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Synthesis and *in vitro* evaluation of ^{18}F-β-carboline alkaloids as PET ligands

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A one-step ^{18}F-labelling strategy was used to prepare four ^{18}F-labelled analogues of 7-methoxy-1-methyl-9*H*-β-carboline (harmine): 7-(2-[^{18}F]fluoroethoxy)-1-methyl-9*H*-β-carboline (5), 7-(3-[^{18}F]fluoro-propoxy)-1-methyl-9*H*-β-carboline (6), 7-[2-(2-[^{18}F]fluoroethoxy)ethoxy]-1-methyl-9*H*-β-carboline (7), and 7-{2-[2-(2-[^{18}F]fluoroethoxy)ethoxy]-ethoxy}-1-methyl-9*H*-β-carboline (8). These were synthesized as potential positron emission tomography ligands for monoamine oxidase A (MAO-A). A solution of pure labelled compound in buffer was obtained in $<$70 min from end of radionuclide production, with a decay-corrected yield of up to 23%. The average specific binding to MAO-A in rat brain, determined by autoradiography experiments, was highest for compounds 7 and 8 (89$\pm$2 and 96$\pm$1%, respectively), which was obtained at $<$1 nM radioligand concentration.

Keywords: harmine analogues; nucleophilic ^{18}F-fluorination; one-step labelling; β-carboline alkaloids; MAO-A

Introduction

Two β-carbolines, harmine and harmaline, which are based on an indole nucleus fused with a pyridine ring, can be isolated from *Penganum harmala*.[1] Harmine derivatives have various biological activities, such as interaction with benzodiazepine receptors,[2] inhibition of cyclin-dependent kinase,[3] and inhibition of monoamine oxidase (MAO),[4] a membrane-bound mitochondrial enzyme[5] with two subtypes, MAO-A and -B[6]. [^{11}C]Harmine has been used and validated *in vivo* in monkey brain to bind to MAO-A.[7] This tracer was also used in humans in a competition study with other MAO-A active drugs,[8] and its binding to MAO-A has been quantified in the human brain.[9] [^{11}C]Harmine has as well been used in a dual tracer positron emission tomography (PET) study together with [^{11}C]raclopride in pig[10] and its biodistribution and radiation dosimetry evaluated in baboons.[11] Recently we observed that the high expression of MAO-A could be exploited for characterization of neuroendocrine gastroenteropancreatic tumours by using [^{11}C]harmine as a tracer.[12] Thus, four ^{18}F-labelled analogues of harmine (Scheme 1) have been synthesized to explore their potential as selective MAO-A tracers using PET, taking advantage of the longer half-life of ^{18}F (110 min) compared with ^{11}C (20.3 min). The ^{18}F-label was placed in the side chain, to investigate the biological properties conferred by changes in its chemical properties (i.e. by modifying lipophilicity using a polyethylene glycol (PEG) strategy[13]). The binding properties of the four ^{18}F-labelled analogues were evaluated using autoradiography on sections from the rat brain, an organ that is known to have high density of MAO-A.[7]

Results and discussion

A number of tracers such as 7-[^{11}C]methoxy-1-methyl-9*H*-β-carboline ([^{11}C]HAR),[7,14] *N*-[3-(2,4-dichlorophenoxy)propyl]-*N*-[^{11}C]methylprop-2-yn-1-amine ([^{11}C]CLG),[7] and *N*-[3-(2,4-dichlorophenoxy)-2-[^{18}F]fluoropropyl]-*N*-methylprop-2-yn-1-amine ([^{18}F]CLG)[15] have previously been used and explored as PET tracers for characterization of MAO-A enzyme. Harmine was chosen as the basis of the ^{18}F-labelled analogues because attaching a side chain to its phenol group does not significantly alter its chemical properties, especially the pK_a (Table 2).

The syntheses of 7-(2-[^{18}F]fluoroethoxy)-1-methyl-9*H*-β-carboline (**5**), 7-(3-[^{18}F]fluoro-propoxy)-1-methyl-9*H*-β-carboline (**6**), 7-[2-(2-[^{18}F]fluoroethoxy)ethoxy]-1-methyl-9*H*-β-carboline (**7**), and 7-{2-[2-(2-[^{18}F]fluoroethoxy)ethoxy]ethoxy}-1-methyl-9*H*-β-carboline (**8**) as the target compounds from the corresponding 2-[(1-methyl-9*H*-β-carbolin-7-yl)oxy]ethyl 4-methylbenzenesulfonate (**1**), 3-[(1-methyl-9*H*-β-carbolin-7-yl)oxy]propyl 4-methylbenzenesulfonate (**2**), 7-[2-(2-bromoethoxy)ethoxy]-1-methyl-9*H*-β-carboline (**3**), and 7-{2-[2-(2-chloroethoxy)ethoxy]ethoxy}-1-methyl-9*H*-β-carboline (**4**) are outlined in Scheme 1.

