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Synthesis and biodistribution of both (±)-5-[^{18}F]-fluoroethoxy and (±)-5-[^{18}F]-fluoropropoxy piperazine analogs of benzovesamicol as vesicular acetylcholine transporter ligands (VAChT)

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Abstract. The radiosynthesis and preliminary biological evaluation in rats' brain of two novel piperazine analogs of benzovesamicol as ligands for the vesicular acetylcholine transporter (VAChT) have been carried out. The novel benzovesamicol derivatives 5-(2-fluoroethoxy)-3-(4-phenylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-2-ol and 5-(3-fluoropropoxy)-3-(4-phenylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-2-ol [(±)-[^{18}F]-6 and (±)-[^{18}F]-7] were successfully labelled with fluorine-18 from their tosylate precursors, with radiochemical purities greater than 98% and with radiochemical yield in the range of 5–6%. To determine whether these compounds could have potential advantage compared to [^{125}I]-iodo benzovesamicol (IBVM), IBVM was also labelled and used as the reference compound in all *in vivo* experiments. Both (±)-[^{18}F]-6 and (±)-[^{18}F]-7 showed lower specific binding in all brain areas explored 2 h post injection when compared to reference compound (-)-[^{125}I] IBVM. Furthermore, defluorination indicated that (±)-[^{18}F]-6 and (±)-[^{18}F]-7 are not suitable as radioligands to explore the VAChT *in vivo* by PET. Moreover, it is well known that interaction at the VAChT binding site is enantioselective, and therefore, working with enantiomerically pure compounds, could improve the compound activity.

Key words: Alzheimer's disease (AD) • benzovisamicol derivatives • brain biodistribution • enantiomeric resolution • radiofluorination • vesicular acetylcholine transporter (VAChT)

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Introduction

Alzheimer's disease (AD) is a progressive and neurodegenerative disorder which affects the cholinergic system and leads to cognitive deficits [1, 4, 8, 12, 15]. Exploration of cholinergic neurotransmission and especially the detection and quantification of the VAChT *in vivo* should provide important and useful information for the early diagnosis of this disease. In recent times, considerable effort has been devoted to the development of both single photon emission computed tomography (SPECT) [2, 3, 7, 16] and positron emission tomography (PET) radioligands suitable for *in vivo* monitoring. Several [^{18}F]-labelled VAChT radioligands have been synthesized including [^{18}F]-NEFA [14], [^{18}F]-FBT [9], (2*R*,3*R*)-5-[^{18}F]-FEOBV [10], (2*R*,3*R*)-5-[^{18}F]-FPOBV [11] and [^{18}F]-FAMV [6]. Both [^{18}F]-NEFA and [^{18}F]-FBT show high affinity for the VAChT. Nicolas G *et al*. [11] demonstrated that the biological (*ex vivo* and *in vivo*) properties of [^{18}F]-FEOBV and [^{18}F]-FPOBV showed low (less than 0.1% of the injected dose) and homogeneous brain uptake. Also the *in vivo* PET study of (2*R*,3*R*)-5-[^{18}F]-FPOBV performed in Baboon revealed rapid defluorination as the main problem. Therefore, [^{18}F]-FEOBV and [^{18}F]-FPOBV appear to be unsuitable for *in vivo* imaging of the VAChT using PET. The piperidine ring of these compounds is an important skeleton

