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Research Article

Synthesis and biological evaluation of *S*-[^{11}C]methylated mercaptoimidazole piperazinyl derivatives as potential radioligands for imaging 5-HT$_{1A}$ receptors by positron emission tomography (PET)

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Summary

The novel 2-mercaptoimidazole derivatives, 1-[4-((2-methoxyphenyl)-1-piperazinyl)-butyl]-2-mercaptoimidazole (**3**) and methyl[4-((2-methoxyphenyl)-1-piperazinyl))butyl] (2-mercapto-1-methylimidazol-5-yl)methanamide (**8**), were efficiently labelled with ^{11}C through methylation of the thioketone function with [^{11}C]methyl iodide. The resulting radioligands 1-[4-((2-methoxyphenyl)-1-piperazinyl))butyl]-2-thio[^{11}C]-methylimidazole ([^{11}C]**9**) and methyl[4-((2-methoxyphenyl)-1-piperazinyl))butyl] (2-thio[^{11}C]methyl-1-methylimidazol-5-yl)-methanamide ([^{11}C]**10**) were synthesized in radiochemical yields of 20–30% (decay-corrected, related to [^{11}C]CO_2) at a specific radioactivity of 0.2–0.4 Ci/μmol within 40–45 min including HPLC-purification. The radiochemical purity exceeded 99%. The reference compounds **9** and **10** were tested in a competitive receptor binding assay to determine their affinity toward the 5-HT$_{1A}$ receptor. Both compounds exhibit excellent sub-nanomolar affinities ($IC_{50} = 0.576 \pm 0.008$ nM (**9**); $IC_{50} = 0.86 \pm 0.02$ nM (**10**)) for the 5-HT$_{1A}$ receptor while displaying a high selectivity towards the 5-HT$_{2A}$ subtype of receptors ($IC_{50} > 480$ nM). By contrast, compound **9** also shows substantial binding for the alpha1-adrenergic receptor ($IC_{50} = 3.00 \pm 0.02$ nM) when compared with compound **10** ($IC_{50} = 54.5 \pm 0.6$ nM). Preliminary biodistribution studies in rats showed an initial

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brain uptake of 1.14 ± 0.11 and 0.37 ± 0.04% ID/g after 5 min, which decreased to 0.18 ± 0.04 and 0.16 ± 0.01% ID/g after 60 min for compounds [^{11}C]**9** and [^{11}C]**10**, respectively. For both compounds, the cerebellum and rest of the brain uptake are very similar at the different time points. Unlike [^{11}C]**9**, the radioligand [^{11}C]**10** has significant uptake and retention in the adrenal glands. Due to their washout from the brain compounds [^{11}C]**9** and [^{11}C]**10** seem not to be good candidates as radioligands for imaging 5-HT$_{1A}$ receptors by PET.

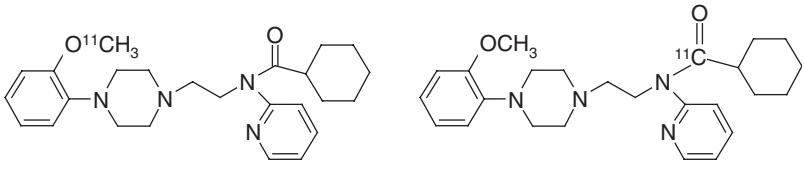
Key Words: carbon-11; mercaptoimidazoles; piperazines; 5-HT$_{1A}$ receptors; positron emission tomography

Introduction

Central 5-hydroxytryptamine (5-HT$_{1A}$) receptors are implicated in the pathophysiology of major neuropsychiatric disorders such as schizophrenia, anxiety and depression. The functional imaging of 5-HT$_{1A}$ receptors by positron emission tomography (PET) represents an innovative approach for the non-invasive assessment of their role in the development of those neuropsychiatric disorders. Moreover, PET imaging is also a useful tool for evaluating the action of new 5-HT$_{1A}$ receptor targeting drugs, allowing the design of more efficient therapeutic strategies.[1,2]

The identification of WAY-100635 as a potent and selective antagonist for the 5-HT$_{1A}$ receptor was accompanied by numerous attempts to label the compound with the short-lived positron emitter ^{11}C ($t_{1/2}$ = 20.4 min) to study 5-HT$_{1A}$ receptors in the human brain (Scheme 1).

Originally, the radiolabelling of WAY-100635 with ^{11}C was accomplished via *O*-methylation with [^{11}C]methyl iodide of the corresponding desmethyl precursor.[3] However, the resulting radioligand [*O*-methyl-^{11}C]WAY-100635 undergoes hepatic metabolism, which involves cleavage of the amide moiety and results in the formation of a lipophilic radioactive metabolite. This metabolite is accumulated in the brain, and it displays high affinity for both 5-HT$_{1A}$ and alpha1-adrenergic receptors. These facts complicate an exact quantification of 5-HT$_{1A}$ brain receptors based on [*O*-methyl-^{11}C]WAY-100635.[4,5] In order to overcome this drawback, [carbonyl-^{11}C]WAY-100635



