransmitter dopamine, the agonist 7-hydroxy-dipropylaminotetraline (7-OH-DPAT) binds preferentially to the D3 subtype (K_d = 0.8 nM). Employing autoradiography, D3 receptors can be distinguished from D2 receptors by using both selective dopaminergic antagonists and agonists. With this approach, the specific distribution of the D3 receptor to limbic areas such as to the ventromedial shell of the nucleus accumbens and the islands of Calleja has been repeatedly demonstrated in the rat.[4–8] In this species, low expression of the D3 receptor was found in the hippocampus, in the septal area, and in subregions of the medial temporal lobe.[4] Autoradiographical studies with (+)-[^{3}H]PD128907 (K_i = 1.08 nM for D3) yielded a quite similar cerebral distribution in the human.[9,10] Using specific antagonists, a role of the D3 receptor in cognition and motivated behavior has been established in experimental animals.[7,9–10] Some evidence suggests that D3 inhibition activates the mesocorticolimbic dopaminergic system.[11] This underlines the potential relevance of disturbances in neurotransmission via the D3 receptor for psychiatric disease. Nevertheless, the physiological role of the D3 receptor has as yet not been fully elucidated. The dopamine D3 receptor has been suggested to be of importance for the therapeutic effects of antipsychotic drugs.[1,12] As D3 is also involved in several neuropsychological disorders, selective D3 ligands may have therapeutic potential for the treatment of drug addiction and Parkinson's disease. Thus, neuroprotective effects during the induction phase of Parkinson's disease have been described for selective D3 agonists such as pramipexole, BP 897 (**1**), and FAUC 329 (**2c**).[13–16] Neurobiological studies revealed an upregulation of the dopamine D3 receptor in the striatum of alcohol-preferring rats after one year of alcohol consumption. A repeated administration of the D3 agonist BP 897 (**1**) and the antagonist SB-277011A caused a significant dose-dependent decrease in the expression of behavioral effects induced by alcohol deprivation in rats.[17]

4-Phenylpiperazines can be regarded as a privileged GPCR-binding structure.[18] This class has been the subject of chemical optimization and thorough pharmacological investigation[19–24] when FAUC 346 (**2a**) proved to be a high affinity (K_i = 0.23 nM), potent (EC_{50} = 1.5 nM) and highly selective D3 partial agonist (~50% maximal intrinsic activity). On the other hand, the 2,3-dichlorophenyl analogue FAUC 365 turned out to act as a neutral antagonist with subnanomolar affinity (K_i = 0.50 nM) and very high subtype selectivity (~7200 versus D2). Recently, 5-iodo derivatives of FAUC 365 have been introduced as potential radioiodine labeled ligands for single photon emission computer tomography (SPECT).[25] Radiosyntheses of ^{11}C-labeled ligands including [^{11}C]FAUC 365 have also been reported.[26–29] However, the results published do not confirm the

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suitability of this radioligand for in vivo use. Aiming at the development of D3 radioligands suitable for diagnostic use in vivo by positron emission tomography (PET), we synthesized ^{18}F-labeled candidate ligands derived from our selective antagonist FAUC 365.[30] As an extension of this work, we successfully generated CoMFA and CoMSIA models, allowing a precise prediction of D3 affinity and selectivity over both congeners $D2_{Long}$ and D4 and thus facilitating the development of D3 PET ligands.[31,32]

The aim of the present study has been the development of fluorine-labeled PET ligands using FAUC 346 (**2a**) as lead compound when the methoxy function should be displaced by a fluoroethoxy bioisostere. Taking advantage of CoMFA and CoMSIA based calculations of selectivity contour maps for D3 over both congeners $D2_{Long}$ and D4, we synthesized the fluoroalkylated analogues **4a–d** and investigated their receptor binding profiles indicating high D3 affinities and selectivities. Using a nucleophilic two-step radiofluorination of the hydroxyl-substituted precursors with 2-[^{18}F]fluoroethyltosylate, we synthesized the N-[4-[4-(2-fluoroethylphenyl)piperazine-1-yl]butyl]benzothiophene-2-carboxamide [^{18}F]**4a**, and the benzofuran [^{18}F]**4b**, the pyrazolo[1,5-a]pyridine [^{18}F]**4c**, and the naphthalene [^{18}F]**4d**, respectively, in high yield, high specific activity, and purity as potential radiotracers.

