Journal of Labelled Compounds and Radiopharmaceuticals
J Label Compd Radiopharm 2007; **50**: 139–145.
Published online in Wiley InterScience
(www.interscience.wiley.com). DOI: 10.1002/jlcr.1244

JLCR

Research Article

Synthesis and *ex vivo* evaluation of aza-trozamicol analogs as SPECT radiotracers for exploration of the vesicular acetylcholine transporter

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Received 27 October 2006; Revised 22 December 2006; Accepted 28 December 2006

Abstract: Several vesamicol derivatives have already been proposed for SPECT exploration of the vesicular acetylcholine transporter (VAChT) which is localized on the nerve endings of cholinergic neurons which are known to degenerate in the early stages of Alzheimer's disease. However, most of these tracers have disadvantages such as *in vivo* non-specific binding, slow brain kinetics, or high toxicity. We present in this study the synthesis, the radiolabelling and the cerebral biodistribution in rats of two enantiomeric pairs of new trozamicol derivatives, potential imaging agents for the VAChT. Radiolabelled compounds were obtained with high purity and specific radioactivity. However, after i.v. injection in rats, they distributed homogeneously throughout the brain, in contrast to the reference compound IBVM which showed in the same experimental conditions a high striatum/cerebellum fixation ratio. These results demonstrate that these new compounds are unsuitable for the *in vivo* imaging of the VAChT using SPECT. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: vesicular acetylcholine transporter; vesamicol derivatives; radioiodination

Introduction

Alzheimer's disease is one of the most frequent human neurodegenerative diseases. One of the characteristics occurring early in this disease is the degeneration of cholinergic neurons of the basal forebrain.[1] The quantification of vesicular acetylcholine transporters (VAChT) localized on the nerve endings of these neurons could therefore be a relevant index of this neuronal loss. The most suitable method for this type of exploration consists of SPECT or PET imaging using specific tracers of the molecular targets labelled with γ or $\beta+$ emitters. For this aim, several labelled vesamicol derivatives have been developed,[2–4] such as (–)-5-iodobenzovesamicol (IBVM) (Figure 1) which has been used in humans volunteers[5] and subjects suffering from Alzheimer's or Parkinson's disease.[6] Examples of additional vesamicol derivatives that have been proposed are meta-iodobenzyltrozamicol (MIBT)[7] and DRC140[8] (Figure 1). However, to date few studies in humans have been reported with these tracers. This may be due either to the unfavorable pharmacokinetic properties of these highly lipophilic compounds or to their potential toxicity. In our continuing effort to map the VAChT and to develop more potent and selective radiotracers for studying the cholinergic system *in vivo*, we have synthesized iodo-aza-vesamicol derivatives. MIBT, a trozamicol analog[7,9] (Figure 1), demonstrated high affinity for the VAChT which could be attributed to the modification of the piperidine ring being substituted by a benzyl group. However, this compound lacks specificity, as it also binds to σ receptors,[10,11] limiting its usefulness as an imaging agent as shown in

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Contract/grant sponsor: Aid scientific research from the AECS (Atomic Energy Commission of Syria); contract grant number: 82/2001 and 582/2003

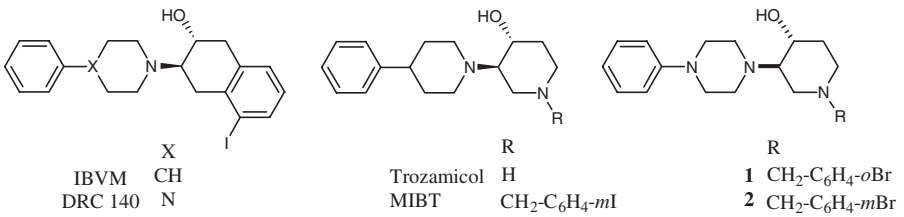
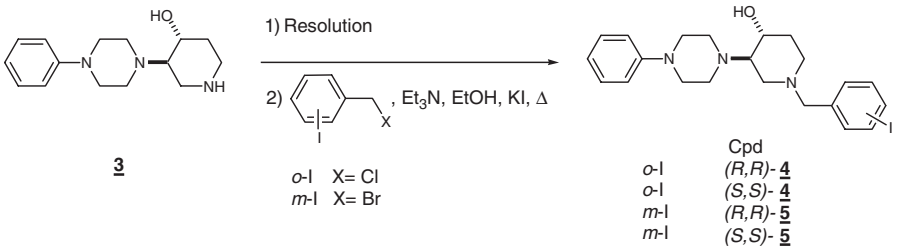


Figure 1 Vesamicol analogs.