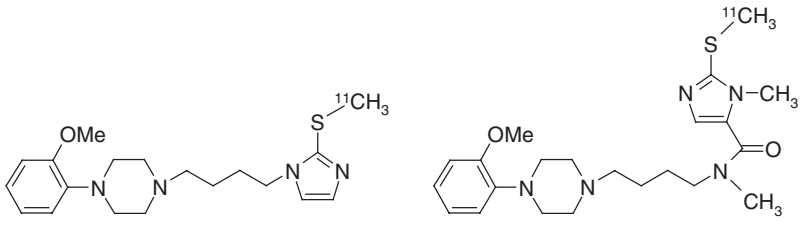
[O-methyl-^{11}C]WAY-100635 **[carbonyl-^{11}C]WAY-100635**

Scheme 1. ^{11}C-labelled 5-HT$_{1A}$ receptor ligands

was synthesized and evaluated.[6] Here the compound is labelled in the carbonyl position with ^{11}C. Although suffering from hepatic metabolism, [carbonyl-^{11}C]WAY-100635 is transformed into polar radioactive cyclohexanecarboxylic acid fragments which are unable to cross the blood brain barrier.[4,5] Thus, [carbonyl-^{11}C]WAY-100635 can be considered to be an effective radioligand for the mapping of 5-HT_{1A} receptors in the human brain.[7–10] However, [carbonyl-^{11}C]WAY-100635 was prepared via a laborious ^{11}C acylation with [carbonyl-^{11}C]cyclohexanecarbonyl chloride. Consequently, the search for alternative radioligands which can be prepared more easily while showing improved metabolic resistance is still a relevant issue.[4,5,11–15]

Several studies highlighted the tolerance of the WAY-100635 lead structure to replacement of the pyridinyl amide group by other substituents, including bulky metallic fragments, such as $[TcO]^{3+}$, $[TcN]^{2+}$ and *fac*-$[Tc(CO)_3]^{+}$, anchored by adequate bifunctional chelators.[13,16–18] Recently, our group introduced the novel organometallic building blocks *fac*-$[M\{\kappa^3\text{-}R(\mu\text{-}H)B(tim^{Me})_2\}(CO)_3]$ (R = H, alkyl, aryl; tim^{Me} = 1-methyl-2-mercaptoimidazolyl; M = Re, ^{99m}Tc), which are anchored by tripodal ligands of the *bis*(mercaptoimidazolyl)borate type.[19,20] These building blocks were functionalized with piperazinyl fragments, which were incorporated at the 5-position of the azole ring.[21] Some of the resulting functionalized complexes displayed excellent sub-nanomolar affinities for 5-HT_{1A} receptors, and their biodistribution is currently under evaluation.[22] Based on these findings, we expected that mercaptoimidazoles bearing piperazinyl moieties would also retain, by themselves, good affinity and selectivity for 5-HT_{1A} receptors. Benefiting from the easy alkylation of thione functions with [^{11}C]methyl iodide, we decided to evaluate the usefulness of ^{11}C-labelled mercaptoimidazoles as radioligands for mapping brain 5-HT_{1A} receptors by means of PET.

Herein, we report on the radiosynthesis and biological evaluation of two *S*-^{11}C-methylated mercaptoimidazole piperazinyl derivatives ([^{11}C]**9** and [^{11}C]**10**). Attachment of the 1-(2-methoxyphenyl)piperazinyl part of WAY-100635 to the azole moiety was accomplished via a butyl spacer (Scheme 2).



Scheme 2. Novel *S*-^{11}C-methylated mercaptoimidazole piperazinyl derivatives [^{11}C]9 and [^{11}C]10

The biological evaluation included the determination of 5-HT_{1A} receptor binding affinities of compounds **9** and **10**, as well as preliminary biodistribution studies of [^{11}C]**9** and [^{11}C]**10** in male Wistar rats.

Results and discussion

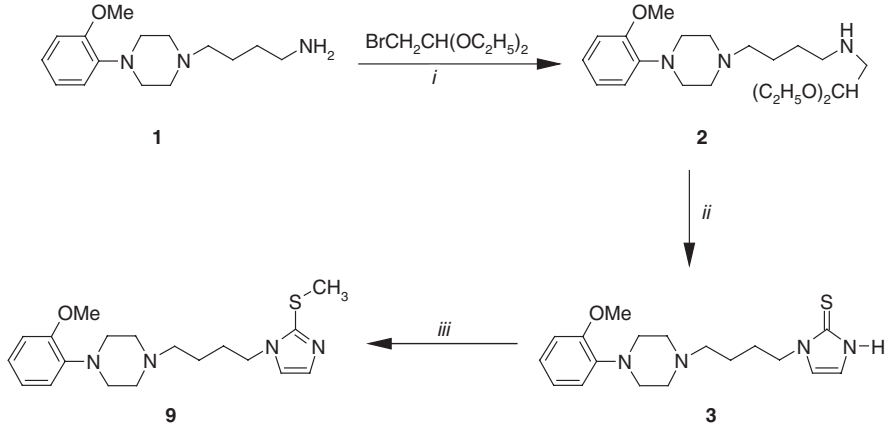
Chemistry

Coupling of the 1-(2-methoxyphenyl)piperazinyl moiety to the 2-mercaptoimidazole ring was carried out at positions 1 or 5 of the azole, and involved the synthesis of the mercaptoimidazole precursors 1-[4-((2-methoxyphenyl)-1-piperazinyl))butyl]-2-mercaptoimidazole (**3**) and methyl[4-((2-methoxyphenyl)-1-piperazinyl)butyl] (2-mercapto-1-methylimidazol-5-yl)methanamide (**8**) as depicted in Schemes 3 and 4, respectively. In both cases, we started from 4-[(2-methoxyphenyl)-1-piperazinyl]butylamine (**1**), which was synthesized according to literature methods.[23]