Results and Discussion

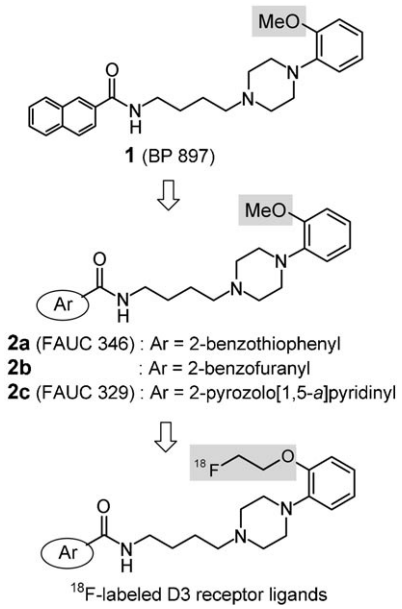
CoMFA and CoMSIA: Taking advantage of our recently described 3D-QSAR models,[31,32] the prediction of D3 selectivities over both congeners D2 and D4 was a crucial part of our strategy. Based on the affinity differences of a training set of 79 ligands, the putative selectivities of the test compounds **4a–d** were calculated. The data depicted in Table 1 indicate pK_i differences expressed as $-\log(K_i(D3)/K_i(D2_{Long}))$ and $-\log(K_i(D3)/K_i(D4))$ values. When the target compounds **4a–d** were predicted employing the CoMFA and CoMSIA models, respectively, the benzothiophene- and benzofuran-2-carboxamides **4a** and **4b** were suggested to have CoMFA/CoMSIA derived $D3/D2_{Long}$ differences of 2.47/2.16 and 2.03/2.03, respectively, and pK_i differences of 1.86/1.55 and 1.08/1.10 for D3 over D4. The pyrazolo[1,5-a]pyridine **4c** and the naphthylcarboxamide **4d** were supposed to give $D3/D2_{Long}$ selectivities of 1.68/1.38 and 1.87/1.91 and D3/D4 selectivities of 0.68/0.63 and 1.27/1.48, respectively. The underestimation of D3 selectivities over D4 might be due to the fact that D4 differs more from D3 than D2 and the predicted test compounds show a lower similarity to the test set that was used for the generation of the D4 based QSAR data. The predicted selectivities of the test compounds **4a–d** suggested a subtype-selective profile that is further improved or at least comparable with that of previously investigated fluoro-substituted aryl carboxamides.[30,32] According to these encouraging data, the synthesis of the calculated ligands was envisioned.

Table 1. Calculated and experimentally derived selectivity data.

	$D3/D2_{Long}$			D3/D4		
compd	CoMFA[a]	CoMSIA[a]	exp.[b]	CoMFA[a]	CoMSIA[a]	exp.[b]
4a	2.47	2.16	2.12	1.86	1.55	2.45
4b	2.03	2.03	1.91	1.08	1.10	2.27
4c	1.68	1.38	1.68	0.68	0.63	2.35
4d	1.87	1.91	2.05	1.27	1.48	2.31

[a] calculated as $-\log(K_i(D3)/K_i(D2_{Long}))$ and $-\log(K_i(D3)/K_i(D4))$, respectively. [b] see also Table 2.

Synthesis: Our initial investigations were directed to the preparation of the methoxyphenylpiperazines **2a–d** and subsequent exchange of the methyl unit by a fluoroethyl substituent (see Scheme 1). The synthesis of the desired fluoroethoxy substituted derivatives was accomplished starting from aminobutyl-(2-methoxyphenyl)piperazine. *N*-acylation with heteroarene carboxylic acid chlorides gave the respective carboxamides **2a–d**, which underwent demethylation among treatment with BBr_3. Following the procedure of Wilson et al.,[33] the obtained *N*-(hydroxyphenyl)piperazines **3a–d** were alkylated using fluoroethoxytosylate in presence of tetrabutylammonium hydroxide to give the fluoroethyl ethers **4a–d** in 50–60% yield (see Scheme 2.



Scheme 1. Development of D3 radioligands [^{18}F]**4a–d**.

Biological Investigation: The test compounds **4a–d** were evaluated for their affinities toward the cloned human dopamine receptor subtypes $D2_{Long}$, $D2_{short}$, D3, and D4.4 by determining their ability to displace [^{3}H]spiperone and also served as authentic reference compounds in analytical radio-HPLC to confirm chemical identity of [^{18}F]**4a–d**.[34–37] D1 receptor affinities were measured utilizing porcine striatal membranes and the D1 selective radioligand [^{3}H]SCH 23390.[37] Because of the observation that the lead compound FAUC 346 (**2a**) reveals serotonergic and adrenergic activity, **4a–d** were investigated for their potency to displace [^{3}H]WAY600135, [^{3}H]ketanserin, and