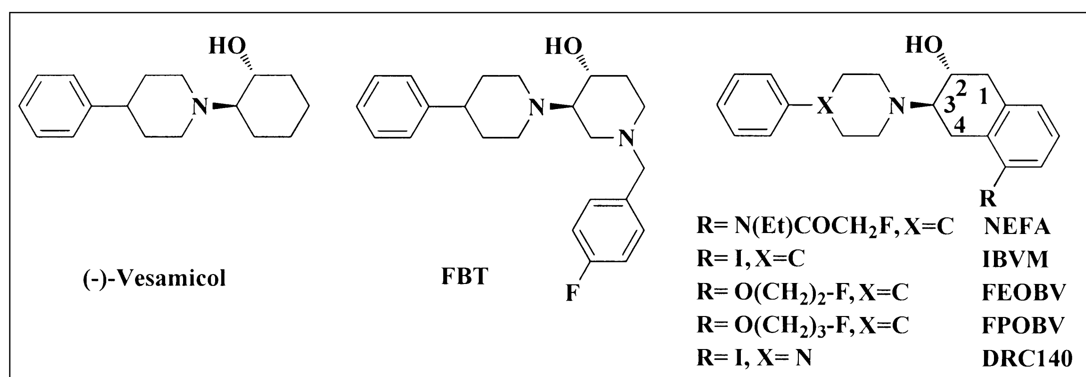


Fig. 1. Vesamicol analogs.

for high affinity to vesamicol receptor on VAChT [13]. Bando *et al.* [5] have reported that the replacement of the piperidine ring of IBVM by a piperazine ring resulting in the formation of DRC140 derivative (Fig. 1) which affords a high affinity compound with high selectivity for VAChT over σ_1 and σ_2 receptors.

We aim to find analogs with potential use as radiolabelled probes for PET to be applied in the detection of density changes at the pre-synaptic level, useful in early detection and can be utilized to follow-up Alzheimer's disease in humans. To that effect, we hypothesized that substitution of a piperazine ring with a piperidine ring in both [^{18}F]-FEOBV and [^{18}F]-FPOBV skeleton could induce specificity towards the VAChT. On the basis of this hypothesis and in order to develop a new tracer for PET exploration of the VAChT, we describe in this paper, the synthesis, radiolabelling and biodistribution studies of two novel racemic analogs of benzovesamicol derivatives.

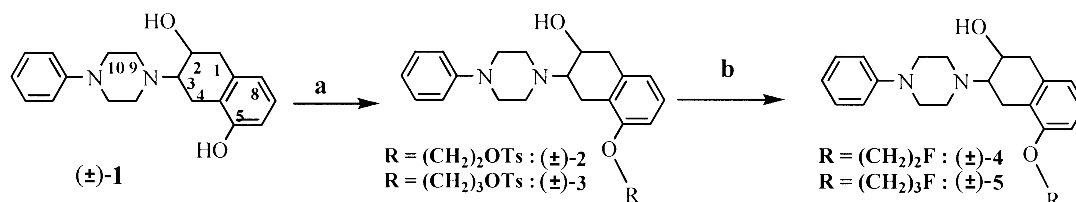
Materials and methods

General remarks

All chemical reagents and solvents were of commercial quality and used as received. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Bio spin 400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C). Chemical shifts (δ) were expressed in ppm relative to TMS (tetramethylsilicane) as an internal standard. Infrared (IR) spectra were recorded on FTIR-JASCO 300E. Thin-layer chromatography (TLC) was performed using Merck 60F$_{254}$ silica gel plates. Flash chromatography was used for routine purification of reaction products using silica gel (70–230 mesh). Visualization was accomplished under UV or in an iodine chamber. The paper chromatographic analysis was performed using Whatman 3MM paper chromatography strips (2 × 10 cm). The radioactive spot migration was determined by a gamma scanner purchased from Bioscan equipped with a sodium iodide detector.

