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Evaluation of [^{18}F]VUF 5000 as a Potential PET Ligand for Brain Imaging of the Histamine H_3 Receptor

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Abstract—[^{18}F]VUF 5000 was evaluated as a potential PET ligand for the histamine H_3 receptor. In the rat a high uptake of [^{18}F]VUF 5000 was observed in liver, lung and kidney and a low uptake in the brain. In order to explain these findings we determined the Log$D_{oct,7.2}$ of [^{18}F]VUF 5000, studied the biodistribution in the presence of carrier VUF 5000, modified [^{18}F]VUF 5000 chemically and studied the binding of [^{18}F]VUF 5000 to human serum albumin. From the results of these experiments it was concluded that [^{18}F]VUF 5000 is not suitable as a PET ligand for brain imaging of the histamine H_3 receptor, since [^{18}F]VUF 5000 hardly penetrates into the brain.

Introduction

The histamine H_3 receptor has been firstly characterized as an autoreceptor[1] in the central nervous system (CNS) of the rat, regulating the release and synthesis of histamine. Subsequently, it has become clear that the H_3 receptor also acts as an heteroreceptor, regulating the release of, for example, noradrenaline,[2] serotonine,[3] acetylcholine,[4] and dopamine.[5] Consequently, the histamine H_3 receptor might be a therapeutic target in CNS disorders, in which these neurotransmitters are involved, as has been extensively reviewed.[6–9]

For in-depth studies of the possible therapeutic applications of histamine H_3 receptor drugs in CNS disorders, there is a great need for a non-invasive in vivo method to establish the localization and density of the H_3 receptor. Positron emission tomography can provide such means if a suitable radiolabeled ligand would be available. So far two compounds, FUB 272 **1** and UCL 1829 **2** (Scheme 1), have been evaluated as potential PET ligands by Ponchant et al.[10] Biodistribution of [^{18}F]FUB 272 showed high initial uptake in the lung with a rapid washout and a low uptake in the brain of 0.4% of the injected dose per gram of tissue (% ID/g), with a homogenous distribution. The second compound, [^{11}C]UCL 1829, showed a very high uptake in the lung of 25% ID/g which was not washed out and again a low brain uptake of 0.5% ID/g, with a homogenous brain distribution. The authors concluded that these two compounds were not suitable as PET ligands for brain imaging of the H_3 receptor.

Thioperamide **3** (Scheme 1), first described by Arrang et al.,[11] can be considered as the classical histamine H_3 antagonist. It has a high potency (pA_2 value of 8.9,[12] $K_i = 5.1$[13]) and is a relatively selective H_3 antagonist.[14] Furthermore, it was clearly shown in ex vivo binding studies that thioperamide penetrates the brain[15,16] after iv or ip administration in the rat and mouse. For these reasons we chose thioperamide as a template for the development of a PET ligand for the H_3 receptor. We fluoromethylated thioperamide at the 4-position of the cyclohexane moiety giving VUF 5000 **4** (Scheme 1). The resulting compound has an in vitro histamine H_3 activity which is comparable to that of thioperamide (pA_2 value of 9.0 ± 0.2, $K_i = 2.3 \pm 0.5$ nM) and can be labeled with ^{18}F.[17] In this paper we report on the biological evaluation of [^{18}F]VUF 5000 **4** to explore its use as a PET ligand for brain imaging of the histamine H_3 receptor.

Key words: Histamine H_3 receptor; VUF 5000; thioperamide; ^{18}F; biodistribution; positron emission tomography
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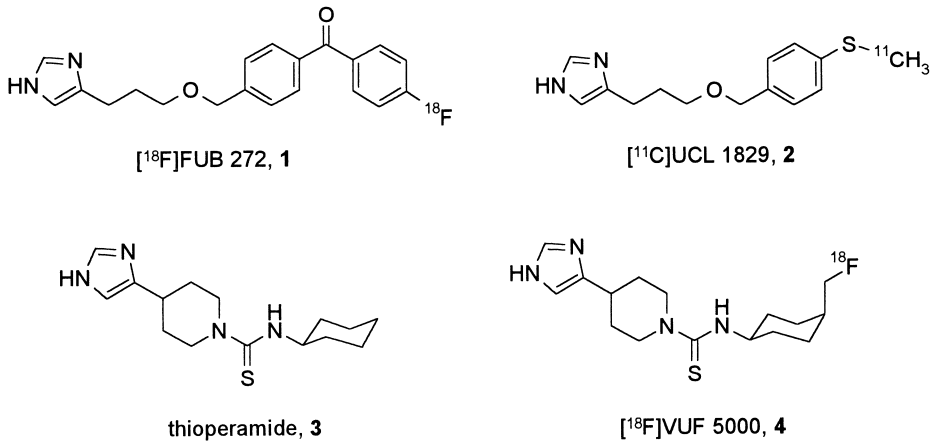


Scheme 1. Chemical structures.

