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Synthesis and radioiodination of selective ligands for the dopamine D3 receptor subtype

Carsten Hocke,[a,*] Olaf Prante,[a] Stefan Löber,[b] Harald Hübner,[b] Peter Gmeiner[b] and Torsten Kuwert[a]

[a]*Department of Nuclear Medicine, Krankenhausstrasse 12, D-91054 Erlangen, Germany*
[b]*Department of Medicinal Chemistry, Emil Fischer Center, Schuhstrasse 19, D-91052 Erlangen, Friedrich-Alexander University, Germany*

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Abstract—Starting from FAUC 365, a series of iodine substituted heteroaryl carboxamides has been synthesized revealing high affinity and selectivity for the dopamine D3 receptor. Binding data showed a 15–560-fold selectivity for the dopamine D3 over D2. A 2,3-dichloro substitution pattern on the phenylpiperazine moiety led to the highest subtype selectivity, whereas the 2-methoxy substituted compounds showed superior D3 affinity. Suitable precursors were radioiodinated with high radiochemical yields (53–85%) leading to potential imaging agents for the D3 receptor by SPET.

Since the dopamine D3 receptor was identified by Sokoloff et al.[1] great progress in the validation of this putative drug target has been made. As a result of these investigations, a couple of disorders are believed to be associated with the D3 receptor signaling pathway, including schizophrenia, Parkinson's disease and cocaine craving. Very recently, BP 897 (**1**), a preferential D3 receptor partial agonist (Fig. 1), was found to inhibit cocaine-seeking behavior without revealing any intrinsic, primary rewarding effect.[2] Based on these findings, we adopted an interactive drug discovery process leading to the super-potent benzothiophene and benzofuran derivatives FAUC 346 (**3**), FAUC 365 (**4**) and **5**, **6**, respectively, and to the pyrazolo[1,5-*a*]pyridine analog

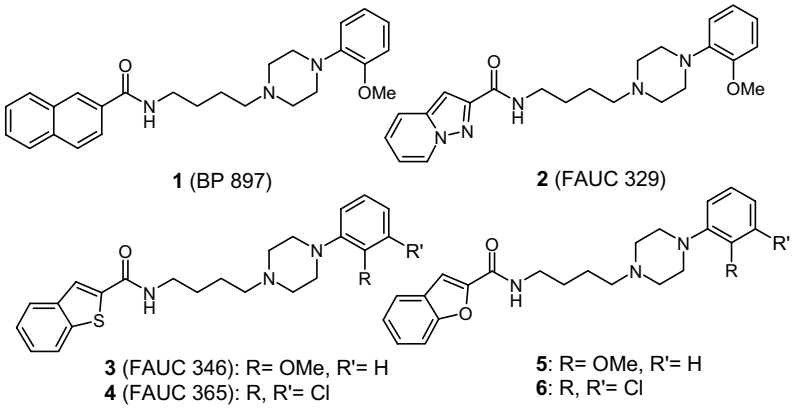

Figure 1. Potent dopamine D3 receptor ligands.

Keywords: Dopamine; D3 receptor; Radioligand; SPET.
* Corresponding author. Tel.: +49-9131-8533699; fax: +49-9131-8539262; e-mail: carsten.hocke@nuklear.imed.uni-erlangen.de

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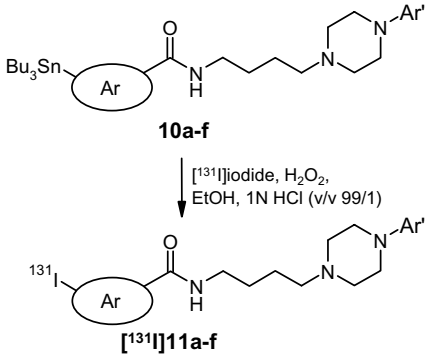
FAUC 329 (**2**),[3] which proved to be protective against MPTP-induced dopamine depletion.[4] Aspiring to utilize these type of potent dopamine D3 receptor ligands for noninvasive drug target imaging procedures, we aimed for the development of radiolabeled piperazinylbutyl substituted heteroaryl carboxamides. Although, several radioligands are at present available to evaluate the D2 receptor mediated neurotransmitter system by single-photon emission tomography (SPET), there is still need for the development of suitable radiopharmaceuticals to investigate the CNS located dopamine D3 receptors in vivo.

In view of the high selectivity of the benzothiophene and benzofuran derivatives **3**–**6**, these ligands served as lead compounds for the development of radioligands. In analogy to radiolabeling procedures previously developed in our laboratory for the synthesis of potential D4 receptor tracers using iodine-131,[5] we aimed for the synthesis and biological in vitro evaluation of new ^{131}I-substituted derivatives of **3**–**6** to evaluate their suitability for application as SPET ligands.