General procedure for the preparation of (±)-2 and (±)-3

A sample of 3-(4-phenylpiperazin-1-yl)-1,2,3,4--tetrahydronaphthalen, 2,5-diol (±)-1 (50.29 mg, 0.155 mmol) (Scheme 1) was dissolved in 3 ml of CH_3CN (acetonitrile) under inert atmosphere and subsequently 0.18 ml of TBAOH (tetrabutylammonium hydroxide 1 M solution in methanol) was added. The solvent was removed by rotary evaporation at room temperature. 3 ml of dry CH_3CN was added to the residue and then evaporated to azeotropically remove traces of moisture (this process was repeated). Under inert atmosphere, the resulting phenoxide was resuspended in 11 ml of dry CH_3CN and the ethane-1,2-diyl bis(4-methylbenzenesulfonate) (718.16 mg, 1.88 mmol) or propane-1,3-diyl bis(4-methylbenzenesulphonate) (738.8 mg, 1.88 mmol) was added. The mixture was then heated under inert atmosphere at 70°C for 4 h. After evaporation of the solvent, the excess of ethylene glycol ditosylate was separated by crystallization in CCl_4 (per chloromethane). The resulting solution was then partitioned between CH_2Cl_2 (dichloromethane) and water (20 and 10 ml, respectively) and the CH_2Cl_2 layer was washed with 0.5 M NaOH (sodium hydroxide), dried over Na_2SO_4 (anhydrous sodium sulphate), concentrated and purified by flash chromatography on silica gel (ethyl acetate EtOAc:hexane, 5:5). The chemical purity of (±)-2 and (±)-3 was determined using an analytical HPLC column (C-18 reverse-phase column (3 × 250 mm; 5-μm particle)) and ammonium acetate (0.1 M):EtOH 95%, (50/50, v/v) as mobile phase at a flow rate of 1 ml/min ultraviolet absorption was monitored at 254 nm. Under these condition, both compounds were obtained with chemical purity greater than 97%.



Scheme 1. Synthesis of (±)-2, (±)-3 and (±)-4, (±)-5. (a) Ethane-1,2-diyl bis(4-methylbenzenesulphonate) or propane-1,3--diyl bis(4-methylbenzenesulphonate), TBAOH, CH_3CN, 70°C, 4 h; (b) TBAF, THF, 80°C, 15 min.

2-(2-hydroxy-3-(4-phenylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-5-yloxy)ethyl 4-methylbenzenesulphonate (±)-2

Orange oil. Yield 65.3%. **^{1}H-NMR (CDCl$_3$)**: δ 2.07–2.48 (m, 2H, 2H-10), 2.37 (s, 3H, CH$_3$), 3.15–3.74 (m, 10H, 4H-9, 2H-4, 2H-1, 2H-10), 3.96–4.75 (m, 7H, H-2, H-3, OH, 2CH$_2$-O, 2CH$_2$-OTs), 6.62–7.19 (m, 8H, 8H-Ar), 7.28–7.82 (m, 4H, 4H-Ar tosylate). **^{13}C-NMR (CDCl$_3$)**: δ 14.13 (CH$_3$); 21.35 (C-4); 22.70, 24.90 (2C, 2CH$_2$); 27.30 (C-1); 29.37 (2C-10); 29.61 (2C-9); 31.93, 34.16 (2CH$_2$); 63.42 (CH-N); 55.48 (C-OH); 106.63–149.63 (16C$_{Ar}$). **IR (KBr, ν cm^{-1})**: 3423.4 (-OH), 3010–3050 (-CH, aromatic), 2956.2 (CH$_3$), 2921.1 (-CH$_2$-S-), 2850.8 (-CH$_2$, aliphatic), 1175.9 (-SO$_2$), 1256.9 (-CH$_2$).

3-(2-hydroxy-3-(4-phenylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-1-yloxy)propyl 4-methylbenzenesulphonate (±)-3

Yellow oil. Yield 48%. **^{1}H-NMR (CDCl$_3$)**: 1.89–2.20 (m, 2H, CH$_2$), 2.37 (s, 3H, CH$_3$), 2.78–3.32 (m, 12H, 4H-9, 2H-4, 2H-1, 4H-10), 3.73-4.30 (m, 7H, H-2, H-3, OH, 2CH$_2$-O, 2CH$_2$-OTs), 6.92–7.39 (m, 8H, 8H-Ar), 7.75–7.84 (dd, 4H, 4H-Ar tosylate). **^{13}C-NMR (CDCl$_3$)**: δ 21.63 (CH$_3$); 28.95 (C-4); 31.64 (CH$_2$); 37.86 (C-1); 48.11, 50.0 (2C-10); 58.36, 62.93 (2C-9); 66.09, 67.04 (2CH$_2$); 65.28 (CH-N); 67.40 (C-OH); 109.87–156.13 (18C$_{Ar}$). **IR (KBr, ν cm^{-1})**: 3450 (-OH), 3015–3059 (-CH, aromatic), 2965 (CH$_3$), 2931.3 (-CH$_2$-S-), 2888 (-CH$_2$, aliphatic), 1181.5 (-SO$_2$), 1268.4 (-CH$_2$).