Experimental

An important requirement of PET radiopharmaceuticals is their selectivity. VUF 5000 proved to be a selective H_3 ligand, since it has only measurable affinity for the α_{2a} and $5HT_3$ receptors, the δ and σ binding sites and the Na^+-channel (Table 1). The highest affinity for receptors other then the H_3 receptor is found at the σ binding site (214 nM), still leaving VUF 5000, with a K_i of 2.3 nM for the H_3 receptor,[17] to be 93 times more selective for the H_3 receptor.

Table 1. Affinity of VUF 5000 for various binding sites in the central nervous system

Binding site	Radioligand	pIC_{50}	K_i (nM)
H_3	Iodophenpropit		2.3 ± 0.5
H_1	Pyrilamine	< 5	
α_1	Prozasin	< 5	
α_2	Clonidine	< 5	
α_{2a}	Rauwolscine	5.47	1894
α_{2b}	Rauwolscine	< 5	
α_{2c}	Rauwolscine	< 5	
β_1	Iodocyanopindolol	< 5	
β_2	Iodocyanopindolol	< 5	
Muscarine	Dexetimide	< 5	
D_1	SCH23390	< 5	
D_{21}	Spiperone	< 5	
D_3	Iodosulpride	< 5	
D_{4v2}	Spiperone	< 5	
$5HT_{1a}$	8-Hydroxy-DOPAT	< 5	
$5HT_{1b}$	8-Hydroxy-DOPAT	< 5	
$5HT_{1c}$	Mesulergine	< 5	
$5HT_{1d_\alpha}$	R91274	< 5	
$5HT_{1d_\beta}$	Alnitidan	< 5	
$5HT_{1e}$	Serotonine	< 5	
$5HT_{2a}$	R91150	< 5	
$5HT_3$	GR6530	5.03	4231
μ	Sufentanil	< 5	
κ	U69563	< 5	
δ	DPDPE	5.16	2874
σ	Haloperidol	6.28	214
Ca^{2+}-channel	Nitrendipine	< 5	
Na^+-channel	BTX	5	9774
Leukotriene	LTD_4	< 5	
PAF	PAF	< 5	
CCK_a	CCK-8	< 5	
CCK_b	CCK-8	< 5	
Substance P	Substance P	< 5	
BK_1	Bradykinine	< 5	
GABA uptake	GABA	< 5	

Experimental

An important requirement of PET radiopharmaceuticals is their selectivity. VUF 5000 proved to be a selective H_3 ligand, since it has only measurable affinity for the α_{2a} and $5HT_3$ receptors, the δ and σ binding sites and the Na^+-channel (Table 1). The highest affinity for receptors other then the H_3 receptor is found at the σ binding site (214 nM), still leaving VUF 5000, with a K_i of 2.3 nM for the H_3 receptor,[17] to be 93 times more selective for the H_3 receptor.

Also important for a potential brain imaging PET ligand is the $\text{Log}D_{\text{oct},7.2}$ value. This physico-chemical property may be used as indication for the capacity of the compound to pass the blood brain barrier. Hansch et al.[18] suggested this parameter for ionizable drugs instead of the distribution coefficient for neutral compounds, Log*P*, and calculated $\text{Log}D_{\text{oct},7.2}$ from the Log*P* value and the pK_a of the compound. We have measured the $\text{Log}D_{\text{oct},7.2}$ value by determining the partition of the radiolabeled compound between 1-octanol and 0.2 M phosphate buffer with a pH of 7.2. A value for $\text{Log}D_{\text{oct},7.2}$ between ca. 0 and ca. 3 is an indication that the lipophilicity of the compound is suited to allow crossing over the blood brain barrier.[19] We found the $\text{Log}D_{\text{oct},7.2}$ value of [^{18}F]VUF 5000 to be 1.88 ± 0.02.