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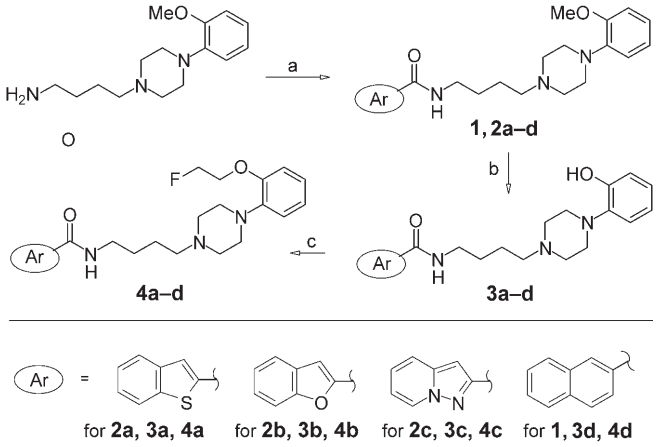


Scheme 2. Syntheses of labeling precursors **3a–d** and the target compounds **4a–d**. Reagents and conditions: a) Ar-COCl, Et_3N, CH_2Cl_2, RT, 16 h; b) BBr_3, CH_2Cl_2, −78 °C, 5 h; c) $TosOCH_2CH_2F$, NBu_4OH, DMF, RT, 16 h.

[^{3}H]prazosin when employing porcine 5-HT_{1A}, 5-HT_2, and α_1-receptors, respectively.[23] The K_i values were calculated on the basis of three competition binding experiments. For comparison, the reference compound FAUC 346 (**2a**) was investigated under the same conditions (Table 2). In fact, the fluoroethoxy substituted test compounds **4a–d** revealed K_i values in the subnanomolar range for the D3 receptor reflecting that the enhanced steric demand of the fluoroethoxy group was tolerated well by the D3 binding pocket. Table 2 displays also the excellent selectivity profiles over the dopamine receptor subtypes D2 and D4 when our QSAR derived predictions were even exceeded. Again, the benzothiophene **4a** showed the most exciting properties. High affinity differences to D1, HT_{1A}, and 5-HT_2 could also be observed. On the other hand, there is still space for improvement with regard to the D3 selectivities over α_1 (12-fold for **4a**). As a measure of functional activity, ligand efficacy of **4a–d** was determined by a mitogenesis assay measuring the rate of [^{3}H]thymidine incorporation into growing CHO dhfr$^-$ cells stably expressing the human D3 receptor. Table 3 clearly indicates substantial ligand efficacy (43–52%; EC_{50}: 2.3–2.8 nM) for all test compounds investigated which is comparable to the partial agonist activity of the reference ligands **1** (BP 897) and **2a** (FAUC 346). Predicted lipophilicities give an indication of brain uptake. The calculated values (log*P*) of the compounds **4a–d** lie between 3.93 and 4.63 (see Table 3). The ideal lipophilicity (log*P* octanol/water) of the radioligand should be between 2–2.5. Considering that the piperazine nitrogen is protonated at physiological pH, the calculated log*P* valules do not suggest increased nonspecific binding.

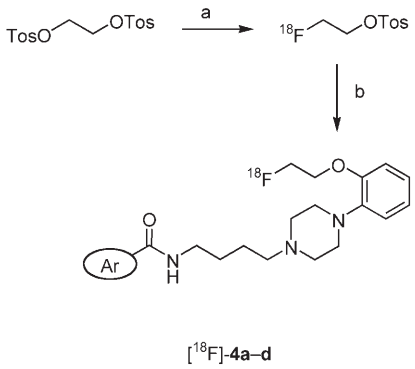
Table 3. Intrinsic activities of **4a–d** and of the reference compounds FAUC 346 and BP 897 derived from the D3 stimulating effect on mitogenesis.

compd	[^{3}H]thymidine uptake (mitogenesis) for D3 receptor[a] agonist effect [%][b]	EC_{50} [nM][c]
4a	51	2.8
4b	43	2.5
4c	50	2.6
4d	52	2.3
1 (BP 897)	38	2.7
2a (FAUC 346)	53	1.5

[a] Determined with CHO dhfr$^-$ mutant cells stably expressing the human D3 receptor. [b] Rate of incorporation of [^{3}H]thymidine as evidence for mitogenesis activity relative to the maximal effect of the full agonist quinpirole (=100%) used as a reference. [c] EC_{50} values derived from the mean of three for four independent experiments.

Radiosynthesis: The methodology for the radiosynthesis of the PET candidates **[^{18}F]4a–d** is shown in Scheme 3.