The labelled compounds **5**–**8** were synthesized from the precursors **1**–**4** using no-carrier-added [^{18}F]fluoride in a one-step nucleophilic substitution (Scheme 1). Heating the reaction mixture containing a precursor and [K/K2.2.2.]$^{+}$^{18}F^{-} in 400 µL *N*,*N*-dimethylformamide (DMF) at 150°C for 15 min gave the

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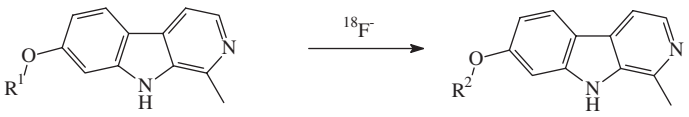


1: $R^1 = (CH_2)_2OTs$

2: $R^1 = (CH_2)_3OTs$

3: $R^1 = (CH_2)_2O(CH_2)_2Br$

4: $R^1 = (CH_2)_2O(CH_2)_2O(CH_2)_2Cl$

5: $R^2 = (CH_2)_2{}^{18}F$

6: $R^2 = (CH_2)_3{}^{18}F$

7: $R^2 = (CH_2)_2O(CH_2)_2{}^{18}F$

8: $R^2 = (CH_2)_2O(CH_2)_2O(CH_2)_2{}^{18}F$

Scheme 1. ^{18}F-Labelling reaction to yield compounds **5**–**8**.

Table 1. Radiochemical yields and specific activity in labelling reactions

Compound	RCY (%)[a]	Spec. act. (GBq/μmol)[b]
5	23 ± 3 (n = 4)	605 ± 110 (n = 4)
6	10 ± 2 (n = 2)	744 ± 30 (n = 2)
7	12 ± 3 (n = 5)	508 ± 160 (n = 5)
8	14 ± 4 (n = 4)	440 ± 100 (n = 2)

[a]Isolated decay-corrected radiochemical yield, calculated from the amount of radioactivity at the start of synthesis and radioactivity of LC purified product, n = number of experiments.
[b]Ratio of radioactivity to amount of substance at end of synthesis, n = number of experiments.

corresponding labelled product. The crude product was purified by semi-preparative high-performance liquid chromatography (HPLC). The decay-corrected radiochemical yield was in the range 10–23% (Table 1). Compound **5** was prepared with the highest radiochemical yield. The unlabelled reference substances to compounds **5**–**8** were synthesized by reacting the corresponding precursor with tetrabutylammonium fluoride in tetrahydrofuran (THF) at 90°C. These were used for identification in the analysis of the ^{18}F-labelled compounds in all the liquid chromatographic (LC) runs. The identities of compounds **5**, **7**, and **8** were confirmed by liquid chromatography mass spectrometry (LC-MS) analysis. In a typical experiment (compound **7**) starting with 7.60 GBq of [^{18}F]fluoride, 0.93 GBq of purified product was obtained within 70 min from end of radionuclide production. Specific activity was determined for compounds **5**–**8** as illustrated in Table 1. The radiochemical purity exceeded 98% in all labelling reactions. In an attempt to increase the radiochemical yield *tert*-butanol was added to the labelling reaction,[16] but in this case it actually decreased the yield.

The precursors **1**–**4** were synthesized from 1-methyl-9*H*-β-carbolin-7-ol (harmol) and either the corresponding alkyl di(*p*-toluenesulfonate), 1-bromo-2-(2-bromoethoxy)ethane, or 1-chloro-2-[2-(2-chloroethoxy)ethoxy]ethane (Scheme 2). These were combined with caesium carbonate in dry DMF at 0°C or, in some cases, at -78°C. After the reagents were added, the temperature was slowly raised to room temperature. Attempts to perform the reactions at higher temperature resulted in very poor yields, or even in exclusive formation of side products. The structures of **1**–**4** were elucidated using ^{1}H–^{13}C heteronuclear multiple bond coherence nuclear magnetic resonance (^{1}H–^{13}C HMBC NMR) experiments to confirm that the precursors were *O*-alkylated and not *N*-alkylated. It should be pointed out that **1**–**4** are not stable when stored at -20°C for more than approximately 1 month. Compounds **3** and **4** are stable for 6 months when stored at -70°C, but **1** and **2** are not. The order of magnitude of the logP values calculated using ACD-labs[17] for compounds **5**–**8** were in agreement with the retention times of the respective compounds using a standardized analytical HPLC program (Table 2).

Several approaches for the preparation of **1**, the precursor to the ethyl analogue **5**, were explored. Three different bases, pyridine, potassium *tert*-butoxide, and caesium carbonate, were used. Caesium carbonate was successful in creating the phenoxide, and was therefore used in all precursor syntheses.