The results of the biodistribution experiments in male Wistar rats are presented in Figures 1 and 2. The peripheral distribution of [^{18}F]VUF 5000, shows that [^{18}F]VUF 5000 is rapidly cleared from the blood, has a high uptake in the liver and kidney and a moderate uptake in lungs, stomach and intestine. The high uptake in the liver and kidney accounts for ca. 75% of the total injected dose. Given the measured $\text{Log}D_{\text{oct},7.2}$ of 1.88, the cerebral distribution (Fig. 2) shows a surprisingly low uptake in the brain. This is an indication that care must be taken with the interpretation of $\text{Log}D_{\text{oct},7.2}$ as has been suggested by Ter Laak et al.[18] and that the $\Delta\text{Log}P_{\text{oct-alk}}$ also must be taken into consideration in order to predict the BBB crossing characteristics of a compound more properly, especially when the Log$D_{\text{oct},7.2}$ value is ca. 2. However, for thioperamide it is known from ex vivo binding studies[15,16] that it penetrates into the brain. Since VUF 5000 is chemically strongly related to thioperamide, it is unlikely that the small chemical modification in VUF 5000 is responsible for this totally different in vivo behavior. Apparently the pharmacokinetic properties of a compound which is administered in tracer amounts can be rather different from its pharmacokinetic behavior when the same compound is administered in several mg/kg body weight.

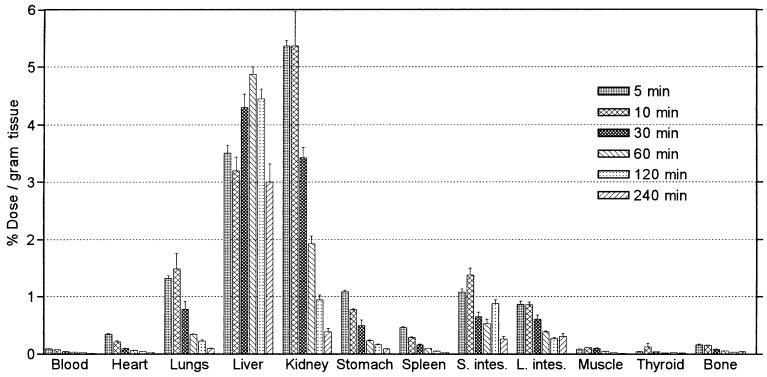


Figure 1. Biodistribution in peripheral tissues of [^{18}F]VUF 5000 in rats. Uptake is expressed as the % of the total injected dose divided by the weight of the organ ($n=4$).

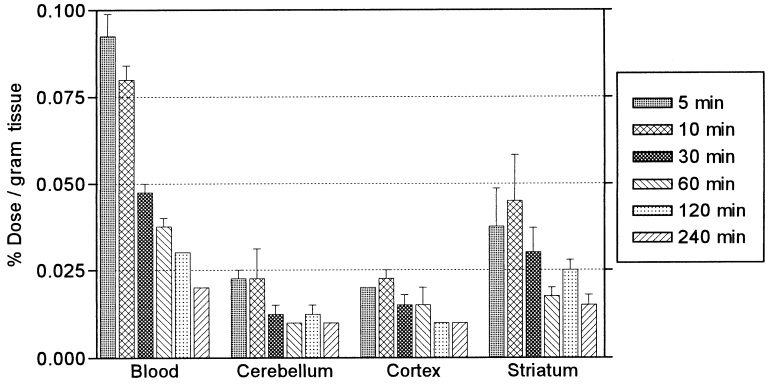


Figure 2. Biodistribution in cerebral tissues of [^{18}F]VUF 5000 in rats. Uptake is expressed as the % of the total injected dose divided by the weight of the organ ($n=4$).

To verify whether the total administered amount of compound is of influence on the biodistribution of [^{18}F]VUF 5000, we performed an experiment where male Wistar rats were administered with carrier added [^{18}F]VUF 5000. The control group was administered nca [^{18}F]VUF 5000, one group was administered carrier added [^{18}F]VUF 5000 at a dose of 0.4 μg/kg body weight and the other group was administered [^{18}F]VUF 5000 at a dose of 34 μg/kg body weight. The results are presented in Figures 3 and 4. From these results it can be concluded that the addition of carrier does not influence the biodistribution of [^{18}F]VUF 5000. Administering a higher dose of VUF 5000 is of no use, since in that case the dose gets into the pharmacologically active range of VUF 5000, which, in general, has to be avoided with PET.