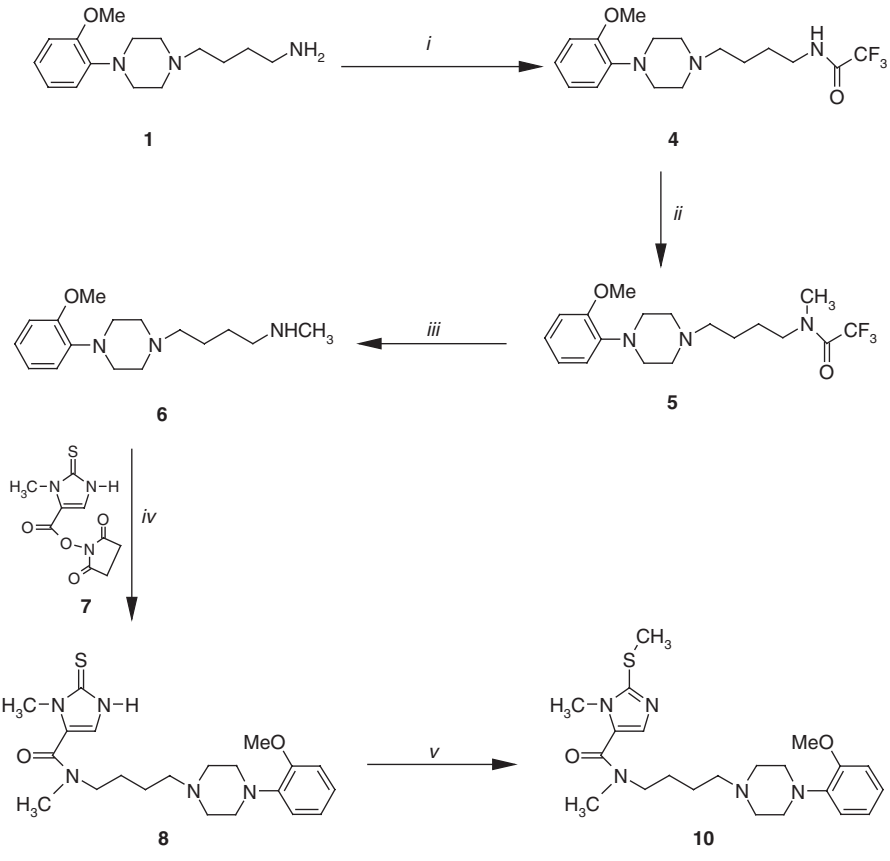
The synthesis of the labelling precursor **3** was accomplished in 50% yield by cyclization of a diethyl acetal derivative (**2**) with potassium thiocyanate under acidic conditions, which is a common method for the synthesis of 2-mercaptoimidazoles (Scheme 3).[24,25]

Amino acetal **2** has been obtained by alkylation of compound **1** with bromoacetaldehyde diethyl acetal. The reaction was performed using an excess of primary amine **1** to avoid overalkylation.[26]

As depicted in Scheme 4, the labelling precursor **8** was obtained by a multistep synthesis, starting from [(2-mercapto-1-methylimidazol-5-yl)methyloxy]-succimide (**7**), which has been synthesized by methods reported in the



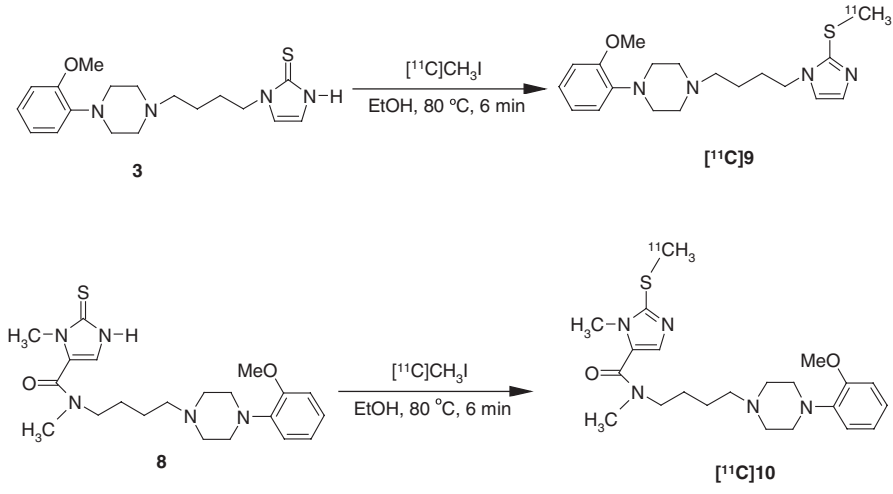
Scheme 3. Synthesis of the labelling precursor 3 and reference compound 9: (i) K_2CO_3, acetonitrile, reflux, 48 h; (ii) KSCN, HCl 2 N, ethanol, reflux, overnight; (iii) NaOH, CH_3I, ethanol, rt, 3 h



Scheme 4. Synthesis of labelling precursor 8 and reference compound 10: (i) $CF_3COOC_2H_5$, dichloromethane, −8°C to rt, 4 h; (ii) NaH, $(CH_3)_2SO_4$, DMF, rt, overnight; (iii) K_2CO_3, methanol/water (30:1 v/v), reflux, 2 h; (iv) DMF, rt, 24 h; (v) NaOH, CH_3I, ethanol, rt, overnight

literature.[27] Direct coupling of activated ester **7** with compound **6** provided labelling precursor **8** in 33% yield. The moderate to low yield can be explained by the low solubility of the activated ester **7** and to the low reactivity of the secondary amine **6**. Secondary amine **6** was prepared by protection of the primary amine group as a trifluoroacetylamide followed by mono methylation with dimethylsulfate and subsequent removal of the amine protecting group under moderate basic conditions.[28] The overall chemical yield for the three steps was 68%.