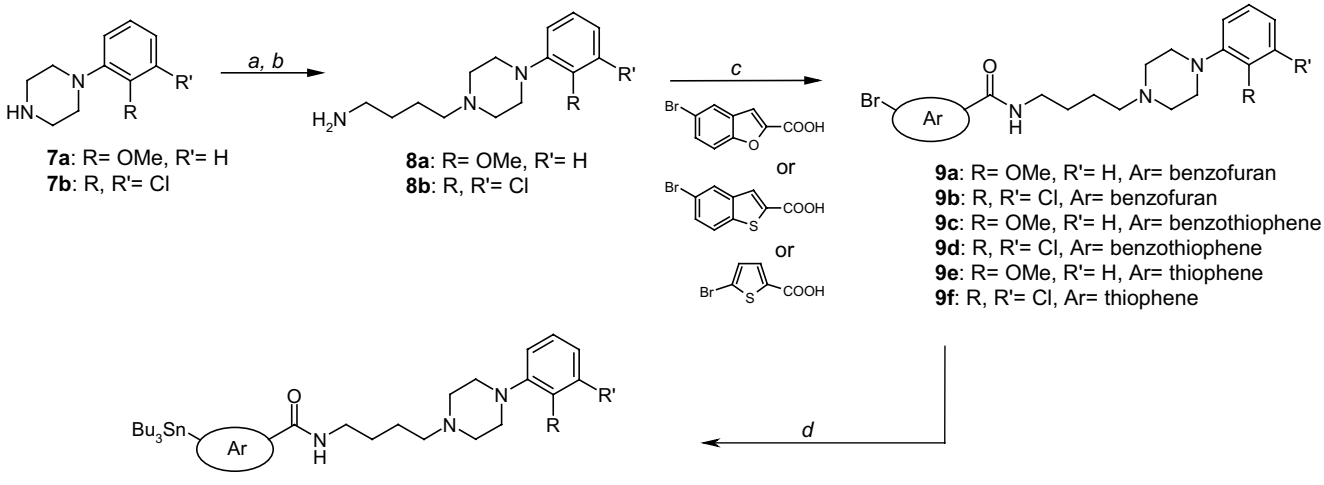
For the synthesis of SPET tracers, aryl stannanes are known as very powerful precursors, allowing regiospecific radioiodination and, thus, giving access to the corresponding iodo standards. For the synthesis of these organo tin derivatives, we started from the commercially available phenylpiperazines **7a**,**b**. Alkylation with 4-bromobutyronitrile and subsequent reduction with $LiAlH_4$ in THF afforded the primary amines **8a**,**b** in more than 60% over-all yield (Scheme 1). Amide bond formation with either 5-bromothiophene carboxylic acid, 5-bromobenzothiophene-2-carboxylic acid[6] or 5-bromobenzofuran-2-carboxylic acid[7] by thionyl chloride induced activation in CH_2Cl_2 and addition of the primary amine in the presence of triethylamine afforded the bromo substituted amides **9a**–**f** in 74–87% yield. Stannyl precursors were prepared from **9a**–**f** by the use of Pd(0) as the catalyst and hexabutyldistannane via tin-for-bromo exchange reaction.[8] The resulting tributylstannyl derivatives **10a**–**f** were readily reacted with 1 M iodine solution in tetrahydrofuran at room temperature to give the iodo derivatives **11a**–**f** in yields of 35–71%.[9,10] These compounds were subjected to receptor binding studies as described below.

Starting from the aryl stannanes **10a**–**f**, radioiodinations were achieved by iododestannylation reactions using hydrogen peroxide as the oxidant giving access to the desired radioiodinated ligands [^{131}I]**11a**–**f**[11] (Scheme 2) in high radiochemical yields (RCY) (53–87%) and with greater than 95% radiochemical purities.[12]

In Table 1, the resulting radiochemical yields were shown for the various compounds. Alternative solvent systems (methanol/acetic acid or phosphate buffer systems) and oxidants (chloramine-T) were examined, but best results were obtained employing the reaction con-

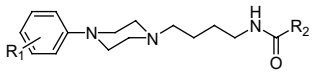


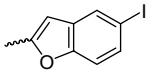
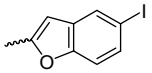
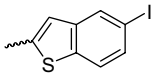
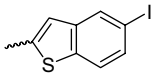
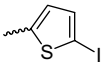
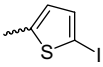
Scheme 2. Radiosynthesis of [^{131}I]**11a**–**f** starting from the stannyl precursors **10a**–**f**.