Striking in the distribution of [^{18}F]VUF 5000 is the high liver uptake. If this uptake would be reduced, the clearance from the blood might become slower and because of its longer availability, the brain uptake of [^{18}F]VUF 5000 could become higher. As can be concluded from Figures 3 and 4, a higher dose of VUF 5000 does not influence the high liver uptake or the fast clearance from

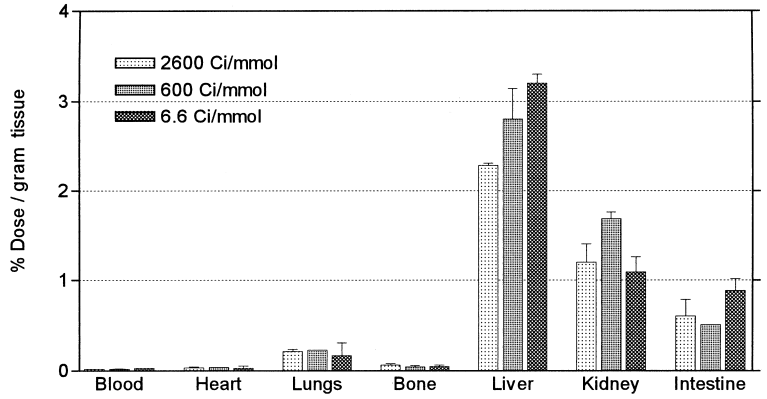


Figure 3. Biodistribution in peripheral tissues after 30 min of [^{18}F]VUF 5000 with three different specific activities in rats: 2600 Ci/mmol (nca), 600 Ci/mmol (0.4 μg/kg), and 6.6 Ci/mmol (34 μg/kg). Uptake is expressed as the % of the total injected dose divided by the weight of the organ ($n=2$).

the blood. An explanation for the high liver uptake can be an aspecific binding of [^{18}F]VUF 5000 to proteins and enzymes through the thiocarbonamide moiety of [^{18}F]VUF 5000. This moiety has two tautomeric forms, one with the proton on the nitrogen atom and one with the proton on the sulfur atom (Scheme 2). The tautomeric form with the thiol function is very nucleophilic, and is capable of reacting with proteins or enzymes. This could be the explanation why GT 2016, a thioperamide analogue where the thiocarbonamide moiety is substituted for an amide moiety, is in vitro less active than thioperamide, but shows a better in vivo profile with good brain penetration.[20] To verify whether protein binding via the thiocarbonamide can be an explanation for the biodistribution of [^{18}F]VUF 5000 we synthesized VUF 5182, an analogue of VUF 5000 with, instead of the sulfur, an oxygen atom, according to Scheme 3. VUF 5182 also has two tautomeric forms, but the resulting alcohol is only weakly nucleophilic and will not react as readily with proteins or enzymes as the thiol moiety of VUF 5000. We have synthesized VUF 5182 in a low yield of 12%, being acceptable for our purpose. The histamine H_3 receptor activity of VUF 5182 was determined, the pA_2 value was 7.0 ($\pm$0.1), which is 100 times less active then VUF 5000.

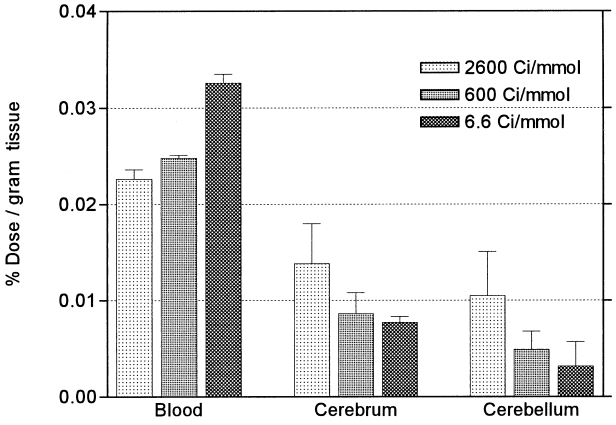
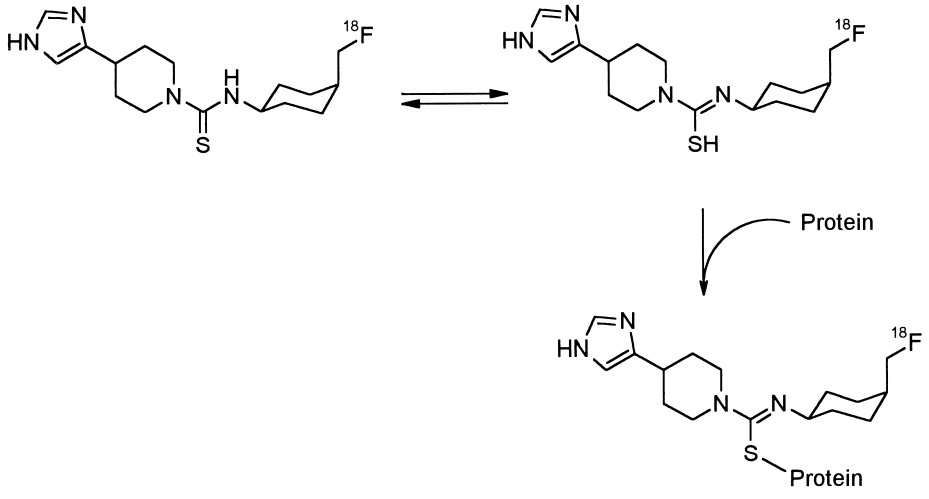
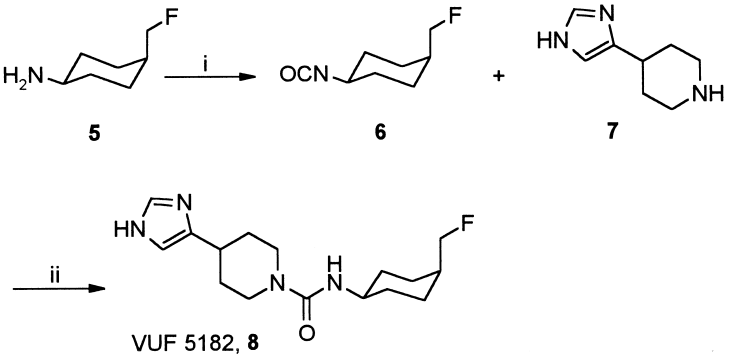


