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^{18}F-Labeled sufentanil for PET-imaging of μ-opioid receptors

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Abstract—The synthesis of an ^{18}F-labeled sufentanil analogue with apparent high μ-opioid receptor selectivity is reported. Intravenous injection of *N*-[4-(methoxymethyl)-1-[2-(2-thienyl)ethyl]-4-piperidinyl]-*N*-phenyl-2-(±)-[^{18}F]fluoropropan-amide in mice resulted in high brain uptake and a regional brain activity distribution corresponding to the μ-opioid receptor expression pattern. The developed ligand is a promising tracer for extended protocols in μ-opioid receptor mapping and quantitation with positron emission tomography.

[^{11}C]Carfentanil ([^{11}C]caf) has been used for positron emission tomography (PET) imaging of μ-opioid receptors (μOR) in studies addressing pain,[1,2] addiction,[3–6] affective disorders,[7] as well as epilepsy.[8,9] [^{11}C]caf is a demanding radiotracer since its inherent agonistic activity requires labeling at very high specific activities for safety reasons. Radiotracers based on ^{11}C ($t_{1/2}$ = 20.3 min) are suitable only for short lasting imaging protocols ($\lesssim$1 h after a bolus injection), but can be injected repeatedly in the same day in the same subject. In longer imaging protocols, the low activity at the end of the scanning procedure leads to low sensitivity for small signal changes, as encountered for instance during ligand-displacement studies. For these applications bolus + infusion protocols are used which increases the concentration of pharmacological active carfentanil. This imposes less safety of the application which is of particular concern for human studies. Compared to ^{11}C, an ^{18}F-labeled ($t_{1/2}$ = 109.7 min) μ-selective ligand it can improve signal intensity and thus potentially allow in vivo competition studies by using a single, bolus injection protocol. Moreover, the ^{18}F-label may make such a tracer available for PET satellite systems. Furthermore, a ^{18}F-labeled μOR ligand would be less limited than [^{11}C]caf by a long time interval for reaching peak equilibrium, and would also make it easier to reproduce the applied specific activity for specific imaging protocols, for example, in small animals.[10]

Initial studies of ^{18}F-labeled analogues of carfentanil [^{18}F]**1** and [^{18}F]**2** (Fig. 1) showed a high initial brain activity uptake in mice, but the applicability of the compounds was hampered by an unsuitable metabolite profile.[11] Removing the 4-carboxymethyl group and shifting the position of the ^{18}F-label to the 2-position of the propionyl group of the *N*-phenyl-amide (Fig. 1) yielded compound [^{18}F]**3** ([^{18}F]fpr-fen), which displayed high brain uptake after iv injection in rodents (Table 1). Extraction and HPLC analysis of the radioactivity in brain after injection of [^{18}F]**3** revealed that >91% of the activity was the intact compound, thus indicating a high metabolic stability of the compound in brain and a low brain uptake of peripherally generated metabolites (unpublished data). However, a low binding selectivity for the μOR was observed in in vitro binding studies using rat brain sections (unpublished data). A study of the biological properties of a series of [^{18}F]fluorinated 4-anilidopiperidines will be published separately. Here we report the results of structure-affinity and brain uptake studies demonstrating that *N*-[4-(methoxymethyl)-1-[2-(2-thienyl)ethyl]-4-piperidinyl]-*N*-phenyl-2-(±)-[^{18}F]fluoropropan-amide ([^{18}F]**4**; [^{18}F]fpr-suf) has more promising characteristics than

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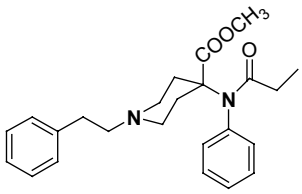
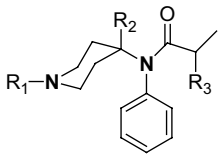


Carfentanil



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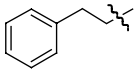
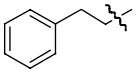
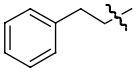
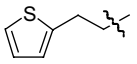
1		CO_2CH_2F	H
2		$CO_2CH_2CH_2F$	H
3		H	F
4		CH_2OCH_3	F

Figure 1. Structural representation of carfentanil and fluorinated 4-anilidopiperidines.