In vitro autoradiography experiments were carried out to investigate the binding properties of labelled compounds **5**–**8** to MAO-A in rat brain (Figure 1). All compounds showed some degree of specific binding to the cerebral cortex and striatum; both areas have high densities of the target enzyme MAO-A. The uptake was similar in the different brain regions, with slightly higher binding observed in the anterior cingulate cortex and dorsal striatum. The average percentage of specific binding was highest for compounds **7** and **8**, as illustrated in Table 3. For comparison, [^{11}C]HAR has specific binding of 78–86%.[14]

The ^{18}F label was placed in different side chains in order to obtain a set of compounds with a range of lipophilicities. PEG segments of different lengths were used in compounds **7** and **8** to give different lipophilicities[13] relative to the ethyl and propyl analogues **5** and **6**. The lower lipophilicity of **7** and **8** (Table 2) might explain their higher specific binding.

Compounds **7** and **8** were chosen for metabolite analysis because of their high specific binding and the relative stability of the corresponding precursors. The fraction of unmetabolized tracer in rat blood plasma was 26 and 52% after 5 min, and 4 and 12% after 30 min for compounds **7** and **8**, respectively. The use of a pegylated side chain in which the ^{18}F label was placed might also decrease the metabolism, but this has not been investigated in this study.

Experimental

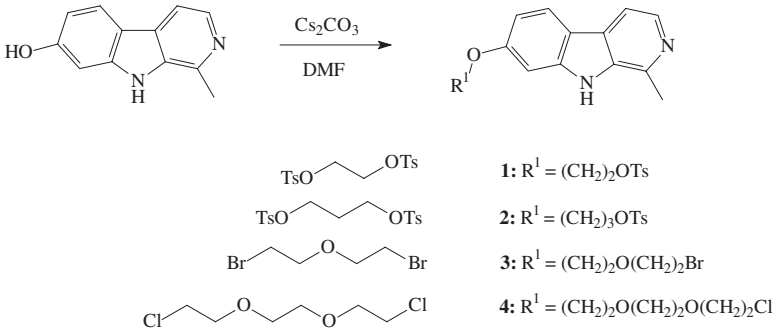
Radiosyntheses

No-carrier-added aqueous [^{18}F]fluoride was produced from water 95% enriched in ^{18}O (Rotem Industries Ltd., Israel or Taiyo Nippon Sanso Corporation) by the nuclear reaction

Table 2. Retention times on analytical HPLC and calculated log *P* and pK_a values for compounds **5**–**8**

Compound	log *P*[a]	Retention time[b] (min)	pK_a[c] Indole	pK_a[c] Pyridine
5	3.40 ± 1.08	7.6	15.61 ± 0.40	8.12 ± 0.40
6	3.69 ± 1.07	8.1	15.52 ± 0.40	8.04 ± 0.40
7	3.00 ± 1.11	7.3	15.57 ± 0.40	8.08 ± 0.40
8	2.64 ± 1.14	7.2	15.57 ± 0.40	8.09 ± 0.40

[a]Calculated using ACD-labs.[17]
[b]Retention time on analytical HPLC column.
[c]Approximated apparent pK_a values of indole and pyridine nitrogen, calculated using ACD-labs.[17]



Scheme 2. Synthesis of precursors **1**–**4**.

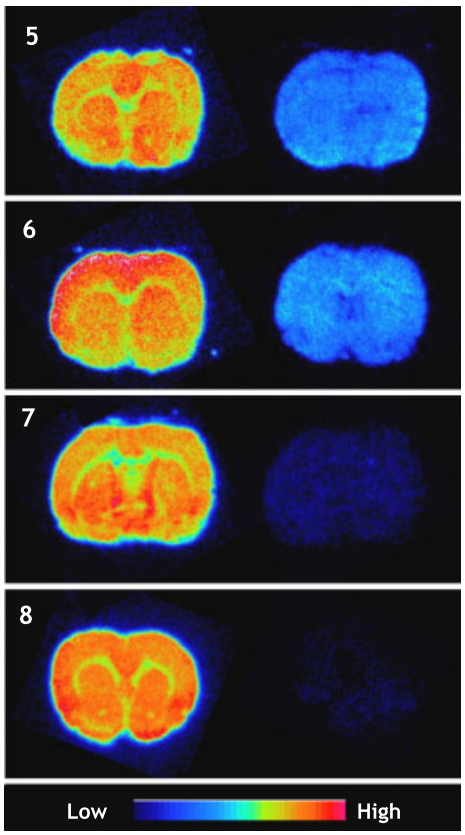


Figure 1. Colour-coded images of total and non-specific binding of the four harmine analogues **5**–**8**.