Figure 4. Biodistribution in cerebral tissues after 30 min of [^{18}F]VUF 5000 with three different specific activities in rats: 2600 Ci/mmol (nca), 600 Ci/mmol (0.4 μg/kg) and 6.6 Ci/mmol (34 μg/kg). Uptake is expressed as the % of the total injected dose divided by the weight of the organ ($n=2$).

[^{18}F]VUF 5182 was synthesized according to the same method as for [^{18}F]VUF 5000, but instead of 1,1′-thiocarbonyldi-2(1H)pyridone, trichloromethylchloroformate was used. Biodistribution experiments revealed that there is a significant difference in the distribution between [^{18}F]VUF 5000 and [^{18}F]VUF 5182 (Fig. 5). The uptake of [^{18}F]VUF 5281 by the liver and the kidney



Scheme 2. Tautomeric equilibrium of [^{18}F]VUF 5000 and hypothesized covalent binding to protein.



Scheme 3. Synthesis of VUF 5182. (i) trichloromethylchloroformate, CH_3CN, diisopropylethylamine, rt, 30 min; (ii) diisopropylethylamine, CH_3CN, rt, 18 h. Yield: (i + ii) 12%.

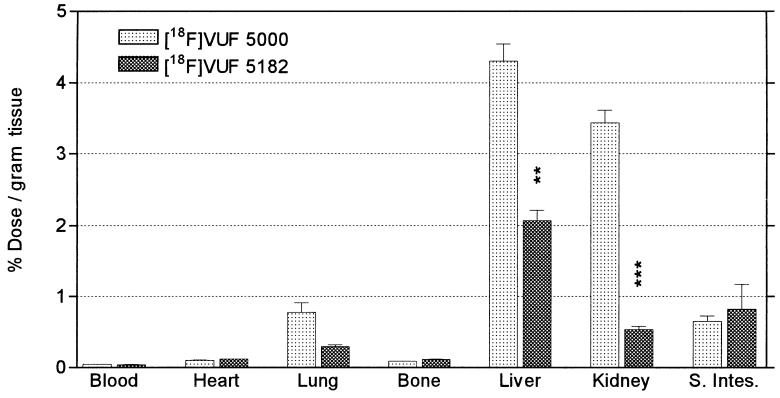


Figure 5. Biodistribution in peripheral tissues after 30 min of [^{18}F]VUF 5182 compared to [^{18}F]VUF 5000. Uptake is expressed as the % of the total injected dose divided by the weight of the organ. (**: $p < 0.01$; ***: $p < 0.001$, $n = 2$).