Table 1. Comparison of the in vitro and in vivo properties of carfentanil and fluorinated 4-anilidopiperidine analogues

	Compound		
	[^{11}C]caf	[^{18}F]fpr-fen ([^{18}F]**3**)	[^{18}F]fpr-suf ([^{18}F]**4**)
log $P_{(oct/PBS)}$	3.42	2.90	3.32
K_i (nM)	0.024[a]	2.1[17]	0.1[17]
5 minutes mouse brain uptake[b]	3.8 ± 0.4	3.9 ± 0.6	5.3 ± 0.8
30 minutes mouse brain uptake[b]	2.2 ± 0.6	1.9 ± 0.4	2.7 ± 0.6
Striatum/cerebellum ratio 5 min p.i.[c]	1.7 ± 0.3	1.4 ± 0.3	1.9 ± 0.5
Striatum/cerebellum ratio 20 min p.i.[c]	2.7 ± 0.4	1.7 ± 0.2	2.5 ± 0.3

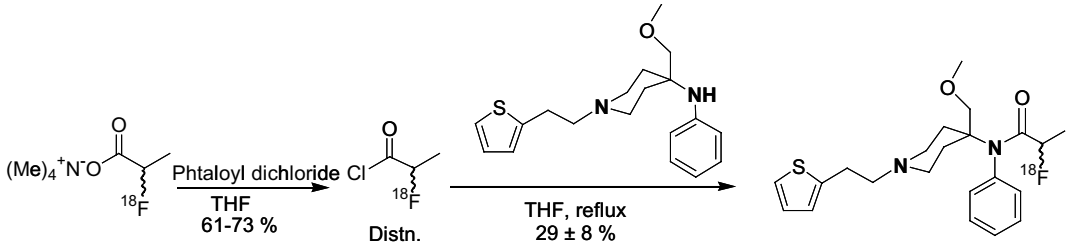
[a] Literature value,[12] added for reference.
[b] Male Balb-C mice, values represent mean ± SD (n = 3–4).
[c] Values represent mean ± SD (n = 3).

previously reported compounds. A comparison of the regional brain uptake kinetics to that of [^{11}C]caf[13,14] is given.

The synthesis of **4**[15] and [^{18}F]**4**[16] was carried out starting from des-propionyl sufentanil. The radiosynthesis of [^{18}F]**4** (Scheme 1) was achieved by reaction of no-carrier-added (n.c.a.) (±)-2-[^{18}F]fluoropropionic acid chloride, formed in situ from treatment of the corresponding acid with phthaloyl chloride and subsequent acylation of the des-propionyl precursor of sufentanil at elevated temperature.[16] Although not optimized, the syntheses yielded [^{18}F]**4** in an overall decay corrected yield averaging 19%, an average specific activity of 40 GBq/μmol, and a total syntheses time of ~110 min. Work towards a simplified and automated syntheses of [^{18}F]**4** is in progress.

Determination of the lipophilicity was performed by measuring octanol–water partition coefficients (P) at pH 7.4 (PBS) (log P in Table 1). Although the log P for [^{18}F]**4** is somewhat lower than that of [^{11}C]caf, the overall brain uptake of [^{18}F]**4** in male Balb-C mice is superior to that of [^{11}C]caf.

In separate experiments, measurement of the regional brain distribution of [^{18}F]**4** in vivo[21] showed that activity accumulation was high in cortex, striatum, and



Scheme 1. Procedure used for radiosynthesis of [^{18}F]fpr-suf ([^{18}F]**4**).