Reference compounds **9** and **10** were synthesized in a straightforward way by the reaction of compounds **3** and **8** with methyl iodide (Schemes 3 and 4). Compounds **9** and **10** were used to identify the radioligands [^{11}C]**9** and [^{11}C]**10**, and they were used in the competitive binding assays to determine their affinity toward the 5-HT_{1A} receptor.



Scheme 5. Radiosynthesis of [^{11}C]9 and [^{11}C]10

Radiochemistry

Efficient *S*-[^{11}C]-methylation reactions of thioketones have already been reported.[29] However, to the best of our knowledge, the labelling of 2-mercaptoimidazoles with [^{11}C]methyl iodide has not been applied yet.

S-Methylation of labelling precursors **3** and **8** with [^{11}C]methyl iodide was easily accomplished in ethanol at 80°C for 6 min using NaOH as a base, giving the desired radioligands 1-[4-((2-methoxyphenyl)-1-piperazinyl))butyl]-2-thio[^{11}C]methylimidazole ([^{11}C]**9**) and methyl[4-((2-methoxyphenyl)-1-piperazinyl)butyl] (2-thio[^{11}C]methyl-1-methyl-imidazol-5-yl)methanamide ([^{11}C]**10**) (Scheme 5).

The radioligands [^{11}C]**9** and [^{11}C]**10** were purified by semi-preparative HPLC using a Kromasil-100 C_{18} column and isocratic elution with CH_3CN/0.2% aqueous NEt_3 (70:30, v/v) at a flow rate of 3 ml/min. Under these conditions, radioligands [^{11}C]**9** and [^{11}C]**10** were easily separated from the excess of 2-mercaptoimidazole precursors **3** and **8** and very low amounts (<5%) of non-reacted [^{11}C]methyl iodide. Both radiotracers were obtained chemically and radiochemically pure (>99%), as checked by analytical HPLC. The identity of the radioligands [^{11}C]**9** and [^{11}C]**10** was confirmed by comparison of their HPLC profiles with those of reference compounds **9** and **10**.

Typically, the radioligands [^{11}C]**9** and [^{11}C]**10** were obtained with radiochemical yields of 20–30% (decay-corrected, related to [^{11}C]CO_2) at a specific radioactivity of 0.2–0.4 Ci/μmol, within 40–45 min including HPLC separation.

Table 1. Inhibition constants (IC_{50}) and log *P* values of 9, 10 and WAY-100635

	IC_{50} (nM)			
Compound	5-HT_{1A} [^{3}H]8-OH-DPAT rat hippocampus homogenate	5-HT_{2A} [^{3}H]Ketanserin rat cortex homogenate	alpha-1 [^{3}H]prazosin rat cortex homogenate	Log *P*[a]
9	0.576 ± 0.008	1050 ± 16	3.00 ± 0.02	4.2
10	0.86 ± 0.02	480 ± 10	54.5 ± 0.6	2.4
WAY 100635	6.0[b]			4.7[b]

[a] Calculated log *P* values have been calculated based on ACDLabs predictions.
[b] Literature value.[11]

In vitro binding assays

Reference compounds **9** and **10** were subjected to *in vitro* competitive receptor binding assay using rat brain homogenates as the source for the receptor proteins to determine their IC_{50} values for the 5-HT_{1A}, 5-HT_{2A} and alpha1-adrenergic receptors. The IC_{50} values obtained for both compounds and WAY-100635 along with log *P* values are depicted in Table 1.

Compounds **9** and **10** exhibit excellent sub-nanomolar affinities for the 5-HT_{1A} receptors, comparable to that found for WAY-100635.[11] Moreover, both compounds display good selectivities against 5-HT_{2A} receptors, with 5-HT_{2A}/5-HT_{1A} ratios of 1553 and 558 for **9** and **10**, respectively. By contrast, much lower selectivities were found to the alpha1-adrenergic receptor. This finding is consistent with reports in the literature on compounds bearing a 2-methoxyarylpiperazine moiety showing substantial affinity toward alpha1-adrenergic receptor.[23] However, compound **9** displays an 18-fold higher affinity to the alpha1-adrenergic receptor when compared with compound **10**, being 3 and 54 nM, respectively. This difference in the binding affinity and consequently in the selectivity is likely to be influenced by the position at which the arylpiperazine pharmacophore is coupled to the 2-mercaptoimidazole ring and/or the presence of the tertiary amide function in the molecule.

Biodistribution studies

Preliminary biodistribution studies of [^{11}C]**9** and [^{11}C]**10** were performed in male Wistar rats to evaluate brain uptake and brain activity retention. Table 2 summarizes the biodistribution data obtained for [^{11}C]**9** and [^{11}C]**10**, expressed in percent injected dose per gram tissue (% ID/g).