General procedure for the preparation of (±)-4 and (±)-5

Under inert atmosphere, (±)-**2** or (±)-**3** (50 mg, 0.09 mmol) was added to a solution of TBAF (tetrabutylammonium fluoride, 1 M solution in THF, 80 μl) in 2 ml of THF (tetrahydrofuran). The mixture was heated, under inert atmosphere, at 80°C for 15 min. The solvent was removed by rotary evaporation. The crude product was partitioned between CHCl$_3$ and 2 M Na$_2$CO$_3$ (sodium carbonate) solution, the organic layer was washed with water, dried over Na$_2$SO$_4$, concentrated and purified by flash chromatography on silica gel (EtOAc/n-Hexan (1/1)).

5-(2-fluoroethoxy)-3-(4-phenylpiperazin-1-yl)-1,2,3,4--tetrahydronaphthalen-2-ol (±)-4

White oil. Yield 90%. **^{1}H-NMR (CDCl$_3$)**: δ 2.04–2.07 (m, 2H, 2H-10), 2.36–3.51 (m, 10H, 4H-9, 2H-4, 2H-1, 2H-10), 3.60–4.85 (m, 7H, H-2, H-3, OH, 2CH$_2$-O, 2CH$_2$-F), 6.70–6.78 (dd, 2H, H-6, H-8), 6.90–6.94 (t, 1H, H-7), 6.98–7.33 (m, 5H, 5H-Ar). **^{13}C-NMR (CDCl$_3$)**: δ 14.14 (C-4); 20.10 (CH$_2$); 22.71 (C-1); 24.92 (2C-10); 29.71, 31.94 (2C-9); 65.33 (CH-N); 66.12 (C-OH); 108.26–129.17 (12C$_{Ar}$).

5-(3-fluoropropoxy)-3-(4-phenylpiperazin-1-yl)-1,2,3,4--tetrahydronaphthalen-2-ol (±)-5

Yellow oil. Yield 48%. **^{1}H-NMR (CDCl$_3$)**: δ 2.15–2.30 (m, 2H, CH$_2$), 2.48–3.35 (m, 12H, 4H-9, 2H-4, 2H-1, 4H-10), 3.77–4.74 (m, 7H, H-2, H-3, OH, 2CH$_2$-O, 2CH$_2$-F), 6.70–7.33 (m, 8H, 8H-Ar). **^{13}C-NMR (CDCl$_3$)**: δ 20.05 (C-4); 30.58 (CH$_2$); 37.87 (C-1); 41.64, 48.14 (2C-10); 49.99, 63.35 (2C-9); 65.29 (CH-N); 66.14 (C-OH); 79.95, 81.59 (2CH$_2$); 108.07–156.44 (12C$_{Ar}$).

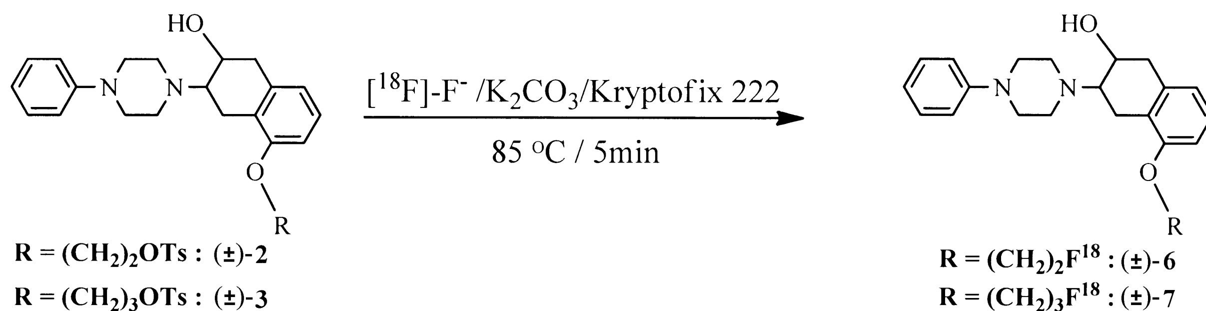
Synthesis and formulation of (±)-[^{18}F]-6 or (±)-[^{18}F]-7