Table 3. Percent specific binding of the four harmine analogues determined from *in vitro* autoradiography

Compound	Specific binding (%)	Ligand concentration (nM)	*n*[a]
5	48 ± 6	0.212 ± 0.064	12
6	48 ± 8	0.188 ± 0.081	7
7	89 ± 2	0.161 ± 0.059	10
8	96 ± 1	0.170 ± 0.063	10

[a]*n* = number of experiments.

$^{18}O(p,n)^{18}F$ using a Scanditronix MC-17 cyclotron at Uppsala Imanet. The produced solution of [^{18}F]fluoride in water was transferred from the cyclotron target by HPLC pump and trapped on a QMA filter (ABX, advanced biochemical compounds, pre-conditioned Sep-PAK®, light QMA cartridge with CO_3^{2-} as counter ions, Radeberg). The QMA filter was purged with helium for 3 min and thereafter the [^{18}F]fluoride was released with a 2 mL solution of 96:4 (by volume, total volume 12 mL) acetonitrile–water mixture containing 55.9 mg of Kryptofix 2.2.2 (K2.2.2) and 12.7 mg K_2CO_3. The eluted ^{18}F/Kryptofix/K_2CO_3 solution was dried under N_2 (g) at 110°C and then with 2 × 1 mL dry acetonitrile. Synthia,[18] an automated synthesis system, was used for the handling of the reagents. LC analysis was performed with a VWR Hitachi Pump L-2130 and a VWR Hitachi UV Detector L-2400 UV detector in series with a β^+-flow detector. A Discovery® C18, Supelco, 25 cm × 4.6 mm, 5 µm HPLC column was used. The following mobile phases were used: 25 mM KH_2PO_4 in water (A) and acetonitrile/water 50:7 (B). Program: 10–100% (B) over 5 min, then 100% for 10 min, flow rate 1.5 mL/min. For semi-preparative LC an ACE 5C18HL, Scantec Lab, 250 mm × 10 mm, 5 µm column was used at a flow rate of 5 mL/min. Acetonitrile (25%) and 0.05 M ammonium formate pH 3.5 (75%) were used as eluent. The identities of the

^{18}F-labelled compounds were confirmed by co-injection of isotopically unmodified reference substances in all the LC runs. LC-MS analyses of ^{18}F-labelled compounds were performed using a Micromass VG Quattro mass spectrometer with electrospray ionization (ESI), in series with a β^+-flow detector. A Jones Chromatography Genesis® C18, Scantec Lab, 10 cm × 4 mm, 4 μm HPLC column was used. The following mobile phases were used: 5 mM ammonium acetate (A) and acetonitrile (B). Program: 10–90% (B) over 10 min, then 90% for 5 min, flow rate 1 mL/min. The identities of compounds **5**, **7**, and **8** were confirmed by LC-MS analysis. Radioactivity was measured in an ion chamber, Veenstra Instrumenten BV, VDC-202. All chemicals were obtained from commercial suppliers and used without further purification.

General labelling procedure

The precursor (**1**–**4**) (≈15 μmol) was dissolved in 200 μL dry DMF and added to a solution of the dried [K/K2.2.2.]$^+$^{18}F$^-$ in 200 μL dry DMF. The reaction mixture was heated at 150°C for 15 min in a heating block, then diluted with 2 mL water and purified by semi-preparative HPLC. Prior to autoradiography experiments, the isolated compound was dissolved in 3 mL of a solution containing phosphate buffer (pH 7.4)/PEG 4:1. In metabolite experiments, only phosphate buffer (2 mL) was used.

LC-MS data of labelled compounds

(**5**): Retention time = 7.1 min, *m/z* = 245 $[M+H]^+$; (**7**): retention time = 6.7 min, *m/z* = 289 $[M+H]^+$; (**8**): retention time = 6.5 min, *m/z* = 333 $[M+H]^+$.

Chemical synthesis

^{1}H NMR, ^{13}C NMR, ^{19}F NMR, and HMBC spectra were recorded on a Varian Unity (^{1}H at 400 MHz, ^{13}C at 100 MHz, ^{19}F at 376 MHz) or Varian Inova (^{1}H at 500 MHz, ^{13}C at 126 MHz) spectrometer using $CDCl_3$ or CD_3OD as solvent (solvent peak used as reference, except in the case of ^{19}F where $CFCl_3$ was used as reference). All NMR experiments were conducted at 25°C. Thin-layer chromatography (TLC) was performed on Merck silica gel F-254 aluminium plates. Silica gel 60, particle size 0.040–0.063 mm (Merck), was used for column chromatography. One millimetre pre-coated plates of silica gel 60, F-254 (Merck), were used for preparative TLC. LC-MS analyses were performed using a Gilson HPLC and Finnigan AQA mass spectrometer in ESI mode.