Both compounds were able to cross the BBB, showing an initial brain uptake of 1.14 ± 0.11% ID/g ([^{11}C]**9**) and 0.37 ± 0.11% ID/g ([^{11}C]**10**) after 5 min. The initial higher brain uptake of compound [^{11}C]**9** seems to correlate with a higher lipophilicity of compound [^{11}C]**9** when compared to compound

Table 2. Biodistribution of [^{11}C]9 and [^{11}C]10 in Wistar rats (mean of % ID/g tissue ± SD, $n = 4$)

	% ID/g			
	[^{11}C]**9**		[^{11}C]**10**	
Organ	5 min	60 min	5 min	60 min
Blood	0.11 ± 0.02	0.10 ± 0.03	0.12 ± 0.03	0.04 ± 0.00
Cerebellum	0.82 ± 0.13	0.14 ± 0.03	0.29 ± 0.04	0.14 ± 0.01
Rest of brain	1.14 ± 0.11	0.18 ± 0.04	0.37 ± 0.04	0.16 ± 0.01
Pancreas	4.16 ± 0.51	5.85 ± 0.84	3.34 ± 0.57	1.86 ± 0.13
Spleen	1.48 ± 0.26	0.92 ± 0.19	1.27 ± 0.17	0.51 ± 0.07
Adrenals	3.77 ± 1.03	1.11 ± 0.16	7.95 ± 3.09	7.32 ± 0.75
Kidneys	2.85 ± 0.43	0.81 ± 0.16	2.56 ± 0.32	1.09 ± 0.15
Heart	0.49 ± 0.08	0.19 ± 0.03	0.41 ± 0.08	0.10 ± 0.01
Lung	1.68 ± 0.19	0.48 ± 0.06	1.14 ± 0.17	0.33 ± 0.07
Liver	0.80 ± 0.18	1.37 ± 0.35	1.33 ± 0.28	2.75 ± 0.72

[^{11}C]**10** (Table 1). However, a washout from the brain was observed for both compounds, reaching 0.18 ± 0.04 and 0.16 ± 0.01% ID/g after 60 min. The blood clearance is fast, and after 5 min the brain/blood ratio is 10 for compound [^{11}C]**9** and 3 for compound [^{11}C]**10**. However, blood activity of compound [^{11}C]**9** is comparable at 5 and 60 min, being 0.11% ID/g and 0.10% ID, respectively. This phenomenon could be explained by a high proportion of protein binding of compound [^{11}C]**9** due to its higher lipophilicity compared to compound [^{11}C]**10**. Despite displaying excellent *in vitro* binding affinities for 5-HT$_{1A}$ receptors compounds [^{11}C]**9** and [^{11}C]**10** show comparable uptake in the cerebellum and in the rest of the brain after 60 min post-injection (p.i). This finding suggests that the found brain uptake is mainly related to high non-specific binding of both compounds. Moreover, the low specific activity of compounds [^{11}C]**9** and [^{11}C]**10** may also lead to a saturation of specific binding sites.

Remarkably, compound [^{11}C]**10** showed a substantial uptake in the adrenals (7.95 ± 3.09% ID/g, at 5 min p.i.) with almost no washout (7.32 ± 0.75% ID/g, at 60 min p.i.). A possible explanation for this uptake could be the moderate affinity ($IC_{50} = 54.5 \pm 0.6$ nM) of [^{11}C]**10** for alpha1-adrenergic receptors, which are active in adrenal glands. However, a much lower retention of activity was observed in other organs bearing adrenergic receptors, like heart, pancreas or spleen. Moreover, radioligand [^{11}C]**9**, having a much higher affinity ($IC_{50} = 3.00 \pm 0.02$ nM) for the adrenergic receptors, showed a lower adrenal uptake (3.77 ± 1.03% ID/g, at 5 min p.i.) with a significant washout (1.11 ± 0.16% ID/g, at 60 min p.i.). Apparently, these data do not support a relationship between the adrenal uptake of compound [^{11}C]**10** and its affinity for the alpha1-adrenergic receptor. Adrenal glands being a receptor-rich

organ, we cannot exclude the involvement of other type of receptors in the high uptake of [^{11}C]**10** by the adrenals.

In summary, we have shown that 2-mercaptoimidazole derivatives bearing 2-methoxypiperazinyl pharmacophores are promptly labelled with [^{11}C]methyl iodide by *S*-alkylation of the thioketone function, giving radioligands [^{11}C]**9** and [^{11}C]**10** in sufficient radiochemical yields and high radiochemical purity. In spite of their excellent *in vitro* affinity for 5-HT_{1A} receptor and good selectivities against the 5-HT_{2A} receptor, compounds [^{11}C]**9** and [^{11}C]**10** are not promising radiotracers for PET imaging of CNS receptors, due to their fast washout from the brain. Surprisingly, [^{11}C]**10** showed a remarkable and prolonged uptake in the adrenals, promoting further studies to clarify this phenomenon. This should include, namely, the determination of the affinity toward other receptors occurring in adrenals, studies on metabolism, as well as some selected blocking experiments to verify specific uptake.

Experimental section

Dichloromethane, ethanol and dimethylformamide were dried and distilled according to described procedures. The compounds 4-[(2-methoxyphenyl)-1-piperazinyl]butylamine (**1**) and [(2-mercapto-1-methylimidazol-5-yl)methyloxy]succimide (**7**) were synthesized as described elsewhere.[23,27] All other solvents and chemicals were used as purchased. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 300 MHz spectrometer; ^{1}H and ^{13}C chemical shifts are given in ppm and were referenced with the residual solvent resonances relative to Me_4Si. Mass spectra were obtained on a Quattro/LC mass spectrometer (MICROMASS) by electrospray ionization.