The production of $^{18}F^-$ was performed by means of the $^{18}O(p,n)^{18}F$ reaction using a 17.5 MeV proton beam from a cyclone-30 cyclotron. A 97% enriched water $H_2^{18}O$ purchased from Isoflex (Russia) was used. The niobium target body with a 25 μm Havar window (from Goodfellow) has the volume of 2 ml. The target was water cooled and the window is helium cooled. The bombardment beam current was fixed at 40 μA giving a target pressure of 34 bars. The bombardment time was 15 min. The average activity of $^{18}F^-$ under these conditions was 900 ± 30 mCi at the end of bombardment. Activities were measured using a calibrated Capintec CRC 15 dose calibrator. $^{18}F^-$ with enriched water $H_2^{18}O$ is transferred to the home-made synthesis module. This automated system is controlled by Klochner Moeller PLC and all production parameters are viewed on PC through SCADA (Supervisory Control and Data Acquisition) software version 7.1. All valves used for this synthesis system are pinch valves. These valves were obtained from Bio-Chem Valve (England).

$^{18}F^-$ is trapped in the QMA (quaternary methyl ammonium) cartridge anion exchange purchased from (Waters). The enriched water is recovered to a separate vial and $^{18}F^-$ is eluted to the reactor with 300 μl of K$_2$CO$_3$ (7 mg of K$_2$CO$_3$ in 300 μl of deionized water) and 22.6 mg Kryptofix 222 dissolved in 300 μl of dry acetonirile. The mixture was evaporated to dryness under vacuum. Then, 1.5 ml of dry acetonitrile was added to the reaction vessel containing the dried residue of (^{18}F, K$_2$CO$_3$, Kryptofix 222) and evaporated to dryness at 95°C under vacuum. Tosylate precursors 2 or 3 (5 mg) was dissolved in 3 ml of dry CH$_3$CN and added to the dry [^{18}F]-potassium--Kryptofix 222 complex in the reactor. The mixture was allowed to react at 85°C for 5 min (Fig. 3, Scheme 2). After completion, the reaction mixture was diluted with 15 ml of water for injection (three aliquots) and passed through alumina B and tC18 Sep-pack cartridges. None reacted ^{18}F-fluoride is trapped in an alumina B cartridge and the radiolabelled product is trapped in a tC18 cartridge. The product was eluted from the tC18 Sep-pack cartridges with EtOH (2 mL) then passed through a sterile Millipore GS 0.22 μm thick filter.

Purification and radiochemical purity determination

To purify the labelled products, (±)-[^{18}F]-6 or (±)-[^{18}F]-7, the resulting alcoholic solution from the synthesis system has been evaporated by a gentle heating and a stream of N$_2$ then dissolved by a mixture of ethyl acetate and hexane (7:1). This mixture has been passed through a preconditioned silica sep-pack cartridge. The pure radiolabelled compound passes through this cartridge and byproducts are retained. The solvent was evaporated using a stream



Scheme 2. Radiosynthesis of (±)-[^{18}F]-6, (±)-[^{18}F]-7.

of N_2 then a 3 ml of 0.9% NaCl isotonic injectable solution was added and passed through a 0.22 μm thick filter to obtain the final radiolabelled product. Determination of the product purity was carried out by TLC analysis using a silica gel TLC strip and a mixture of ethyl acetate and hexane (7:1) as eluent. Previously we know that our labelled product should be the one situated at 6 cm, taking into account that the non-radioactive product gives one spot at 6 cm from the starting point. Effectively, we obtained one radioactive spot at 6 cm which demonstrates that our radiolabelled product is pure. The radiolabelling yield, as not corrected decay, was calculated to be 5%.