*2-[(1-Methyl-9H-β-carbolin-7-yl)oxy]ethyl 4-methylbenzenesulfonate (**1**)*

1-Methyl-9*H*-β-carbolin-7-ol (harmol) (1.497 g, 7.77 mmol) was dissolved in 5 mL dry DMF. Anhydrous caesium carbonate (3.721 g, 11.42 mmol) was added and the suspension was stirred under a nitrogen atmosphere at room temperature for 1 h. After cooling to -78°C, a solution of ethylene di(*p*-toluenesulfonate) (3.093 g, 8.35 mmol) in 10 mL dry DMF was added. The reaction mixture was kept at -78°C for 2 h before the temperature was slowly raised to room temperature. After 14 h more, the reaction mixture was extracted with ethyl acetate (5 × 100 mL) and water (200 mL). The combined organic phases were concentrated under reduced pressure and the residue purified by column chromatography (dichloromethane/methanol 10:1) to yield **1** as a yellow oil (0.246 g, 8%). ^{1}H NMR (400 MHz, CD_3OD, 25°C) δ = 8.08 (d, $^3J_{H,H}$ = 5.5 Hz, 1H), 7.90 (dd, $^5J_{H,H}$ = 0.5, $^3J_{H,H}$ = 8.7 Hz, 1H), 7.76 (AA′XX′, 2H), 7.74 (d, $^3J_{H,H}$ = 5.5 Hz, 1H), 7.32 (AA′XX′, 2H), 6.90 (d, $^4J_{H,H}$ = 2.2 Hz, 1H), 6.71 (dd, $^4J_{H,H}$ = 2.2, $^3J_{H,H}$ = 8.7 Hz, 1H), 4.41–4.38 (m, 2H), 4.24–4.21 (m, 2H), 2.73 (s, 3H), 2.33 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD 25°c) δ = 160.8, 146.4, 143.9, 142.1, 137.9, 136.3, 134.4, 130.9, 130.1, 128.9, 123.5, 116.9, 113.3, 111.0, 96.9, 70.1, 67.2, 21.5, 19.4. ESI-MS: *m/z* = 397 $[M+H]^+$.

*3-[(1-Methyl-9H-β-carbolin-7-yl)oxy]propyl 4-methylbenzenesulfonate (**2**)*

1-Methyl-9*H*-β-carbolin-7-ol (harmol) (0.050 g, 0.25 mmol) was dissolved in 2 mL dry DMF. Anhydrous caesium carbonate (0.124 g, 0.38 mmol) was added and the suspension was stirred under a nitrogen atmosphere at room temperature for 30 min. After cooling to -78°C, a solution of propylene di(*p*-toluenesulfonate) (0.106 g, 0.28 mmol) in 2 mL dry DMF was added. The reaction mixture was kept at -78°C for 2 h before the temperature was slowly raised to room temperature. After another 16 h, the reaction mixture was extracted with ethyl acetate (3 × 100 mL) and water (200 mL). The combined organic phases were concentrated under reduced pressure and the residue was purified by column chromatography (dichloromethane/methanol 10:1) yielding **2** as a yellow oil (0.015 g, 15%). ^{1}H NMR (400 MHz, CD_3OD, 25°C) δ = 8.10 (d, $^3J_{H,H}$ = 5.5 Hz, 1H), 7.94 (d, $^3J_{H,H}$ = 8.7 Hz, 1H), 7.79 (d, $^3J_{H,H}$ = 5.5 Hz, 1H), 7.70 (AA′XX′, 2H), 7.18 (AA′XX′, 2H), 6.87 (d, $^4J_{H,H}$ = 2.0 Hz, 1H), 6.68 (dd, $^4J_{H,H}$ = 2.0, $^3J_{H,H}$ = 8.7 Hz, 1H), 4.27 (t, $^3J_{H,H}$ = 6.0 Hz, 2H), 3.99 (t, $^3J_{H,H}$ = 5.8 Hz, 2H), 2.77 (m, 3H), 2.15 (s, 3H), 2.14–2.09 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD, 25°C) δ = 161.2, 146.2, 143.9, 141.8, 137.5, 136.0, 134.0, 130.8, 128.6, 126.9, 123.3, 116.4, 113.2, 111.1, 96.3, 68.6, 64.6, 29.9, 21.4, 19.4. ESI-MS: *m/z* = 411 $[M+H]^+$.