[^{11}C]CO_2 was produced by the ^{14}N(p,α)^{11}C reaction on a IBA CYCLONE 18/9 cyclotron. The synthesis of [^{11}C]methyl iodide was done by procedures described in the literature,[30] and involved reduction of [^{11}C]CO_2 with $LiAlH_4$, followed by hydrolysis and iodination with hydroiodic acid. All radiochemical syntheses including semi-preparative HPLC-separation were run on the fully automated synthesis module (PET tracer synthesizer, Nuclear Interface GmbH, Münster, Germany). Analytical HPLC was performed using a JASCO HPLC system (JASCO, HPLC-pump PU-1580) equipped with a UV-vis detector (JASCO UV-1575) and a gamma detector (Raytest Gabi).

The animal experiments were carried out in accordance with the guidelines on the use of living animals in scientific investigations and on the German law, and followed the principles of laboratory animal care.

Chemistry

1-(Acetaldehydediethyacetal)-4-((2-methoxyphenyl)-1-piperazinyl)butylamine (**2**). To a solution of compound **1** (1.5 g, 5.7 mmol) in acetonitrile was added

bromoacetaldehyde diethyl acetal (0.43 ml, 2.9 mmol) and 1.57 g (11.4 mmol) of K_2CO_3, and the resulting suspension was refluxed during 48 h. After cooling to room temperature and filtration, the solvent was removed under vacuum to give an oily residue. Column chromatography of this residue on silica gel with CH_2Cl_2/methanol (95:5) afforded compound **2** (567 mg, 1.50 mmol, $\eta = 26\%$) as an orange oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.17 (6H, t, $-OCH_2C\underline{H}_3$), 1.57 (m, 4H, CH_2), 2.42 (t, 2H, CH_2), 2.64–2.72 (m, 6H, $CH_2 + NCH_2$), 3.07 (d, 2H, CH_2), 3.40 (br, 4H, NCH_2), 3.48–3.58 (m, 2H, $OC\underline{H}_2CH_3$), 3.65–3.75 (m, 2H, $OC\underline{H}_2CH_3$), 3.81 (s, 3H, $-OCH_3$), 4.64 (t, 1H, CH), 6.80–6.99 (m, 4H, Ar).

*1-[4-((2-Methoxyphenyl)-1-piperazinyl)butyl]-2-mercaptoimidazole (**3**)*. To an ethanolic solution of **2** (755 mg, 2 mmol) was added 3 ml of 2 N HCl and solid KSCN (400 mg, 4.12 mmol), and the reaction mixture was refluxed overnight. After cooling to room temperature, a white insoluble solid was removed by filtration. To the supernatant was added a saturated $NaHCO_3$ solution, followed by extraction with $CHCl_3$. The solvent from the organic phase was removed, yielding a yellow-brown solid. Column chromatography of this solid on silica gel with methanol afforded compound **3** (360 mg, 1 mmol, $\eta = 50\%$) as a yellowish solid.

^{1}H NMR (300 MHz, CD_3OD) δ: 1.58 (q, 2H, CH_2), 1.82 (q, 2H, CH_2), 2.47(t, 2H, CH_2), 2.64 (br, 4H, NCH_2), 3.05 (br, 4H, NCH_2), 3.84 (s, 3H, $-OCH_3$), 4.06 (t, 2H, CH_2), 6.85 (d, 1H, C<u>H</u>– Simz), 6.86–7.00 (m, 4 H, Ar), 7.05 (d, 1H, C<u>H</u>– Simz). ^{13}C NMR (75.37 MHz, CD_3OD) δ: 24.3, 28.2, 47.6, 51.5, 54.3, 55.9, 59.1, 112.7, 115.7, 119.4, 119.8, 122.5, 124.7, 142.2, 153.9, 161.0. LRMS(ESI positive) (*m*/*z*): 347 $[M + H]^+$.

*4-[((2-Methoxyphenyl)-1-piperazinyl)]butyltrifluoroacetamide (**4**)*. To a solution of compound **1** (612 mg, 2.33 mmol) in CH_2Cl_2 at −8°C was added dropwise a solution of $CF_3COOC_2H_5$ (305 µl, 2.56 mmol) in CH_2Cl_2. After complete addition, the mixture was stirred at −8°C for 2 h and at room temperature for 1.5 h. Removal of the solvent yielded compound **4** as a yellow oil (800 mg, $\eta = 96\%$).

^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.68 (m, 4H, CH_2), 2.47 (t, 2H, CH_2), 2.66 (br, 4H, NCH_2), 3.08 (br, 4H, NCH_2), 3.37 (t, 2H, CH_2), 3.84 (s, 3H, OCH_3), 6.80–7.02 (m, 4H, Ar), 8.35 (br, 1H, NH).

*1-Methyl-4-[(2-methoxyphenyl)-1-piperazinyl]butylamine (**6**)*. To a suspension of NaH (64 mg, 2.67 mmol) in dry DMF at 0°C was added dropwise a solution of **4** (800 mg, 2.2 mmol) in DMF, and after complete addition the mixture was stirred at room temperature for 1 h. Then, a solution of $(CH_3)_2SO_4$ (253 µl, 2.67 mmol) in DMF was added dropwise at 0°C, and the reaction mixture was stirred overnight at room temperature. Removal of solvent under vacuum gave crude methyl[4-((2-methoxyphenyl)-1-piperazi-

nyl))butyl]trifluoroacetamide (**5**), which has been used in the next step without further purification.