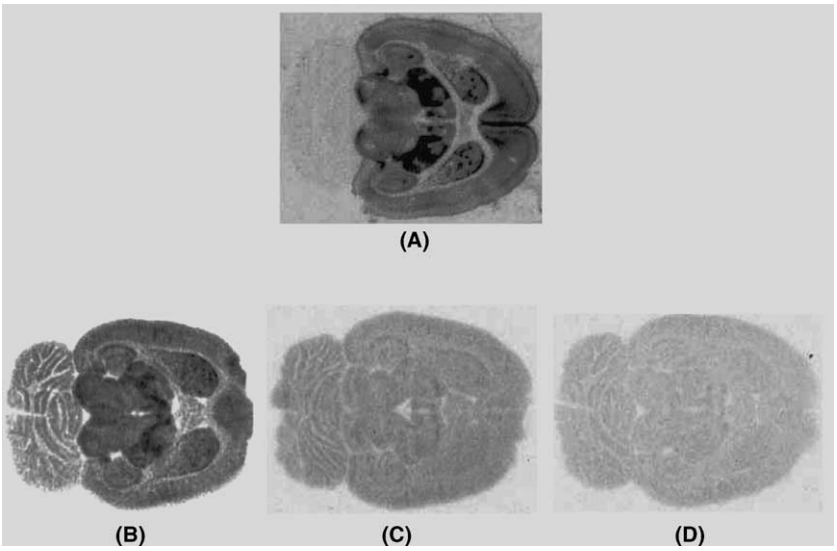


Figure 2. Autoradiography of [^{18}F]fpr-suf ([^{18}F]**4**) binding to rat brain sections under naive and blocking conditions (B–D) compared to [^{3}H][D-Ala^{2}, *N*Me-Phe^{4}, Gly-ol^{5}]-enkephalin. (A) ([^{3}H]DAMGO; log $P_{oct/PBS}$ = 0.5[24]; literature[20] values for opioid receptor affinity $K_i(\mu)$ = 2.0 nM, $K_i(\delta)$ > 1 μM, $K_i(\kappa)$ > 1 μM). (B) [^{18}F]fpr-suf alone. (C) [^{18}F]fpr-suf in the presence of 10 μM naloxone. (D) [^{18}F]fpr-suf in the presence of 1.5 μM sufentanil.

thalamus and low in the cerebellum. This regional variation in the distribution is in accordance with the known regional μOR density. The binding ratio striatum/cerebellum at 5 min and 30 min after injection shows comparable specificity of [^{18}F]**4** and [^{11}C]caf at both time points (Table 1). However, due to the higher overall uptake of [^{18}F]**4**, improved signal statistics can be expected for in vivo studies with this compound.

The binding pattern of [^{18}F]**4** to rat brain sections under naive and receptor blocking conditions in vitro was measured by means of binding autoradiography.[18] [^{18}F]**4** showed highly selective binding to brain regions with known high μOR density[19] (Fig. 2). Co-incubation with naloxone (blocking of μ-, δ-, and κOR) or unlabeled sufentanil (blocking of μOR[20]) nearly completely inhibited binding of [^{18}F]**4** (Fig. 2), further demonstrating selectivity for the μOR.

The peripheral metabolism of [^{18}F]**4** in mice was rapid, with intact compound representing 57 ± 3% and 21 ± 2% of total plasma radioactivity at 5 min and 40 min after injection, respectively.[22] Characterization and quantification[23] of the radiolabeled species extracted from brains at 40 min after injection into mice revealed that more than 92% of the total radioactivity was intact [^{18}F]**4**, indicating a high metabolic stability of the compound in brain and that peripherally generated metabolites do not have a significant brain uptake.