Based on the nucleophilic ^{18}F-for-OTos substitution on ethyleneglycol-1,2-bistosylate, 2-[^{18}F]fluoroethyltosylate was isolated by semipreparative reversed-phase HPLC followed by solid



Scheme 3. Two-step procedure for the radiosyntheses of [^{18}F]**4a–d**. a) [K⊂222]$^+$[^{18}F]F$^-$, ethyleneglycol-1,2-bistosylate, CH_3CN, 80 °C, 3 min; b) **3a–d**, NBu_4OH, DMF, 120 °C, 3 min.

Table 2. Receptor binding data[a] and selectivity ratios for **4a–d** in comparison to the reference compounds FAUC 346 employing human $D2_{Long}$, $D2_{short}$, D3, and D4.4 and porcine D1, $5HT_{1A}$, $5HT_2$ and α_1 receptors.

Compd	[^{3}H]spiperone $D2_{Long}$	$D2_{short}$	D3	D4.4	D3 selectivity $D2_{Long}$/D3	D4/D3	[^{3}H]SCH 23990 D1	[^{3}H]WAY 600135 5-HT_{1A}	[^{3}H]ketanserin 5-HT_2	[^{3}H]prazosin α_1
4a	16	14	0.12	34	133	283	550	15	710	1.4
4b	28	24	0.35	64	80	183	790	7.2	1600	2
4c	32	33	0.68	150	47	220	2000	6.6	1900	2.8
4d	18	14	0.16	33	113	206	350	12	1100	1.6
2a (FAUC346)[b]	87	52	0.23	15	380	65	670	41	350	15

[a] K_i values in nM are based on the means of 2–3 experiments each done in triplicate. [b] Ref. [23].

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phase extraction.[38,39] The reaction parameters under investigation for the following ^{18}F-fluoroethylation step, are listed in Table 4. Starting from isolated 2-[^{18}F]fluoroethyltosylate, the radiofluorinated compounds [^{18}F]**4a**–**d** were obtained in DMF at 120 °C using 10 μmol **3a**–**d** and 1.4 equivalents of $N(Bu)_4OH$ solution. Different reaction parameters were examined, such as temperature, the reaction solvent, base, and reaction time. The resulting radiochemical yields for the various compounds are also listed in Table 4. DMSO as alternative solvent and the bases (NaH and NaOMe) were examined, but the best results were obtained employing the above-mentioned reaction conditions. Using NaH and NaOMe as bases, no radiochemical yield of [^{18}F]**4a** could be detected.

Table 4. Radiochemical yields (RCY) for the ^{18}F-fluoroethylation of **3a**–**d** using 2-[^{18}F]fluoroethyltosylate, log*P*, and retention time (R_t) of **4a**–**d**.

product	solvent system	*T* [°C]	*t* [min]	RCY [%][a]	log*P*[b]	R_t[c]
[^{18}F]**4a**	DMSO/NaOMe	140	1–25	0	–	–
[^{18}F]**4a**	DMF/NaH	120	1–25	0	–	–
[^{18}F]**4a**	DMF/$N(Bu)_4OH$	120	3	65±3	4.67	5.14
[^{18}F]**4b**	DMF/$N(Bu)_4OH$	120	3	53±3	4.01	4.90
[^{18}F]**4c**	DMF/$N(Bu)_4OH$	120	3	24±7	3.93	4.79
[^{18}F]**4d**	DMF/$N(Bu)_4OH$	120	3	45±5	4.62	5.31

[a] determined by radio-HPLC (10.8 μmol precursor, $V=350$ μL, $n=2$-3). [b] Calculated value using the program Clog*P*. [c] HPLC retention time.

Using tetrabutylammonium hydroxide as base to generate the phenoxide of **3a**–**d** in DMF proved to be beneficial. Subsequent conversion of the 2-[^{18}F]fluoroethyltosylate within 3 min at 120 °C gave the final product [^{18}F]**4a**–**d** in 24–65% decay-corrected radiochemical yield. This optimization study for the syntheses of the ^{18}F-labeled radioligands [^{18}F]**4a**–**d** (DMF/$N(Bu)_4OH$, 120 °C, 3 min) provided evidence for the rapid and reliable accessibility of the predicted dopamine D3 ligands by a two-step ^{18}F-fluoroethylation procedure followed by HPLC.

Conclusions

Based on recently described 3D-QSAR models that are able to predict subtype selectivities of dopaminergic test compounds, the highly selective [^{18}F]labeled D3 receptor ligands **[^{18}F]4a**–**d** were synthesized by ^{18}F-fluoroalkylation of the hydroxyphenyl substituted precursors **3a**–**d**. Receptor binding experiments confirmed the computer-assisted molecular design revealing subnanomolar D3 affinities and excellent selectivity profiles.