To a solution of **5** in MeOH (30 ml) and water (1 ml) was added an excess of K_2CO_3 (1:10 molar ratio), and the mixture was refluxed for 2 h. After cooling to room temperature and filtration, the solvent was removed under vacuum. The crude was dissolved in water and the pH adjusted to about pH = 14 with 40% NaOH. The aqueous solution was extracted four times with chloroform and the combined organic phases were dried over $MgSO_4$. Removal of the solvent gave **6** as a yellow oil (416 mg, 1.50 mmol, $\eta = 68\%$).

Compound **5**, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.50–1.72 (m, 4H, CH_2), 2.41 (m, 2H, CH_2), 2.65 (br, 4H, CH_2N), 3.09 (br, 7H, CH_2N and CH_3N), 3.47 (t, 2H, CH_2), 3.84 (s, 3H, OCH_3), 6.83–7.01 (m, 4H, Ar).

Compound **6**, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.50–1.57 (m, 4H, CH_2), 2.38–2.45 (m, 5H, m, $CH_3N + CH_2$), 2.56–2.63 (m, 6H, $CH_2N + CH_2$), 3.09 (br, 4H, CH_2N), 3.84 (s, 3H, OCH_3), 6.82–6.99 (m, 4H, Ar).

Methyl[4-((2-methoxyphenyl)-1-piperazinyl)butyl](2-mercapto-1-methylimidazol-5-yl)methanamide (**8**). To a suspension of compound **7** (976 mg, 3.8 mmol) in DMF at 0°C was added dropwise a DMF solution of **6** (1.060 g, 3.8 mmol). The reaction mixture was allowed to warm to room temperature, and was stirred for 24 h. After this time, the solvent was removed under vacuum. The crude residue was purified by column chromatography (silica gel 60, eluent 15% MeOH in CH_2Cl_2). Removal of the solvent from the collected fractions, followed by washing with aqueous 10% $NaHCO_3$ and prolonged drying under vacuum, gave compound **8** (532 mg, $\eta = 33\%$) as a yellow solid.

^{1}H NMR (300 MHz, CD_3CN) δ: 1.46 (m, 2H, CH_2), 1.60 (m, 2H, CH_2), 2.34 (tr 2H, CH_2), 2.51 (br, 4H, NCH_2), 2.90–3.10 (br, 4 + 3H, $NCH_2 + NCH_3$), 3.45 (tr 2H, CH_2), 3.51 (s, 3H, N-CH_3), 3.78 (s, 3H, –OCH_3), 6.83–6.97 (m, 4H + 1H, Ar + C<u>H</u>– Simz). ^{13}C NMR (75.37 MHz, $CDCl_3$) δ: 23.3, 24.9, 32.6, 36.3, 47.6, 49.9, 52.8, 54.9, 57.4, 110.6, 115.2, 117.7, 120.5, 122.6, 124.0, 140.5, 151.7, 159.6, 162.4. LRMS (ESI-positive) (*m*/*z*): 418 $[M + H]^+$.

1-[4-((2-Methoxyphenyl)-1-piperazinyl)butyl]-2-thiomethylimidazole (**9**). Solid NaOH (12 mg, 0.29 mmol) was added to an ethanolic solution of compound **3** (100 mg, 0.29 mmol), followed by dropwise addition of an ethanolic solution of CH_3I (45 mg, 0.32 mmol). After complete addition, the reaction mixture was stirred at room temperature for 3 h. Removal of the volatiles gave a yellow residue, which was dissolved in CH_2Cl_2. After washing with H_2O, the organic phase was dried over $MgSO_4$, filtered and the solvent removed under vacuum, affording compound **9** (83 mg, 0.23 mmol, $\eta = 80\%$) in the form of a yellow solid.

^{1}H NMR (300 MHz, CD_3CN) δ: 1.54 (q, 2H, CH_2), 1.80 (q, 2H, CH_2), 2.43 (t, 2H, CH_2), 2.51 (s, 3H, –SCH_3), 2.62 (br, 4H, NCH_2), 3.04 (br, 4H, NCH_2),

3.83 (s, 3H, $-OCH_3$), 4.05 (t, 2H, CH_2), 6.82–7.02 (m + d, 4H + 1H, Ar + C<u>H</u>– Simz), 7.19 (d. 1H, C<u>H</u>– Simz). ^{13}C NMR (75.37 MHz, CD_3CN) δ: 17.1, 24.4, 29.7, 47.4, 51.5, 54.2, 55.9, 59.0, 112.7, 119.4, 122.2, 122.8, 124.7, 129.5, 142.1, 143.5, 153.8. LRMS (ESI-positive) (*m*/*z*): 361 $[M + H]^+$.

Methyl[4-((2-methoxyphenyl)-1-piperazinyl)butyl](2-thiomethyl-1-methylimidazol-5-yl)methanamide (**10**). Compound **10** has been synthesized as described above for **9**, but the reaction was allowed to proceed overnight. Starting from 100 mg (0.24 mmol) of compound **8**, were obtained 28 mg ($\eta = 27\%$) of **10** in the form of a colourless oil, after purification by column chromatography (silica gel 60, eluent 5% MeOH in CH_2Cl_2).