*7-[2-(2-Bromoethoxy)ethoxy]-1-methyl-9H-β-carboline (**3**)*

1-Methyl-9*H*-β-carbolin-7-ol (harmol) (0.497 g, 2.51 mmol) was dissolved in 10 mL dry DMF. Anhydrous caesium carbonate (1.135 g, 3.79 mmol) was added and the suspension was stirred under a nitrogen atmosphere at room temperature for 30 min. After cooling to 0°C, 1-bromo-2-(2-bromoethoxy)ethane (0.64 mL, 5.06 mmol) was added. The reaction mixture was kept at 0°C for 1 h before the temperature was slowly raised to room temperature. After another 3 h, the reaction mixture was extracted with ethyl acetate (3 × 100 mL) and water (200 mL). The combined organic phases were concentrated under reduced pressure and the residue was purified by column chromatography (dichloromethane/methanol 10:1.5) to yield **3** as a yellow oil (0.235 g, 25%). ^{1}H NMR (400 MHz, $CDCl_3$, 25°C) δ = 9.60 (bs, 1H), 8.25 (d, $^3J_{H,H}$ = 5.5 Hz, 1H), 7.93 (d, $^3J_{H,H}$ = 8.6 Hz, 1H), 7.72 (d, $^3J_{H,H}$ = 5.5 Hz, 1H), 7.07 (d, $^4J_{H,H}$ = 2.0 Hz, 1H), 6.90 (dd, $^4J_{H,H}$ = 2.0, $^3J_{H,H}$ = 8.6 Hz, 1H), 4.18 (m, 2H), 3.88 (m, 4H), 3.48 (t, $^3J_{H,H}$ = 6.4 Hz, 2H), 2.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 159.6, 142.5, 140.8, 136.5, 134.8, 128.5, 122.3, 115.1, 112.1, 110.1, 95.4, 71.0, 69.2, 67.4, 30.3, 19.9. ESI-MS: *m/z* (%) = 349 (50.5), 351 (49.5) $[M+H]^+$.

*7-{2-[2-(2-Chloroethoxy)ethoxy]ethoxy}-1-methyl-9H-β-carboline (**4**)*

1-Methyl-9*H*-β-carbolin-7-ol (harmol) (0.500 g, 2.52 mmol) was dissolved in 10 mL dry DMF. Anhydrous caesium carbonate

(0.985 g, 3.02 mmol) was added and the suspension was stirred under a nitrogen atmosphere at room temperature for 30 min. After cooling to 0°C, 1-chloro-2-[2-(2-chloroethoxy)ethoxy]ethane (0.943 g, 5.04 mmol) was added. The reaction mixture was kept at 0°C for 2 h before the temperature was slowly raised to room temperature. After another 24 h, the reaction mixture was extracted with ethyl acetate (3 × 100 mL) and water (200 mL). The combined organic phases were concentrated under reduced pressure and the residue was purified by column chromatography (dichloromethane/methanol 10:1.5) to yield **4** as a colourless oil (0.037 g, 4%). ^{1}H NMR (400 MHz, $CDCl_3$, 25°C) δ = 9.21 (bs, 1H), 8.29 (d, $^{3}J_{H,H}$ = 5.6 Hz, 1H), 7.93 (d, $^{3}J_{H,H}$ = 8.7 Hz, 1H), 7.72 (d, $^{3}J_{H,H}$ = 5.6 Hz, 1H), 7.00 ($^{4}J_{H,H}$ = 2.2 Hz, 1H), 6.90 (dd, $^{4}J_{H,H}$ = 2.2, $^{3}J_{H,H}$ = 8.7 Hz, 1H), 4.21–4.18 (m, 2H), 3.91–3.88 (m, 2H), 3.77–3.69 (m, 6H), 3.61 (t, $^{3}J_{H,H}$ = 6.1 Hz, 2H), 2.83 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 160.2, 142.2, 140.7, 137.7, 134.8, 129.0, 122.6, 115.9, 112.3, 110.5, 95.9, 71.4, 70.9, 70.8, 69.8, 68.0, 42.7, 19.8. ESI-MS: *m/z* (%) = 348 (75.5), 350 (24.5) $[M+H]^+$.

*7-(2-Fluoroethoxy)-1-methyl-9*H*-β-carboline (unlabelled reference to **5**)*

2-[(1-Methyl-9*H*-β-carbolin-7-yl)oxy]ethyl 4-methylbenzenesulfonate (**1**) (0.020 g, 0.050 mmol) was dissolved in 0.5 mL dry THF and tetrabutylammonium fluoride (0.03 mL, 1 M in THF) was added. The reaction mixture was heated at 65°C for 2 h and then extracted with ethyl acetate (3 × 50 mL) and water (100 mL). The combined organic phases were concentrated under reduced pressure and the residue was purified by preparative TLC (dichloromethane/methanol 10:1) to yield 7-(2-fluoroethoxy)-1-methyl-9*H*-β-carboline as a colourless oil (0.002 g, 16%). ^{1}H NMR (500 MHz, $CDCl_3$, 25°C) δ = 8.34 (d, $^{3}J_{H,H}$ = 5.3 Hz, 1H), 8.22 (bs, 1H), 7.98 (d, $^{3}J_{H,H}$ = 8.9 Hz, 1H), 7.72 (d, $^{3}J_{H,H}$ = 5.3 Hz, 1H), 6.99 (d, $^{4}J_{H,H}$ = 2.1 Hz, 1H), 6.93 (dd, $^{4}J_{H,H}$ = 2.1, $^{3}J_{H,H}$ = 8.9 Hz, 1H), 4.83 (dm, $^{2}J_{H,F}$ = 47.3 Hz, 2H), 4.33 (dm, $^{3}J_{H,F}$ = 27.6 Hz, 2H), 2.80 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 159.5, 141.4, 141.1, 139.0, 134.7, 128.5, 122.8, 116.4, 112.3, 109.8, 95.9, 81.9 (d, $^{1}J_{C,F}$ = 172.3 Hz), 67.5 (d, $^{2}J_{C,F}$ = 20.7 Hz), 20.2. ^{19}F NMR (376 MHz, $CDCl_3$, 25°C) δ = −222.3 (m). ESI-MS: *m/z* = 245 $[M+H]^+$.