Experimental Section

All reagents were purchased from commercial sources and were used without further purification. [^{18}F]fluoride was obtained from PET Net GmbH (Erlangen, Germany). Proton NMR spectra were recorded on a Bruker Avance 360 or Bruker Avance 600. Chemical shifts were recorded in ppm (δ) from an internal tetramethylsilane (TMS) standard in chloroform-d3, and coupling constants (*J*) are reported in Hz. Chromatographic purification of unlabeled compounds was performed with silica gel (Merck, 70–230 mesh) using the solvent systems indicated in the text. 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. For the isolation of [^{18}F]products, solid phase cartridges (Sep-PakPlus C18 cartridges) were used from Waters (Eschborn, Germany). The radioligands were purified and characterized by HPLC methods. The HPLC (Agilent 1100) system comprised the following equipment: a quaternary pump, variable wavelength detector, and radio-HPLC-detector D505TR (Canberra Packard). Computer analysis of the HPLC data was performed using FLO-One software (Canberra Packard). LC-MS analyses were performed on an Agilent 1100 Series analytic HPLC system with a VWL detector (254 nm) coupled to a Bruker esquire 2000 mass spectrometer with atmospheric pressure chemical ionization (APCI). A Zorbax SB-C8 (4.6 mm ID×250 mm, 5 μm) column was used with a flow rate of 0.5 mL min^{-1} (MeOH/0.1% aq. HCOOH, 10–100% MeOH). All ^{18}F-labeled compounds were identified by retention time (R_t) on the radio-HPLC system and coinjection of the corresponding reference compound.

General Procedure for the Amide Coupling. Triethylamine (2 equiv) and one of the corresponding carboxylic acid chlorides (1.1 equiv) was added to a solution of the aminobutyl-(2-methoxyphenyl)piperazine (1 equiv) in dry CH_2Cl_2 (15 mL) under argon atmosphere. The mixture was stirred at RT overnight. The precipitate was removed and the solution was washed with water (2×20 mL) and brine (20 mL). After separation, the organic layer was dried with Na_2SO_4 and concentrated under reduced pressure. Column chromatography (CH_2Cl_2/MeOH; 9:1) gave **2a**–**d** in very good chemical purity. Compounds **2a**–**d** were characterized by LC/MS, for NMR details of **2a**–**c** see ref. [23].

N-[4-[4-(2-Methoxyphenyl)-piperazin-1-yl]-butyl]-benzothiophene-2-carboxamide (2a): According to the general procedure for the amide coupling, 1-(2-methoxyphenyl-piperazinyl)butylamine (208 mg, 0.8 mmol), triethylamine (281 μL, 2 mmol), and benzothiophene-2-carboxylic acid chloride (197 mg, 1 mmol) were stirred in CH_2Cl_2 (15 mL). After 24 h, **2a** (217 mg, 64%) was obtained as a white solid: MS (EI) *m/z* 424.5 (M^+, 100).

N-[4-[4-(2-Methoxyphenyl)-piperazin-1-yl]-butyl]-benzofuran-2-carboxamide (2b): According to the general procedure for the amide coupling, 1-(2-methoxyphenyl-piperazinyl)butylamine (250 mg, 0.95 mmol), triethylamine (281 μL, 2 mmol), and benzofuran-2-carboxylic acid chloride (181 mg, 1 mmol) were stirred in CH_2Cl_2 (15 mL). After 24 h, **2b** (255 mg, 66%) was obtained as a white solid: MS (EI) *m/z* 408.5 (M^+, 100).

N-[4-[4-(2-Methoxyphenyl)-piperazin-1-yl]-butyl]-pyrazolopyridine-2-carboxamide (2c): According to the general procedure for the amid coupling, 1-(2-methoxyphenyl-piperazinyl)butylamine (263 mg, 1 mmol), triethylamine (281 μL, 2 mmol), and pyrazolo-[1,5-a]pyridinyl-2-carboxylic acid chloride (199 mg, 1.1 mmol) were stirred in CH_2Cl_2 (15 mL). After 24 h, **2c** (91.4 mg, 46%) was obtained as a white solid: MS (EI) *m/z* 408.5 (M^+, 100).