Scheme 1 Synthesis of cold compounds **4** and **5**.

in vivo studies.[12] Recent studies have shown that the replacement of the central piperidine ring of IBVM by a piperazine ring afforded a compound (DRC-140)[8] with reinforced affinity for VAChT, and especially with a strong selectivity for VAChT over $\sigma 1$ and $\sigma 2$ receptors.[8] By combining the information generated by these two structures, MIBT and DRC-140, we hypothesized that substitution of a piperazine ring for a piperidine ring in the trozamicol skeleton could induce specificity towards the VAChT. On the basis of this hypothesis and in order to develop a new tracer for SPECT exploration of the VAChT, we have described more recently[13] a new series of potent new tracer agents derived from both DRC-140 and the trozamicol derivative MIBT. From this series, racemic compounds (±)-**1** and (±)-**2** (Figure 1) displayed good *in vitro* affinity for the VAChT ($IC_{50} = 47$ and 46 nM, respectively) and appeared as suitable candidates for radiolabelling. Furthermore, it is well known that interaction at the VAChT binding site is enantioselective, and so, we have presumed that by working with enantiomerically pure compounds, we could improve the affinity for the VAChT. We report here the synthesis, radiolabelling and *ex vivo* evaluation in the rat brain of two enantiomeric pairs of new derivatives of aza-trozamicol.

Results and discussion

Target compounds **4** and **5** have been prepared as presented in Scheme 1. The synthesis started with compound **3**, prepared as previously described,[13] by reaction of 1-phenylpiperazine with 3-(2,2,2-trichloroethoxycarbonyl)-7-oxa-3-aza-bicyclo[4.1.0]heptane. This reaction led to two regioisomers which were easily separated by flash chromatography. Enantiomers of **3** were then resolved by preparative thin layer chromatography after reaction with (*S*)-(+)-α-methoxy-α-(trifluomethyl)phenylacetyl chloride[14] (MTPA-Cl) (the less polar and the more polar isomers had R_f values of 0.6 and 0.5, respectively). Each derivative was then treated separately with DIBAL-H to produce the resolved enantiomer **3**. Finally, target compounds **4** and **5** were obtained by *N*-alkylation of **3** with 2-iodobenzylchloride and 3-iodobenzylbromide, respectively. Enantiomeric purity of each compound was measured by chiral HPLC and retention times of (*S, S*) and (*R, R*)-**4** were 17 and 20.5 min, respectively, as compared to 18 and 24 min for (*S, S*) and (*R, R*)-**5**, respectively. Enantiomeric purity was 95, 88, 99 and 92% for (*S, S*)-**4** (*R, R*)-**4**, (*S, S*)-**5** and (*R, R*)-**5**, respectively. Finally, analysis of both enantiomers of **4** by X-ray crystallography showed, and confirmed so an (*S, S*) configuration (Figure 2, compound (*S, S*)-**4**) for the enantiomer resulting from the less polar enantiomer **3** and so, the use of the more polar enantiomer **3** as starting material gave the (*R, R*) configuration for compounds **4** or **5**. Stannylated derivatives **6** and **7** which serve as precursors for labelling with radioiodine have been prepared from their corresponding racemic brominated analog **1** and **2** by halogen–tin exchange under palladium catalysis by a general procedure previously described.[15] Radiolabelling of compounds **4** and **5** was performed from their *n*-tributyltin derivatives as a racemic mixture. The reaction was driven at 50°C with hydrogen peroxide as the oxidant and afforded pure enantiomers after chiral HPLC separation (Scheme 2). To check the tracer