Scheme 1. Reagents and conditions: (a) 4-bromobutylnitrile, DMF, 100 °C, 5 h, (85–90%); (b) $LiAlH_4$, THF, 0 °C–reflux, 5 h (80%); (c) $SOCl_2$, CH_2Cl_2, NEt_3, rt (74–87%); (d) hexabutyldistannane, $(Ph_3P_4)Pd$, toluene, rt–reflux, 16 h (12–41%).

Table 1. Radiochemical yields [%] of radioiodinated compound [^{131}I]**11a–f** (100 μL EtOH/1 N HCl (99/1), 10 μL H_2O_2 (30%), nca [^{131}I] iodide ca. 2 MBq, $t = 2$ min)



Compound	R_1	R_2	RCY [%]
[^{131}I]**11a**	2-MeO		56 ± 4
[^{131}I]**11b**	2,3-Dichloro		58 ± 3
[^{131}I]**11c**	2-MeO		53 ± 5
[^{131}I]**11d**	2,3-Dichloro		55 ± 7
[^{131}I]**11e**	2-MeO		83 ± 3
[^{131}I]**11f**	2,3-Dichloro		87 ± 3

ditions described above. The RCYs of radioiodinated benzofuranes and benzothiophenes ([^{131}I]**11a–d**) were about 55% when significant differences in the radiochemical yields between both bicyclic systems could not be observed. Radioiodination of the thiophene derivatives [^{131}I]**11e–f** resulted in RCYs of over 80%. The RCYs were not influenced by the 2-methoxy or 2,3-dichloro substitution of the phenylpiperazine. All radiolabeled compounds ([^{131}I]**11a–f**) could be obtained in sufficient high yields to investigate the tracer behavior in vivo and in vitro for further studies.

Radioligand binding assays were employed to investigate the affinity and selectivity of the target compounds **11a–f** to the different subtypes of dopamine receptors. Binding data were based on the displacement of the radioligand [^{3}H] spiperone from the cloned human dopamine receptors $D2_{long}$, $D2_{short}$,[13] D3,[14] and D4.4[15] all stably expressed in Chinese hamster ovary cells (CHO).[16] D1 receptor affinities were determined utilizing porcine striatal membranes and the D1 selective radioligand [^{3}H]SCH 23390.[16] Affinities to the serotonin receptors $5HT_{1A}$, $5HT_2$, and the adrenergic α1 receptor were evaluated utilizing [^{3}H]8-OH-DPAT, [^{3}H]ketanserin and [^{3}H]prazosin and porcine cortical membranes, respectively.

The resulting K_i values are listed in Table 2 compared to the D3 selective lead compound FAUC 365. Most compounds (**11a**,**c**,**e**, and **11f**) showed high affinities in the low nanomolar range to the different dopamine receptor subtypes preferentially recognizing the D3 receptor with K_i values of 1.4, 0.86, 2.4, and 1.4 nM, respectively. The 2,3-dichlorophenylpiperazinyl substituted derivatives **11b** and **11d** exhibited substantial D3 selectivity when binding only in high nanomolar or micromolar concentrations to $D2_{long}$, $D2_{short}$ and D4 but showing D3 affinities of 5.7 and 4.5 nM, respectively. The dichlorophenylpiperazine moiety induced clear selectivity for the D3 receptor especially emphasized when combined to a spatially demanding heteroaryl moiety as shown for **11b** and **11d**, which are characterized by a D3 selectivity of 560- and 100-fold over D2 and 390- and 120-fold over D4.

With respect to the serotonin receptor binding, the K_i values of 10–130 nM indicate a preferred binding to the $5\text{-}HT_{1A}$ subtype compared to the K_i values of 210–790 nM for $5\text{-}HT_2$.

Whereas serotonergic binding is less affected by the different substitution pattern of the phenylpiperazine moiety, the affinity to the adrenergic α1 receptor is clearly influenced by these residues resulting in K_i values of 5.9, 5.8, and 1.7 nM for the 2-methoxyphenylpiperazinyl substituted compounds **11a**,**c**, and **11e** and 300, 49, and 42 nM for the appropriate 2,3-dichloro substituted derivatives, respectively.

Comparing the binding profile of the test compounds **11a–f** to that of the lead FAUC 365, incorporation of a bulky iodine substituent in position 5 of the heteroaryl moiety decreases affinity and selectivity to the dopamine D3 receptor. The benzofuran derivative **11b** displayed promising binding data justifying an application as a radioligand for further SPET imaging studies.