N-[4-[4-(2-Methoxyphenyl)-piperazin-1-yl]-butyl]-naphthalene-2-carboxamide (1): According to the general procedure for the amide coupling, 1-(2-methoxyphenyl-piperazinyl)butylamine (270 mg, 1.03 mmol), triethylamine (281 μL, 2 mmol), and naphthyl-2-carboxylic acid chloride (190,6 mg, 1 mmol) were stirred in CH_2Cl_2 (15 mL). After 24 h, **1** (305 mg, 73%) was obtained as a white solid: ^{1}H NMR (360 MHz, $CDCl_3$): $\delta=1.709$ (d, 4H), 2.52 (s, 2H), 2.692 (s, 4H), 3.043 (s, 4H), 3.50 (q, 2H), 3.773 (s, 3H), 6.74 (dd, 1H), 6.802 (m, 2H), 6.932 (dt, 1H), 7.012 (d, 1H), 7.474 (dtd, 2H), 7.811 (m, 3H), 7.859 (d, 1H), 8.264 ppm (s, 1H); MS (EI) *m/z* 418.5 (M^+, 100).

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General Procedure for the Dealkylation with BBr_3: Deprotection was accomplished by treatment of **1** and **2a**–c (1 equiv) with boron tribromide (4–5 equiv) in dry CH_2Cl_2 (15 mL) under argon atmosphere for 5 h at −78 °C. After reaction, 20 mL saturated Na_2CO_3 solution was used to neutralize the solution. The aqueous phase was extracted with ethyl acetate (3×25 mL) and the combined organic extracts were washed with water (3×10 mL) and saturated brine (10 mL) and dried over Na_2SO_4. The solvent was removed under vacuum to give a brown solid that was purified (CH_2Cl_2/MeOH; 9:1) to give each compound **3a**–**d** in moderate yield.

N-[4-[4-(2-Hydroxyphenyl)-piperazin-1-yl]-butyl]-benzothiophene-2-carboxamide (3a): According to the general procedure for the deprotection, **2a** (423.5 mg, 1 mmol) and BBr_3 (4 mL, 4 mmol) were stirred in 15 mL CH_2Cl_2 at −78 °C. After chromatography, **3a** (283 mg, 69%) was obtained as a white solid: ^{1}H NMR (360 MHz, ($CDCl_3$): δ = 1.69 (m, 4H), 2.49 (t, 2H), 2.64 (s, 4H), 2.90 (t, 4H), 3.51 (m, 2H), 6.62 (t, 1H), 6.82 (m, 1H), 6.93 (m, 1H), 7.06 (m, 2H), 7.40 (m, 2H), 7.83 ppm (m, 3H); MS (EI) *m/z* 410,2 (M^+, 100) 16.2 min.

N-[4-[4-(2-Hydroxyphenyl)-piperazin-1-yl]-butyl]-benzofuran-2-carboxamide (3b): According to the general procedure for the deprotection **2b** (407 mg, 1 mmol) and BBr_3 (1N) (4 mL, 4 mmol) were stirred in 15 mL CH_2Cl_2 at −78 °C. After chromatography **3b** (248 mg, 63%) was obtained as a white solid: ^{1}H NMR (360 MHz, ($CDCl_3$): δ = 1.68 (m, 4H), 2.49 (dd, 2H), 2.63 (s, 4H), 2.91 (m, 4H), 3.52 (m, 2H), 6.84 (m, 1H), 6.92 (m, 1H), 6.97 (dd, 1H), 7.05 (dt, 1H), 7.13 (dd, 1H), 7.27 (m, 1H), 7.38 (m, 1H), 7.38 (m, 1H), 7.45 (m, 2H), 7.65 ppm (dd, 1H); MS (EI) *m/z* 394.3 (M^+, 100) 14.8 min.

N-[4-[4-(2-Hydroxyphenyl)-piperazin-1-yl]-butyl]-pyrazolo[1,5-a]pyridine-2-carboxamide (3c): According to the general procedure for the deprotection, **2c** (136 mg, 0.33 mmol) and BBr_3 (1N) (2 mL, 2 mmol) were stirred in 12 mL CH_2Cl_2 at −78 °C. After chromatography **5c** (100 mg, 77%) was obtained as a white solid: ^{1}H NMR (360 MHz, ($CDCl_3$): δ = 1.67 (m, 4H), 2.47 (dd, 2H), 2.67 (s, 4H), 3.11 (m, 4H), 3.50 (m, 2H), 6.89 (m, 5H), 7.04 (m, 1H), 7.11 (m, 1H), 7.28 (m, 1H), 7.56 (m, 1H), 8.34 ppm (m, 1H); MS (EI) *m/z* 394.2 (M^+, 100) 13.7 min.