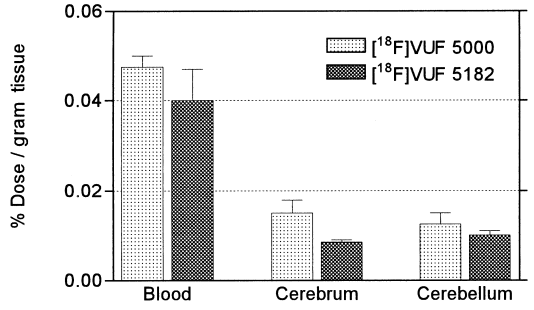


Figure 6. Biodistribution in cerebral tissues after 30 min of [^{18}F]VUF 5182 compared to [^{18}F]VUF 5000. Uptake is expressed as the % of the total injected dose divided by the weight of the organ ($n = 2$).

is indeed greatly reduced compared to [^{18}F]VUF 5000, yet the blood clearance is still fast and not significantly different from [^{18}F]VUF 5000.

The lower peripheral uptake of [^{18}F]VUF 5182 did not lead to a higher brain uptake, as can be seen in Figure 6. Apparently the thiocarbonamide moiety is at least partly responsible for the high liver uptake, but not for the low brain penetration of [^{18}F]VUF 5000.

Plazzi et al.[21] have investigated the binding of thioperamide to rat plasma proteins. They found that thioperamide is strongly bound to plasma proteins, especially at low concentrations. Since VUF 5000 is a chemically close analogue of thioperamide, the binding to plasma proteins could be an explanation for the low brain penetration of [^{18}F]VUF 5000. However, the biodistribution experiments showed a fast blood clearance of [^{18}F]VUF 5000. In order to verify these findings from the biodistribution experiments, we investigated the binding of [^{18}F]VUF 5000 to purified human serum albumin (HSA). After incubation at 37°C we separated bound and unbound [^{18}F]VUF 5000 by gel filtration. The results are shown in Figure 7. The fractions containing protein (measured with UV absorption at 210 nm) did not contain any radioactivity. The fractions containing radioactivity did not show UV absorption at 210 nm. From these data we conclude that at tracer amounts [^{18}F]VUF 5000 is practically unbound to HSA. Also at a concentration of 20 μM of VUF 5000, a concentration where Plazzi et al. found 95% binding to plasma proteins for thioperamide, we did not find any binding of [^{18}F]VUF 5000 to HSA. These results confirm the results from the biodistribution experiments and show that [^{18}F]VUF 5000 is hardly bound to plasma proteins and rapidly cleared from the blood.

Conclusion

In this paper we have evaluated [^{18}F]VUF 5000 for its potential use as a PET ligand for the histamine H_3 receptor. Biodistribution studies in rats showed a low brain uptake and the co-administeration of carrier VUF 5000 did not alter the biodistribution significantly. The high liver uptake can be reduced significantly by changing the thiocarbonamide moiety from [^{18}F]VUF 5000 to an amide moiety in [^{18}F]VUF 5182. However, this modification did not result in a higher brain uptake. The reason why [^{18}F]VUF 5000 does not penetrate the brain remains unrevealed, but it can be concluded that [^{18}F]VUF 5000 is, unfortunately, not suitable as a PET ligand for the histamine H_3 receptor.

Materials and Methods

^{1}H NMR spectra were recorded on a Bruker AC 200 (at 200.13 MHz for ^{1}H). Chemical shifts (δ) are determined relative to the solvent and converted to the TMS scale using $\delta = 2.50$ for DMSO-d_6. The synthesis of [^{18}F]VUF 5000 was performed as described previously,[17] [^{18}F]VUF 5000 having a specific activity (SA) of > 2600 Ci/mmol (determined by HPLC). All chemicals were purchased from Aldrich, solvents from Baker or Riedel–deHaen. Radiochemical reactions were carried out under an argon atmosphere and were monitored by thin layer chromatography (TLC) using Merck TLC plates (silica gel 60, F_{254}, 0.25 mm). The TLC plates were scanned using a MD 425s phosphorimager and quantified using the software package ImageQuant (version 4.2). All animal experiments were approved according to the guidelines of the law on animal experiments of The Netherlands.

Selectivity profile of VUF 5000. In a routine program (Department of Biochemical Pharmacology of the Janssen Research Foundation), the binding of VUF 5000