*7-(2-Fluoropropoxy)-1-methyl-9*H*-β-carboline (unlabelled reference to **6**)*

3-[(1-Methyl-9*H*-β-carbolin-7-yl)oxy]propyl 4-methylbenzenesulfonate (**2**) (0.517 g, 1.26 mmol) was dissolved in 5 mL dry DMF and tetrabutylammonium fluoride (1.26 mL, 1 M in THF) was added. The reaction mixture was heated at 90°C for 2.5 h and then extracted with ethyl acetate (3 × 50 mL) and water (100 mL). The combined organic phases were concentrated under reduced pressure and the residue was purified by column chromatography (dichloromethane/methanol 10:1) to yield 7-(2-fluoropropoxy)-1-methyl-9*H*-β-carboline as a colourless oil (0.070 g, 22%).^{1}H NMR (400 MHz, $CDCl_3$, 25°C) δ = 8.27 (d, $^{3}J_{H,H}$ = 5.4 Hz, 1H), 7.93 (d, $^{3}J_{H,H}$ = 8.7 Hz, 1H), 7.71 (d, $^{3}J_{H,H}$ = 5.4 Hz, 1H), 6.95 (d, $^{4}J_{H,H}$ = 2.2 Hz, 1H), 6.87 (dd, $^{4}J_{H,H}$ = 2.2, $^{3}J_{H,H}$ = 8.7 Hz, 1H), 4.63 (dt, $^{3}J_{H,H}$ = 5.9, $^{2}J_{H,F}$ = 47.0 Hz, 2H), 4.11 (t, $^{3}J_{H,H}$ = 6.2 Hz, 2H), 2.80 (s, 3H), 2.16 (dm, $^{3}J_{H,F}$ = 25.6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 160.1, 142.2, 141.0, 138.0, 135.0, 128.9, 122.6, 115.9, 112.3, 110.1, 95.7, 80.7 (d, $^{1}J_{C,F}$ = 164.6 Hz), 64.0 (d, $^{3}J_{C,F}$ = 5.3 Hz), 30.4 (d, $^{2}J_{C,F}$ = 20.1 Hz), 19.8. ^{19}F NMR (376 MHz, $CDCl_3$, 25°C) δ = −222.5 (m). ESI-MS: *m/z* = 259 $[M+H]^+$.

*7-[2-(2-Fluoroethoxy)ethoxy]-1-methyl-9*H*-β-carboline (unlabelled reference to **7**)*

7-[2-(2-Bromoethoxy)ethoxy]-1-methyl-9*H*-β-carboline (**3**) (0.085 g, 0.29 mmol) was dissolved in 5 mL dry THF. Dry DMF (0.1 mL) and tetrabutylammonium fluoride (0.6 mL, 1 M in THF) were added. The reaction mixture was heated at 65°C for 8.5 h and then extracted with ethyl acetate (3 × 50 mL) and water (100 mL). The combined organic phases were concentrated under reduced pressure and the residue was purified by preparative TLC (dichloromethane/methanol 10:2) to yield 7-[2-(2-fluoroethoxy)ethoxy]-1-methyl-9*H*-β-carboline as a colourless oil (0.004 g, 5 %). ^{1}H NMR (400 MHz, $CDCl_3$, 25°C) δ = 8.31 (d, $^{3}J_{H,H}$ = 5.2 Hz, 1H), 7.97 (d, $^{3}J_{H,H}$ = 8.8 Hz, 1H), 7.73 (d, $^{3}J_{H,H}$ = 5.2 Hz, 1H), 7.02 (d, $^{4}J_{H,H}$ = 2.2 Hz, 1H), 6.94 (dd, $^{4}J_{H,H}$ = 2.2, $^{3}J_{H,H}$ = 8.8 Hz, 1H), 4.62 (dm, $^{2}J_{H,F}$ = 47.6 Hz, 2H), 4.30–4.27 (m, 2H), 4.00–3.97 (m, 2H), 3.86–3.84 (m, 2H), 2.83 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 159.9, 141.7, 140.9, 138.5, 134.7, 128.6, 122.6, 116.0, 112.2, 110.0, 95.7, 84.0, 82.3, 70.7, 70.5, 67.8, 66.3, 20.0 ppm. ^{19}F NMR (376 MHz, $CDCl_3$, 25°C) δ = −222.0 (m). ESI-MS: *m/z* = 289 $[M+H]^+$.