In summary, we report the synthesis of the new ^{18}F-labeled 4-anilidopiperidine *N*-[4-(methoxymethyl)-1-[2-(2-thienyl)ethyl]-4-piperidinyl]-*N*-phenyl-2-(±)-[^{18}F]fluoropropan-amide ([^{18}F]**4**). This new PET tracer exhibits high affinity and has apparent high selectivity for the μOR. Injection of [^{18}F]**4** into mice resulted in high brain uptake at early time points and showed a time dependent regional brain distribution that correlates with the concentration of the μOR for the different brain regions. As a derivative of sufentanil, [^{18}F]**4** is expected to possess a lower pharmacological potency than carfentanil, while it was shown in this study to exhibit a brain uptake kinetics and a specificity for the target comparable to those of [^{11}C]carfentanil. [^{18}F]**4** is therefore a promising compound for further evaluation as a PET-imaging agent for μ-opioid receptors.

Acknowledgements

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15. (±)-2-Fluoropropionyl chloride was prepared according to the procedure reported by Dolbier, W. R.; Lee, S. K.; Phanstiel, O. *Tetrahedron* **1991**, *47*, 2065, Des-propionyl sufentanil was obtained as a gift from the Janssen Research Foundation. The procedure used to prepare reference **4** was as follows: 33 mg (0.1 mmol) of des-propionyl sufentanil was dissolved in 1 mL THF containing 15.3 μL of triethylamine (0.11 mmol). (±)-2-Fluoropropionyl chloride (13.2 mg, 0.12 mmol), dissolved in 2 mL THF, was added dropwise over 1 min at room temperature. The mixture was heated at reflux for 1 h and thereafter concentrated under reduced pressure. The residue was dissolved in 10 mL dichloromethane and partitioned with water (10 mL). The aqueous phase was washed once with 10 mL dichloromethane. The combined organic phases were dried ($MgSO_4$), filtered, and concentrated to an oil which was purified by flash-chromatography (hexane/ethyl acetate 1:1). ^{1}H NMR (250 MHz, $CDCl_3$) δ: 7.12–7.18 (m, 2H); 7.04 (m, 1H); 6.88–6.94 (m, 2H); 6.85 (t, 1H); 6.77–6.82 (m, 2H); 4.51–4.58 (m, 1H, J = 46.2 Hz); 3.36 (s, 2H); 3.32 (s, 3H); 3.01–3.08 (m, 2H); 2.62–2.74 (m, 4H); 2.50–2.58 (m, 2H); 1.94–2.00 (m, 2H); 1.75–1.82 (m, 2H); 1.20–1.28 (m, 3H, J = 22.3 Hz). MS calcd for $C_{22}H_{29}FN_2O_3$: 404.2. Found: 405.2 ($[M+H]^+$).
16. N.c.a. (±)-2-[^{18}F]fluoropropionic acid was obtained by hydrolysis of 9′-anthrylmethyl-(±)-2-[^{18}F]fluoropropionate with tetramethylammonium hydroxide in aqueous methanol according to the procedure reported by Guhlke, S.; Coenen, H. H.; Stöcklin, G. *Appl. Radiat. Isot.* **1994**, *45*, 715. The dry tetramethylammonium salt of (±)-2-[^{18}F]fluoropropionic acid was obtained by azeotropic distillation with four portions of 1 mL MeCN at 90 °C under a stream of argon. The vial was sealed and phthaloyl dichloride (200 μL, 1.33 mmol) in 200 μL anhydrous tetrahydrofuran (THF) was added to the residue. The mixture was heated at reflux for 5 min. The heating source was removed and the vial was left to cool for 2 min. Thereafter, the vial was again heated to 90 °C, connected to a supply of argon gas flow and vented via a capillary PEEK tubing to a second vial containing des-propionyl sufentanil dissolved in THF, which was kept at 0 °C. The (±)-2-[^{18}F]fluoropropionic chloride was distilled at a flow rate of 20 mL /min. After 5 min, vessel B was closed and heated at reflux in order to promote amide formation. After removal of THF by evaporation, the residue was re-dissolved in 300 μL MeCN, diluted with 4 mL ammonium formate (0.1 M), loaded into a 7 mL injection loop, and transferred onto a sample enrichment column (10 mm × 50 mm; PRP-1: CS-Chromatographie Service, Langerwehe, Germany). The enrichment column