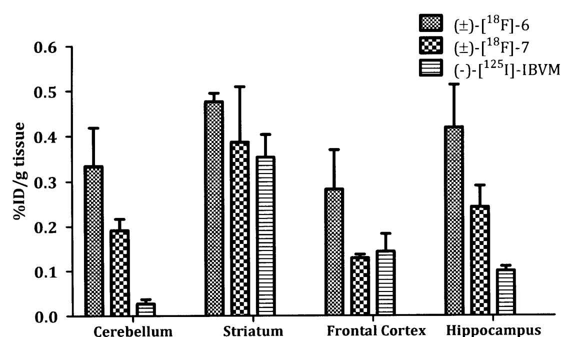


Fig. 2. Cerebral biodistribution of (±)-[^{18}F]-6, (±)-[^{18}F]-7 and (-)-[^{125}I]-IBVM in the rat.

Biodistribution of (±)-[^{18}F]-6 or (±)-[^{18}F]-7 and (-)-[^{125}I]-IBVM in rats

2-months-old male Wistar han rats (175–250 g) were used for *in vivo* experiments. Each rat received an intravenous injection of 2.25–4.20 MBq of (±)-[^{18}F]-6 or (±)-[^{18}F]-7 or (-)-[^{125}I]-IBVM (via the tail vein) in 0.3 ml solution of 0.9% NaCl/EtOH, under ethoxy ethane anesthesia. All animals were sacrificed at 2 h post injection (p.i.) of the radioactive compound. The brain was removed, and samples of the cerebellum, striatum, frontal cortex, and hippocampus were dissected and weighed. Thigh bones were also taken. The radioactivity of each sample was measured in a GAMMA MÜVEK NK-360 counter. Results were expressed as the mean percentage of injected dose/g (%ID/g) tissue ± SD (12 rats) (Fig. 2).

Results and discussion

Radiochemistry

Both compounds (±)-[^{18}F]-6 and (±)-[^{18}F]-7 were obtained with radiochemical purity greater than 98% and with radiochemical yields in the range of 5–6% non decay corrected. The synthesis time was 36 min including purification.

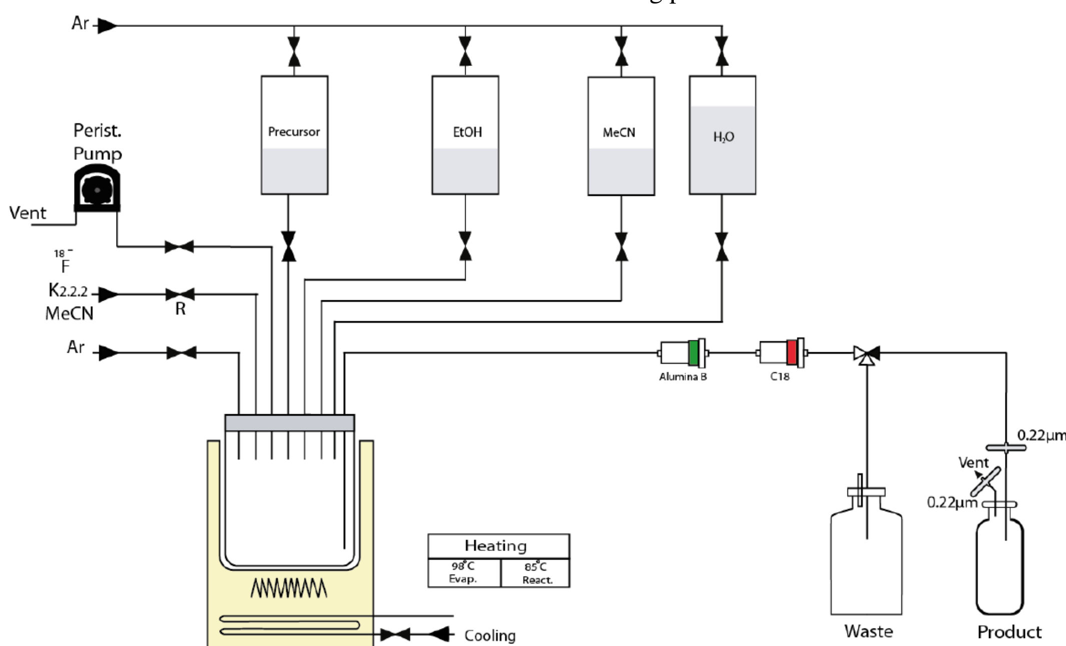


Fig. 3. Schematic diagram of the automated synthesis module of (±)-[^{18}F]-6, (±)-[^{18}F]-7.