*7-{2-[2-(2-Fluoroethoxy)ethoxy]ethoxy}-1-methyl-9*H*-β-carboline (unlabelled reference to **8**)*

7-{2-[2-(2-Chloroethoxy)ethoxy]ethoxy}-1-methyl-9*H*-β-carboline (**4**) (0.020 g, 0.058 mmol) was dissolved in 2 mL dry THF and tetrabutylammonium fluoride (0.5 mL, 1 M in THF) was added. The reaction mixture was heated at 65°C for 48 h and then extracted with ethyl acetate (3 × 50 mL) and water (100 mL). The combined organic phases were concentrated under reduced pressure and the residue was purified by preparative TLC (dichloromethane/methanol 10:1) to yield 7-{2-[2-(2-fluoroethoxy)ethoxy]ethoxy}-1-methyl-9*H*-β-carboline as a colourless oil (0.002 g, 11%). ^{1}H NMR (400 MHz, $CDCl_3$, 25°C) δ = 8.16 (d, $^{3}J_{H,H}$ = 5.0 Hz, 1H), 7.84 (d, $^{3}J_{H,H}$ = 8.8 Hz, 1H), 7.63 (d, $^{3}J_{H,H}$ = 5.0 Hz, 1H), 7.23 (d, $^{3}J_{H,H}$ = 2.1 Hz, 1H), 6.78 (dm, $^{3}J_{H,H}$ = 8.8 Hz, 1H), 4.50 (dm, $^{2}J_{H,F}$ = 47.7 Hz, 2H), 3.78–3.55 (m, 8H), 3.20–3.13 (m, 2H), 2.91 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 159.6, 142.8, 141.5, 136.6, 135.0, 128.2, 121.9, 115.1, 111.7, 110.1, 96.0, 83.9, 82.2, 71.2, 70.6, 70.4, 70.2, 69.6, 67.6, 20.5. ^{19}F NMR (376 MHz, $CDCl_3$, 25°C) δ = −222.9 (m). ESI-MS: *m/z* = 333 $[M+H]^+$.

Biology

Autoradiography

Brains from male Sprague–Dawley rats were sectioned at 25 μm using a microtome (Microm HM 560, Microm, Germany) and stored at -20°C until used. Sections with striatum were incubated in 0.01–5 nM tracer in phosphate-buffered saline (PBS) buffer for 40 min at room temperature. For the determination of non-specific binding, 1 μM harmine (~500 × k_d for [^{11}C]HAR, 2.0 ± 0.7 nM[14]) was added to the incubate. The sections were washed 3 times in PBS buffer on ice for 3 min, briefly washed in distilled water, dried for approximately 10 min at 37°C, and placed on a phosphor imager plate (Amersham Biosciences, USA) for exposure for 2 h (approximately one half-life). The plates were scanned at 100 μm resolution using a Phospho imager Model 400S (Molecular Dynamics, USA). The autoradiograms were analyzed using ImageQuant 5.1 (Molecu-

lar Dynamics, USA). Aliquots (20 μL) of the incubation solution were dropped onto a filter paper and scanned together with the sections to be used as a calibration. Two to five experiments, each with sections from one or two different rats, were performed for each tracer. Four concentrations were used in each experiment.

Metabolite study

After the radioactive compound in the buffer solution was injected into a Sprague–Dawley rat, blood samples were withdrawn at predefined times and centrifuged. After addition of acetonitrile containing isotopically unmodified **7** or **8**, respectively, to the plasma samples, centrifugation, and filtering, the samples were analyzed using a Beckman Coulter Ultraphere ODS 250 × 10 mm, 5 μm LC column at a flow of 5 mL/min. Acetonitrile (20%) and 0.05 M ammonium formate pH 3.5 (80%) were used as eluent.

Conclusion

Four ^{18}F-labelled harmine analogues have been synthesized using a one-step nucleophilic ^{18}F-fluorination strategy. Pure labelled compound was obtained in less than 70 min from the end of radionuclide production, with a decay-corrected yield up to 23%. Specific radioactivity was in the range 400–700 GBq/μmol. The two analogues **7** and **8**, having PEG side chains, showed higher specific binding to MAO-A in *in vitro* experiments than did the corresponding tracer [^{11}C]HAR.[14] The fraction of unmetabolized labelled tracer in rat blood plasma was highest for compound **8**, when compared with the other pegylated analogue **7**, and will be used in further studies.

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