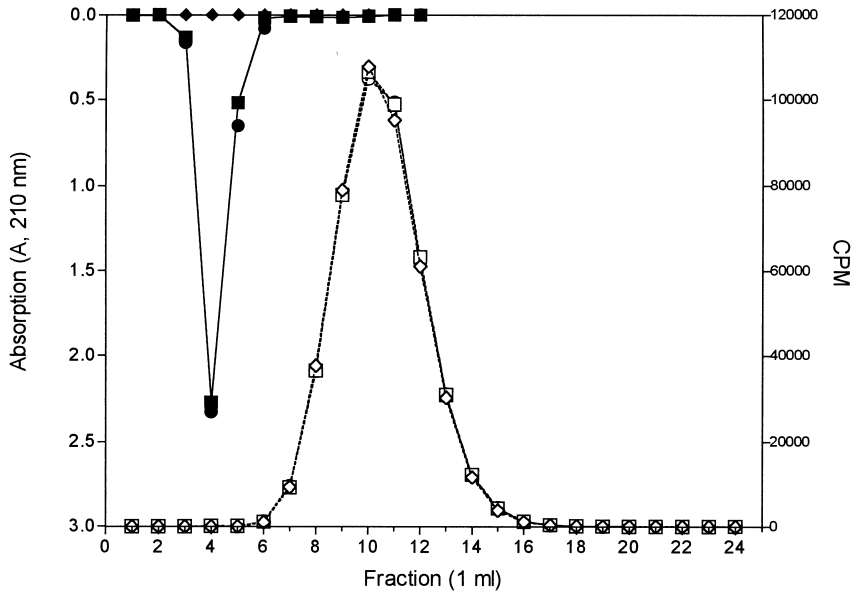


Figure 7. Gelfiltration separation of bound and unbound [^{18}F]VUF 5000 to human serum albumin (HSA). Experiment a: 148 μCi [^{18}F]VUF 5000 and no HSA added to the incubation (blanco experiment); experiment b: 149 μCi [^{18}F]VUF 5000 and 1 mg HSA; experiment c: 151 μCi [^{18}F]VUF 5000, 1 mg HSA and 20 μM VUF 5000. A = UV absorption at 210 nm; CPM = counts per minute. Recovery of the radioactivity >97%. (Legend: ◆, absorption experiment a; ■, absorption experiment b; ●, absorption experiment c; ◇, CPM experiment a; □, CPM experiment b; ○, CPM experiment c).

was tested for a series of 34 binding sites. The assays which were used, have been published previously.[22]

Synthesis of VUF 5182 (Scheme 3). *cis*-4-Fluoromethylcyclohexylamine **5** (116 mg, 0.5 mmol),[23] trichloromethyl-chloroformate (60.3 μL, 0.5 mmol) and diisopropylethylamine (0.52 mL, 3.0 mmol) were dissolved in 10 mL CH_3CN and stirred at room temperature under a nitrogen atmosphere for 30 min, resulting in the in situ formation of isocyanate **6**. Subsequently 4-[1(*H*)-imidazol-4-yl]piperidine dihydrobromic acid **7** (156 mg, 0.5 mmol)[24] was added. The reaction mixture was stirred for 18 h at room temperature under a nitrogen atmosphere. After evaporation of the solvent under reduced pressure, the residue was dissolved in 25 mL of ethyl acetate and washed with 15 mL of a saturated $NaHCO_3$ solution in water. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The product was isolated with flash column chromatography (silica 60, eluent: ethyl acetate/methanol = 4/1 + 2% (v/v) triethylamine). This yielded 85 mg of VUF 5182, which was still contaminated with unknown products. A second purification on a Merck Lobar RP-18 column (eluent H_2O/methanol 85/15) yielded 19 mg (0.06 mmol, 12%) of VUF 5182 **8**. R_f (ethyl acetate/methanol 7/3 + 2% (v/v) triethylamine) = 0.35; mp 134–136°C. ^{1}H NMR (DMSO-d_6) δ 1.45–1.68 (m, 10H, cyclohexane-H2,3,5,6 and piperidine-H3$_{ax}$,5$_{ax}$), 1.77–2.00 (m, 3H, piperidine-H2$_{ax}$,6$_{ax}$ and cyclohexane-H4), 2.61–2.76 (m, 1H, cyclohexane-H1), 3.20 (m, 2H, piperidine-H3$_{eq}$,5$_{eq}$), 4.52 (dd, $^3J_{FH}$ = 48 Hz, $^3J_{HH}$ = 7.0 Hz, 2H, CH_2F), 4.66 (m, 1H, piperidine-H4), 4.75 (m, 2H, piperidine-H2eq, H6eq), 6.80 (s, 1H, imidazole-H4), 7.63 (s, 1H, imidazole-H2).

Synthesis of [^{18}F]VUF 5182. The synthesis of [^{18}F]VUF 5182 was performed according to the same method as for [^{18}F]VUF 5000,[17] except that for the synthesis of [^{18}F]VUF 5182 trichloromethyl-chloroformate was used instead of 1,1′-thiocarbonyldi-2(1*H*)pyridone. This yielded [^{18}F]VUF 5182 in a radiochemical yield of 12% (corrected for decay), with a radiochemical purity >99.9% (assessed by HPLC) in a total synthesis time of 4 h.