Cerebral biodistribution studies in rats

Cerebral biodistribution study of both (±)-[^{18}F]-6, (±)-[^{18}F]-7 and (-)-[^{125}I]-IBVM was performed 2 h p.i. in rats. The histogram reveals that levels of [^{125}I]-IBVM found in brain areas of interest were in agreement with those already published using the same conditions. This pattern of uptake corresponds to the known biodistribution of VAChT density, since the highest level was found in striatum, then in cortex and finally in hippocampus. The cerebellum, which is a low VAChT density area, displayed the lowest [^{125}I]-IBVM uptake. The region/cerebellum ratios of (-)-[^{125}I]-IBVM were 13, 5.29 and 3.7 for the striatum, frontal cortex and hippocampus, respectively.

(±)-[^{18}F]-6 showed an overall homogeneous brain distribution as no significant difference in activity uptake was measured between the cerebellum and other cerebral regions. By contrast, (±)-[^{18}F]-7 showed a higher accumulation in striatum than in the other studied regions. The striatal binding was found to be higher by a factor of 2, 3 and 1.59 compared to those of cerebellum, cortex and hippocampus, respectively. The high uptake of (±)-[^{18}F]-7 in striatum could be associated with VAChT binding. By contrast, the high concentration found in the cerebellum could be associated with non-specific binding to the VAChT.

The cerebral biodistribution of (-)-[^{125}I]- IBVM, has shown a different profile compared to (±)-[^{18}F]-6 or (±)-[^{18}F]-7. However, the uptake in the cerebellum, which could be considered as non-specific binding, was seven times as high as for IBVM. Thus, regions of interest (ROIs)/cerebellum ratios, which characterize the specific binding, were higher for IBVM compared to (±)-[^{18}F]-6 or (±)-[^{18}F]-7 with a maximum striatum/cerebellum ratio of 13 for IBVM vs. 1.4, 2 for (±)-[^{18}F]-6 and (±)-[^{18}F]-7, respectively. Furthermore, we observed the accumulation of radioactivity in bone (0.77 and 1.27% ID/g for (±)-[^{18}F]-6 and (±)-[^{18}F]-7, respectively) which indicates the presence of free [^{18}F]-fluoride resulting from defluorination of (±)-[^{18}F]-6 and (±)-[^{18}F]-7.

Conclusion

We have developed two novel analogs of benzovesamicol as ligands for the VAChT and evaluated them in the rat brain. Compounds 4 and 5 were radiolabelled with ^{18}F from their tosylate precursors (2 and 3, respectively) and evaluated *in vivo* in rat. (±)-[^{18}F]-6 and (±)-[^{18}F]-7 were produced with 5 and 6% radiochemical yield, respectively. Biodistribution studies of (±)-[^{18}F]-6 showed an overall homogeneous brain distribution as no significant difference in activity uptake was found between the cerebellum and the other cerebral regions. Higher level uptake of (±)-[^{18}F]-7 was found in striatum compared to the other studied regions. However, when compared to (-)-[^{125}I]-IBVM, both (±)-[^{18}F]-6 and (±)-[^{18}F]-7 showed lower specific binding in all brain areas explored 2 h p.i. As no preferential localization was observed in regions known to contain high levels of the VAChT, the uptake was characterized as nonspecific. Furthermore, defluorination indicated that (±)-[^{18}F]-6 and (±)-[^{18}F]-7 are not suitable as radioligands for VAChT. Although it is well known that interaction at the VAChT binding site is stereoselective, only racemic mixtures have been used. We can expect that enantiomeric resolution of (±)-[^{18}F]-6 and (±)-[^{18}F]-7 could provide compounds with improved results.