Table 2. Receptor binding data (K_i values [nM] based on the for the human $D2_{long}$, $D2_{short}$, D3, and D4 and the bovine D1 receptors in correlation to the reference compound **4** (FAUC 365) (2–4 experiments each performed in triplicate)

Compound	K_i values (nM)								
	[^{3}H]spiperone					[^{3}H]SCH 23990	[^{3}H]8-OH-DPAT	[^{3}H]ketanserin	[^{3}H]prazosin
	$D2_{long}$	$D2_{short}$	D3	D4.4	$D2_l$/D3–$D2_s$/D3–D4/D3	D1	$5\text{-}HT_{1A}$	$5\text{-}HT_2$	α1
11a	59	27	1.4	46	42–19–33	420	33	230	5.9
11b	3200	430	5.7	2200	560–75–390	3300	130	790	300
11c	48	15	0.86	42	56–17–49	520	27	250	5.8
11d	450	140	4.5	550	100–31–120	1400	46	290	49
11e	75	35	2.4	31	31–15–13	600	10	690	1.7
11f	89	32	1.4	130	64–23–93	820	24	210	42
4	3600	2600	0.50	340	7200–5200–680	8800	360	3000	370

In conclusion, highly selective dopamine D3 receptor radioligands were synthesized using FAUC 365 as lead compound. Using radiodestannylation reactions the obtained radiochemical yields of [^{131}I]**11a**–**f** were sufficiently high. These compounds are potential radioligands for imaging of the D3 receptor by SPET. Especially, the selectivity and affinity of [^{131}I]**11b** for the D3 receptor stimulate further studies to determine its biodistribution and metabolic stability in vivo.

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9. To a solution of **10a**–**f** (0.19 mmol) in THF (5 mL) was added a solution of iodine in THF (0.5 mL, 1 M) at room temperature. The mixture was stirred at room temperature over night. After addition of aqueous $NaHSO_3$ solution, the mixture was stirred for 5 min and the organic layer was separated. The organic phase was dried (Na_2SO_4), filtered, and concentrated to give **11a**–**f** (12–45% yield).
10. ^{1}H NMR ($CDCl_3$, 360 MHz) **11a**: δ (ppm): 1.7 (4H), 2.5 (2H), 2.7 (4H), 3.15 (4H), 3.55 (2H), 3.85 (3H), 6.8–7.0 (4H), 7.1 (1H), 7.25 (2H), 7.7 (1H), 8.05 (1H). Compound **11b**: δ (ppm): 1.7 (4H), 2.5 (2H), 2.65 (4H), 3.1 (4H), 3.55 (2H), 6.95 (1H), 7.0 (1H), 7.15 (2H), 7.25 (1H), 7.5 (1H), 7.65 (1H), 8.0 (1H). Compound **11c**: δ (ppm): 1.65 (4H), 2.45 (2H), 2.6 (4H), 3.05 (4H), 3.5 (2H), 3.65 (3H), 6.75–6.95 (4H), 7.0 (1H), 7.55 (1H), 7.65 (2H) 8.15 (1H). Compound **11d**: δ (ppm): 1.65 (4H), 2.45 (2H), 2.6 (4H), 3.0 (4H), 3.5 (2H), 6.65 (2H), 7.1 (1H), 7.15 (1H), 7.5 (1H), 7.65 (2H), 8.15 (1H). Compound **11e**: δ (ppm): 1.5 (4H), 2.3 (2H), 2.9 (4H), 3.2 (2H), 3.45 (4H), 3.75 (3H), 6.8–7.0 (4H), 7.4 (2H), 8.5 (1H). Compound **11f** δ (ppm): 1.5 (4H), 2.35 (2H), 2.95 (4H), 3.2 (2H), 3.45 (4H), 7.1 (1H), 7.25 (2H), 7.35 (1H), 7.45 (1H), 8.55 (1H).
11. $Na^{131}I$, code no. IBSSO, was obtained from Amersham Buchler (Braunschweig, Germany). Typical radiolabeling procedures were as follows: To the stannyl precursor (150 nmol) dissolved in 100 μL ethanol/HCl (v/v; 99/1), no carrier added (nca) [^{131}I]iodide (typically 2 MBq) and 10 μL hydrogen peroxide solution (30%) as oxidant at room temperature were added. After 2 min maximal RCY were observed to be 53–89% (radio-HPLC and radio-TLC) of substituted product, exclusively.
12. The radioiodinated compounds [^{131}I]**11a**–**f** were identified by reversed-phase chromatography (RP 18 Select B5 column (250 × 4 mm) eluted with methanol/aqueous buffer (70/30 v/v, 0.1 N ammonium formiate buffer, pH 6.8) and a flow rate of 1 mL/min). Analytical HPLC was performed on the following system: HPLC Hewlett Packard (HP 1100) with a quarternary pump and variable wavelength detector (HP 1100) connected to a radio-HPLC detector D505TR (Canberra Packard). Computer analysis of the HPLC data was performed using FLO-One software (Canberra Packard). Electronic autoradiography (Instant-Imager™, Canberra Packard) was used to analyse radio-TLC data.
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