N-[4-[4-(2-Hydroxyphenyl)-piperazin-1-yl]-butyl]-naphthyl-2-carboxamide (3d): According to the general procedure for the deprotection **1** (160 mg, 0.38 mmol) and BBr_3 (1 N) (1.5 mL, 1.5 mmol) were stirred in 12 mL CH_2Cl_2 at −78 °C. After chromatography **3d** (110 mg, 71%) was obtained as a white solid: ^{1}H NMR (360 MHz, ($CDCl_3$): δ = 1.68 (m, 4H), 2.45 (dd, 2H), 2.59 (m, 2H), 2.63 (m, 2H), 2.8 (t, 2H), 3.01 (m, 2H), 3.51 (m, 2H), 6.75 (m, 1H), 6.91 (m, 4H), 7.5 (m, 2H), 7.84 (m, 4H), 8.25 ppm (s, 1H); MS (EI) *m/z* 404.4 (M^+, 100) 15.2 min.

General Procedure for alkylation with 2-fluoroethyltosylate. A solution of the hydroxyl precursor **3a**–**d** (1 equiv), 2-fluoroethyltosylate (1.5 equiv) and tetrabutylammonium hydroxide solution (1.4 equiv, 1.4 N in methanol) in dry DMF was stirred at RT overnight. The solution was quenched with aqueous NaOH (0.05 N; 10 mL) and extracted with ethyl acetate (2× 15 mL). After separation, the organic layer dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (CH_2Cl_2/MeOH; 9:1) gave **4a**–**d** in very good chemical purity.

N-[4-[4-(2-Fluoroethoxyphenyl)-piperazin-1-yl]-butyl]-benzothiophene-2-carboxamide (4a): According to the general procedure, **3a** (70 mg, 0.17 mmol), 2-fluoroethyl tosylate (56.0 mg, 0.263 mmol) tetrabutylammonium hydroxide solution (0.17 mL, 0.24 mmol, 1.4 N in methanol) in dry DMF (5 mL) was stirred at RT over night. **4a** was obtained as a white solid (59 mg, 76%): R_t = 5.14 min., ^{1}H NMR (360 MHz, ($CDCl_3$): δ = 1.71 (m, 4H), 2.51 (t, 2H), 2.7 (s, 4H), 3.11 (t, 4H), 3.5 (q, 2H), 4.24 (d, 2H), 4.77 (d, 2H), 6.86 (m, 2H), 6.95 (m, 3H), 6.93 (m, 1H), 7.39 (m, 2H), 7.83 ppm (m, 3H); MS (EI) *m/z* 456.6 (M^+, 100) 16.4 min.

N-[4-[4-(2-Fluoroethoxyphenyl)-piperazin-1-yl]-butyl]-benzofuran-2-carboxamide (4b): According to the general procedure, **3b** (120 mg, 0.3 mmol), 2-fluoroethyltosylate (96 mg, 0.45 mmol) tetrabutylammonium hydroxide solution (0.29 mL, 0.41 mmol, 1.4N in methanol) in dry DMF (5 mL) was stirred at RT over night. **4b** was obtained as a white solid (69 mg, 52%): R_t = 4.90 min., ^{1}H NMR (360 MHz, ($CDCl_3$): δ = 1.72 (m, 4H), 2.53 (t, 2H), 2.73 (s, 4H), 3.19 (s, 4H), 3.53 (q, 2H), 4.26 (d, 2H), 4.78 (d, 2H), 6.86 (m, 1H), 6.95 (m, 3H), 7.08 (m, 1H), 7.28 (m, 2H), 7.39 (m, 1H), 7.48 (m, 1H), 7.67 ppm (m, 1H); MS (EI) *m/z* 440.5 (M^+, 100) 16.1 min.

N-[4-[4-(2-Fluoroethoxyphenyl)-piperazin-1-yl]-butyl]-pyrazolo-[1,5-a]pyridin-2-carboxamide (4c): According to the general procedure, **3c** (75 mg, 0.19 mmol), 2-fluoroethyltosylate (61 mg, 0.285 mmol) tetrabutylammonium hydroxide solution (0.19 mL, 0.265 mmol, 1.4N in methanol) in dry DMF (5 mL) was stirred at RT over night. **4c** was obtained as a white solid (27 mg, 32%): R_t = 4.79 min., ^{1}H NMR (360 MHz, ($CDCl_3$): δ = 1.72 (m, 4H), 2.51 (s, 2H), 2.71 (s, 4H), 3.18 (s, 4H), 3.54 (q, 2H), 4.26 (m, 2H), 4.77 (m, 2H), 6.86 (m, 2H), 6.96 (m, 3H), 7.06 (s, 1H), 7.14 (ddd, 1H), 7.31 (m, 1H), 7.59 (d, 1H), 8.37 ppm (dd, 1H); MS (EI) *m/z* 440.5 (M^+, 100) 15.0 min.