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References

1. Arvidsson U, Riedl M, Elde R, Meister B (1997) Vesicular acetylcholine transporter (VAChT) protein: a novel and unique marker for cholinergic neutrons in the central and peripheral nervous systems. J Comp Neurol 378;4:454–467
2. Assaad T (2011) Synthesis and characterization of novel benzovesamicol analogs. Turk J Chem 35:189–200
3. Assaad T, Mavel S, Emond P *et al*. (2007) Synthesis and *ex vivo* evaluation of *aza*-trozamicol analogs as SPECT radiotracers for exploration of the vesicular acetylcholine transporter. J Labelled Compd Radiopharm 50:139–145
4. Auld DS, Kornecook TJ, Bastianetto S, Quirion R (2002) Alzheimer's disease and the basal forebrain cholinergic system: relations to beta-amyloid peptides, cognition, and treatment strategies. Prog Neurobiol 68:209–245
5. Bando K, Naganuma T, Taguchi K *et al*. (2000) Piperazine analog of vesamicol *in vitro* and *in vivo* characterization for vesicular acetylcholine transporter. Synapse 38:27–37
6. Dietlind S, Matthias S, Udo G, Steffen F (2008) A new ^{18}F-labeled fluoroacetylmorpholino derivative of vesamicol for neuro imaging of the vesicular acetylcholine transporter. Nucl Med Biol 35;2:185–195
7. Emond P, Mavel S, Zea-Ponce Y *et al*. (2007) (E)-[^{125}I]-5-AOIBV: a SPECT radioligand for the vesicular acetylcholine transporter. Nucl Med Biol 34:967–971
8. Ichikawa T, Ajiki K, Matsuura J, Misawa H (1997) Localization of two cholinergic markers, choline acetyltransferase and vesicular acetylcholine transporter in the central nervous system of the rat: *in situ* hybridization histochemistry and immunohistochemistry. J Chem Neuroanat 13:23–39
9. Mach RH, Voytko ML, Ehrenkaufer RL *et al*. (1997) Imaging of cholinergic terminals using the radiotracer [^{18}F](+)-4-fluorobenzyltrozamicol: *in vitro* binding studies and positron emission tomography studies in nonhuman primates. Synapse 25:368–380
10. Mulholland GK, Wieland DM, Kilbourn MR *et al*. (1998) [^{18}F]fluoroethoxy-benzovesamicol, a PET radiotracer for the vesicular acetylcholine transporter and cholinergic synapses. Synapse 30:263–274
11. Nicolas G, Patrick E, Roger R *et al*. (2007) *Ex vivo* and *in vivo* evaluation of (2*R*,3*R*)-5-[^{18}F]-fluoroethoxy- and fluoropropoxy-benzovesamicol, as PET radioligands for the vesicular acetylcholine transporter. Synapse 61:962–970
12. Parsons SM, Bahr BA, Rogers GA, Clarkson ED, Noremberg K, Hicks BW (1993) Acetylcholine transporter-

vesamicol receptor pharmacology and structure. Prog Brain Res 98:175–181
13. Rogers GA, Parsons SM, Anderson DC *et al*. (1989) Synthesis *in vitro* acetylcholine-storage-blockingactivities and biological properties of derivatives and analogues of trans-2-(4-phenylpiperidino) cyclohexanol (vesamicol). J Med Chem 32:1217–1230
14. Rogers GA, Stone-Elander S, Ingvar M, Eriksson L, Parsons SM, Widen L (1994) ^{18}F-labelled vesamicol derivatives: syntheses and preliminary *in vivo* small animal positron emission tomography evaluation. Nucl Med Biol 21:219–230
15. Schafer MK, Weihe E, Varoqui H, Eiden LE, Erickson JD (1994) Distribution of the vesicular acetylcholine transporter (VAChT) in the central and peripheral nervous systems of the rat. J Mol Neurosci 5:1–26
16. Zea-Ponce Y, Mavel S, Assaad T *et al*. (2005) Synthesis and *in vitro* evaluation of new benzovesamicol analogues as potential imaging probes for the vesicular acetylcholine transporter. Bioorg Med Chem 13:745–753