Biodistribution studies with no carrier added [^{18}F]VUF 5000 and [^{18}F]VUF 5182. A solution of either [^{18}F]VUF 5000, synthesized with a radiochemical purity >99.9% (assessed by HPLC)[17] or [^{18}F]VUF 5182 in 0.2 M phosphate buffer (pH 7.2) was prepared with a concentration of 0.3 mCi/mL. Male wistar rats (250–300 g, Harlan bv, The Netherlands) were anaesthetized with diethyl ether and injected with 300 μl of the radiolabeled compounds into the tail vein. At 5, 10, 30, 60, 120 and 240 min for [^{18}F]VUF 5000 and at 30 min for [^{18}F]VUF 5182, the animals were again anaesthetized with diethyl ether and killed by cervical dislocation. The organs of interest were rapidly removed, and weighed. The radioactivity was counted in a LKB-Wallac 1282 compugamma CS. The amount of activity taken up by the organ is expressed as the percentage of the total dose divided by the weight of the dissected organ (% dose/gram tissue).

Biodistribution of carrier added [^{18}F]VUF 5000. Three solutions were prepared. One with no carrier added (nca) [^{18}F]VUF 5000 with a specific activity (SA) estimated at 2600 Ci/mmol, the second with carrier added VUF 5000 leading to a SA of 600 Ci/mmol (determined with HPLC) and the third with a SA of 6.6 Ci/mmol. Male Wistar rats (250–300 g) were injected with 175 μCi of the radiolabeled compound. After 30 min the animals were killed, the organs of interest were rapidly removed, weighed and counted for radioactivity.

In vitro pharmacology. The histamine H_3 activity of VUF 5182 was determined using the method described by Vollinga et al.[12] Briefly, the pA_2 value was determined in an in vitro test system, based on the concentration dependent inhibitory effect of H_3 agonists on electrically evoked contractile responses (acetylcholine release) of guinea pig jejunum preparations. The experiment has been performed four times in duplicate. As agonist [R]-α-methylhistamine was used [$pD_2 = 7.8$ (± 0.2), $n = 12$]; The Schild slopes were not significantly different from unity.

Determination of the Log$D_{oct,7.2}$ value of [^{18}F]VUF 5000. The lipophilicity of an ionizable compound can be expressed, according to Hansch et al.[18] as the Log$D_{oct,7.2}$, which is determined from the calculated Log$P$ and the pK_a of the compound. We have measured the Log$D_{oct,7.2}$ value of [^{18}F]VUF 5000, by the determination of the partitioning of [^{18}F]VUF 5000 between 1-octanol and 0.2 M phosphate buffer (pH 7.2). It was performed by mixing 1 mL of a 0.3 mCi/mL solution of [^{18}F]VUF 5000 in 0.2 M phosphate buffer (pH 7.2) with 1 mL 1-octanol for 1 min. After resting for 30 min, five samples of 100 μL were taken from both layers. As a reference, five samples of 100 μL of the 0.3 mCi/mL solution of [^{18}F]VUF 5000 in 0.2 M phosphate buffer (pH 7.2) were taken to determine the recovery. The amount of radioactivity of all samples was determined in LKB-Wallac 1282 compugamma CS. The Log$D_{oct,7.2}$ was calculated according to the formula: Log$D_{oct,7.2}$ = ^{10}Log((A_{oct}/A_{buffer}), with A_{oct} = average radioactivity of the five octanol samples (cpm/gram octanol) and A_{buffer} = average radioactivity of the five buffer samples (cpm/gram buffer). The experiment was performed in triplicate.

Human serum albumin binding. Five hundred mircrolitres of a 0.3 mCi/mL solution of [^{18}F]VUF 5000 in 0.2 M phosphate buffer (pH 7.2) was added to a solution of 1 mg of human serum albumin (HSA, obtained from the pharmacy of the Vrije Universiteit Hospital) in 1.5 mL of 0.2 M phosphate buffer (pH 7.2). The mixture was incubated for 15 min at 37°C in duplicate. After the incubation 1 mL of the mixture was brought onto a PD10 gel filtration column and eluted with 0.9% NaCl in water. Fractions of 1 mL were collected. As a reference five samples of 100 μL of the incubation solution were taken to determine the recovery of the radioactivity. The amount of radioactivity of all samples was determined with a LKB-Wallac 1282 compugamma CS.

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