N-[4-[4-(2-Fluoroethoxyphenyl)-piperazin-1-yl]-butyl]-naphthalene-2-carboxamide (4d): According to the general procedure, **3d** (90 mg, 0.22 mmol), 2-fluoroethyltosylate (71 mg, 0.33 mmol) tetrabutylammonium hydroxide solution (0.22 mL, 0.31 mmol, 1.4N in methanol) in dry DMF (5 mL) was stirred at RT over night. **4d** was obtained as a white solid (67.2 mg, 67%): R_t = 5.31 min., ^{1}H NMR (360 MHz, ($CDCl_3$): δ = 1.74 (m, 4H), 2.53 (t, 2H), 2.66 (d, 4H), 3.1 (s, 4H), 3.55 (q, 2H), 4.23 (m, 2H), 4.75 (m, 2H), 6.81 (m, 2H), 6.94 (m, 2H), 7.04 (s, 1H), 7.53 (m, 2H), 7.86 (m, 4H) 8.29 ppm (s, 1H); MS (EI) *m/z* 450.6 (M^+, 100) 15.9 min.

Radiochemistry: [^{18}F]4a–d were prepared by a two-step reaction that consisted of ^{18}F-fluorination of ethyleneglycol-1,2-bistosylate and subsequent ^{18}F-fluoroethylation of 2-hydroxy substituted compounds **3a**–**d** (Scheme 3). These precursors were prepared for the substitution by the addition of $N(Bu)_4OH$ solution (14 μL, 0.1 N) in 250 μL DMF as solvent. Quality control analysis by analytical HPLC methods (C-18 Phenomenex Prodigy; mobile phase: 0.1 M ammonium acetate buffer/acetonitrile, 25:75 (*v/v*); flow rate = 1 mL min^{-1}; retention time (R_t) = (4.79–5.31 min) revealed that the radiolabeled product co-eluted with a fully characterized **4a**–**d** standard.

2-[^{18}F]fluoroethyltosylate: Following the procedure of Block et al.,[38] [^{18}F]fluoride (400–650 MBq) was eluted from a QMA cartridge with Kryptofix 2.2.2 (15 mg) and potassium carbonate solution (1 M, 15 μL) in 1 mL acetonitrile/water (8:2, *v/v*) into a 5 mL reaction vial. After evaporation under an argon stream, 0.5 mL acetonitrile was supplied to the reactor. After repeated evaporation, 4.5 mg (12 μmol) ethyleneglycol-1,2-bistosylate in 250 μL acetonitrile was added and the resulting mixture was heated at 80 °C for 3 min. The reaction vial was cooled and the synthesized 2-[^{18}F]fluoroethyltosylate was transferred into another vial. This solution was diluted with water and the product was isolated by reversed-phase HPLC (Lichrosorb RP18, 125×8 mm, 4 mL min^{-1}, CH_3CN/H_2O (40/60) (0.1% TFA)). The 2-[^{18}F]fluoroethyltosylate fraction was diluted with water (1:10) and fixed on a C18-cartridge (Waters Sep-PakPlus). After drying in a nitrogen stream, 2-[^{18}F]fluoroethyltosylate eluted with DMF (1 mL) from the cartridge. The ^{18}F-fluoroethylation of **3a** was optimized by repeating the reaction with varying parameters as indicated in Table 4. The optimized conditions were also used for the radiofluorination of **3b**–**d**.

N-[4-[4-(2-Fluoroethylphenyl)-piperazin-1-yl]-butyl]-benzothiophene-2-carboxamide ([^{18}F]4a): **3a** (4.1 mg 10 μmol) was dis-

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solved in dry DMF (230 μL). Tetrabutylammonium hydroxide (10 μL 1.4 N) in dry MeOH was added to the reaction vial. The reaction mixture was stirred for 3 min at 120 °C under a nitrogen atmosphere. Subsequently, 2-[^{18}F]fluoroethyltosylate solution (50 μL) in dry DMF (10–40 MBq) were added to the reaction mixture. The radiochemical yield of **[^{18}F]4a** was 65 ± 3% after 3 min at a reaction temperature of 120 °C in the solvent system DMF/N(Bu)$_4$OH. The radioligands were proved by the comparison of the retention times to the F-19 compounds **4a–d** by means of HPLC (see above).

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