was washed with 0.1 M ammonium formate at a flow rate of 2 mL/min for 5 min and subsequently eluted in the reverse direction onto a semi-preparative μ-Bondapak C_{18} column (5 μm particle size; 8 mm × 300 mm; CS-Chromatographie Service) using a linear gradient of 15–70% B in 20 min; 0.1% TFA in water (A), 0.1% TFA in MeCN (B), flow rate of 3 mL/min. In-line HPLC detectors (Sykam, Gilching Germany) included a UV detector (254 nm) and a radioactivity detector (Flow-count, Bioscan, Washington DC). For animal experiments, the product eluting at 18.4 min was led into a rotary evaporation flask containing 1 mL of 1% HCl in EtOH and evaporated to dryness under reduced pressure. The product was dissolved in isotonic saline and transferred into a vial containing 8.4% sodium bicarbonate (100 μL). The pH of the final solution was between 7 and 8. Analytical HPLC was performed using reverse-phase chromatography (Nucleosil 100 CN-phase 5 μm; 4.6 mm × 250 mm eluted with acetonitrile/0.1 M ammonium formate (55:45, v/v)). The k' value of [^{18}F]**4** under these conditions was 3.9, and [^{18}F]**4** co-eluted with a sample of authentic **4**. In addition, a C_{18} phase was used to confirm co-elution of [^{18}F]**4** and authentic **4** (5 μm; 4.6 mm × 250 mm; eluted with acetonitrile/0.1 M ammonium formate (60:40, v/v)). The k' value of [^{18}F]**4** and **4** under these conditions was 5.4. The isolated radiochemical yield averaged 19% at end-of-syntheses based on [^{18}F]fluoride, and the specific activity averaged 40.2 GBq/μmol at EOS.
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21. The procedure used to assess regional brain distribution of radioactivity was as follows: Male Balb-C mice with a body weight of 22–27 g was injected into a tail vein with 0.1–0.15 mL of a solution of 2 MBq [^{11}C]caf or 1 MBq [^{18}F]**4**. The mice were sacrificed by cervical dislocation and the various brain regions were dissected. The radioactivity of weighed tissue samples was measured in a γ-counter.
22. The procedure used for plasma analysis was as follows: Whole blood (0.2–0.4 mL) containing 15–30 μL of heparin was centrifuged at 6.000*g* for 5 min and approx. 0.1–0.2 mL of the supernatant plasma was removed. An equal volume of acetonitrile was added, the mixture was vortexed for 1 min and centrifuged at 6.000*g* for 3 min. To calculate the radioactivity balance and extraction efficiency, the radioactivity from the combined liquids was compared to the radioactivity of the extracted material by γ-counter measurements (>91% was

extracted). Approximately 0.1 mL of the supernatant solution was analyzed using HPLC (Nucleosphere 100, 5 μm; 10 × 150 mm; eluted with MeOH/0.1 M ammonium formate (65:45, v/v). The k' values of the radiolabeled metabolites were 0.4–2.7 (k' of [^{18}F]**4** = 5.8).

23. The procedure used to assess brain radioactivity was as follows: Male Balb-C mice were injected via a tail vein with 1.4–4 MBq of high specific activity [^{18}F]**4** (>37 GBq/μmol) contained in 0.1–0.15 mL of isotonic saline solution. The mice were sacrificed at 40 min post-injection and their brains were dissected, snap-frozen in liquid nitrogen, and homogenized, followed by addition of 1 mL of isotonic saline. The mixture was vigorously vortexed and 0.5 mL of MeCN was added. After centrifugation for 5 min at 6.000*g* the supernatant was collected. Samples from the extract and the tissue were counted using a γ well-counter to determine the extraction efficiency (>95% was extracted). The extract was spiked with authentic **4** and analyzed by reverse-phase HPLC using methods described above for the analysis of radioactivity distribution in plasma.
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