^1H NMR (300 MHz, $CDCl_3$) δ: 1.53 (m, 2H, CH_2), 1.64 (m, 2H, CH_2), 2.42 (t, 2H, CH_2), 3.08 (br, 7H, $NCH_3 + CH_2N$), 3.51 (t, 2H, CH_2), 6.64 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 6.80–6.97 (m, 4H, Ar), 7.20 (s, 1H, CH). ^{13}C NMR (75.37 MHz, $CDCl_3$) δ: 15.1, 23.8, 25.6, 32.4, 36.3, 47.7, 50.4, 53.3, 55.3, 58.0, 111.0, 118.1, 120.9, 122.9, 127.0, 131.6, 141.1, 147.5, 152.1, 161.6. LRMS (ESI-positive) (*m*/*z*): 432 $[M + H]^+$.

Radiosynthesis

1-[4-((2-Methoxyphenyl)-1-piperazinyl)butyl]-2-thio[^{11}C]methylimidazole (*[^{11}C]***9**). Methylation with [^{11}C]methyl iodide was carried out by distillation of approximately 100 mCi [^{11}C]CH_3I into a second reaction vessel containing 1.30 mg (0.0036 mmol) of the mercaptoimidazole precursor **3** in 0.6 ml of ethanol and 20 µl of 5 N NaOH. The reaction mixture was heated for 6 min at 80°C. After cooling to room temperature and dilution with 1 ml of acetonitrile, the reaction mixture was injected into a Kromasil-100 C_{18} semi-preparative HPLC column (300 mm × 8 mm, 7 µM). Isocratic elution with CH_3CN: 0.2% aqueous NEt_3 (70:30, v/v) at 3 ml/min flow rate allowed the purification of radioligand [^{11}C]**9**. The effluent from the column containing [^{11}C]**9** was collected in 50 ml of distilled water, and separated by solid-phase extraction by a SEP-Pak C-18 cartridge (Millipore). Elution of the cartridge with 1 ml of ethanol, allowed the recovering of approximately 30 mCi of radioligand [^{11}C]**9**, which was collected into a vial containing 5.0 ml of saline.

The final solution of radioligand [^{11}C]**9** was assayed for total radioactivity and its radiochemical purity evaluated in an analytical HPLC Phenomenex Luna C-18 (250 × 4.6 mm) column, using CH_3CN: 0.2% aqueous NEt_3 (70:30, v/v) as eluent, at 1.0 ml/min flow rate. In these chromatographic conditions, retention times were 4.35 min for [^{11}C]**9** and 3.08 min for the mercaptoimidazole precursor **3**, while $^{11}CH_3I$ is eluted at 5.23 min. The identity of the radiolabelled product [^{11}C]**9** was confirmed by HPLC in comparison with the isolated 'cold' congener **9**. Compound [^{11}C]**9** was obtained in approximate 30% yield (decay-corrected, related to [^{11}C]CO_2), with a radiochemical purity

better than 99% and with a specific activity of 0.2–0.4 Ci/μmol at the end-of-synthesis.

*Methyl[4-((2-methoxyphenyl)-1-piperazinyl)butyl]-(2-thio[^{11}C]methyl-1-methylimidazol-5-yl-)methanamide ([^{11}C]**10**)*. The radiosynthesis and purification of [^{11}C]**10** has been performed using the procedures described above for [^{11}C]**9**, starting from 1.12 mg (0.0027 mmol) of precursor **8**. Compound ([^{11}C]**10**) was obtained in approximate 20% yield (decay-corrected, related to [^{11}C]CO_2), with a radiochemical purity better than 99% and with a specific activity at the end-of-synthesis comparable to [^{11}C]**9**. The identity of [^{11}C]**10** was confirmed by HPLC comparison with the isolated 'cold' congener **10**. Using the chromatographic conditions described above, the retention times were 3.75 min for [^{11}C]**10** and 2.82 min for the mercaptoimidazole precursor **8**.

Receptor binding assays in rat brain homogenates

The receptor binding assays were performed as described elsewhere.[23] For the 5-HT_{1A} receptor binding assay [^{3}H]8-OH-DPAT (NEN, 4.6 TBq/mmol) as specific radioligand and rat hippocampus homogenate were used. Rat cortex homogenates were used for the 5-HT_{2A} receptor binding assay with [^{3}H]ketanserin (NEN, 2.3 TBq/mmol) as specific radioligand. [^{3}H]prazosin (NEN, 2.5 TBq/mmol) was used as specific radioligand to measure the alpha1-adrenergic receptor binding.

The incubation step of binding assays was terminated by rapid filtration through GF/B glass fiber filters (Whatman) using a 30-port Brandell Cell Harvester. The filters were rapidly washed with 4 ml portions of ice-cold buffer, transferred into 4 ml scintillation liquid (Ultima Gold, Packard) and analysed for radioactivity by scintillation counting (Beckmann-LS 600-LL).

Biodistribution studies in rats

Groups of four male Wistar rats, 5–6 weeks old, were injected with [^{11}C]**9** or [^{11}C]**10** (0.5 ml, 1.13 mCi) into the tail vein under ether anaesthesia. The rats were sacrificed by heart puncture at 5 and 60 min post-injection. The organs of interest were removed and weighted. The radioactivity of the different organs was measured in a COBRA II counter (CANBERRA-PACKARD), and the percentage of the injected dose per organ (% ID) and dose per gram organ were calculated (% ID/g).

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