DOI: 10.1002.jlcr

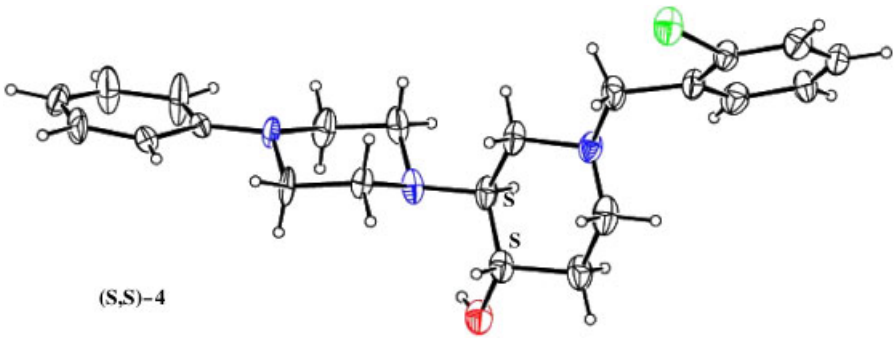
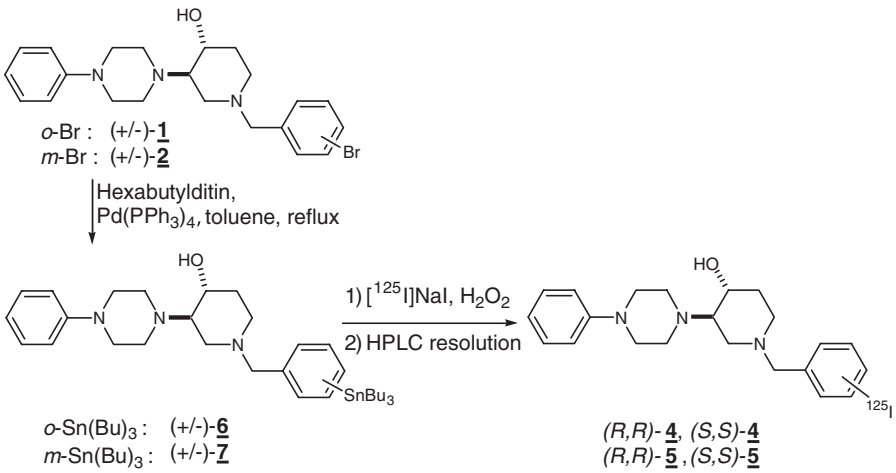


Figure 2 X-ray characterization: ellipsoid representation of (*S*, *S*)-**4**.



Scheme 2 Radiosynthesis of (*S*, *S*)/(*R*, *R*) [^{125}I]-**4** and **5**.

identity, coinjection of the purified radioiodinated enantiomeres with the corresponding cold standard was done. In this way, the radiolabelling of the racemic mixture of tin precursors allowed the preparation of the two radioiodinated enantiomers in a single reaction after chiral HPLC separation. (*S*, *S*)-[^{125}I]-**4**, (*R*, *R*)-[^{125}I]-**4**, (*S*, *S*)-[^{125}I]-**5** and (*R*, *R*)-[^{125}I]-**5** were obtained in 36, 34, 41 and 36% yield, respectively, with a radiochemical purity >98%.

The cerebral biodistribution of the four radioiodinated compounds was studied in rats and the results compared to those obtained with [^{125}I]-IBVM.[2,16] Two hours post-injection, we observed for (*S*, *S*)-**4**, (*R*, *R*)-**4**, (*S*, *S*)-**5** and (*R*, *R*)-**5** an overall homogeneous brain distribution as no significant difference was measured between the cerebellum and other regions (Figure 3). Because calculated log *P* (*c*log *P*) of these compounds are similar (*c*log *P* for **4** and **5** = 4.5, *c*log *P* IBVM = 5.1 (ChemDraw Ultra 10, CambridgeSoft)), the discrepancy between biodistribution studies (low cerebral accumulation of compound **5** compared to the others (Figure 3)) cannot be explained by a lipophilic factor. However, the homogeneous distribution in all brain regions is in agreement with non-selective binding to the VAChT. It could be proposed that this homogeneous brain distribution associated with a displacement in all regions by vesamicol could be attributed to *in vivo* binding to sigma receptors (σ-1, σ-2) as reported for vesamicol and vesamicol analogs[17] or piperazine derivatives.[18] Pre-injection of 0.5 μmol/kg of (-)-vesamicol in order to block the VAChT induced a significant reduction in the accumulation of the four tested compounds in all regions studied, i.e. the striatum, frontal cortex, hippocampus and the cerebellum which is known to be deprived of VAChT (Figure 3). This probably reflects a general effect of vesamicol injected in near saturation quantities on the passage of compounds through the blood–brain barrier and/or peripheral or central effects of it. In similar experimental conditions, the reference compound (-)-[^{125}I]-IBVM showed a lower accumulation in the cerebellum (representing the non-specific binding) than in the other regions studied with region/cerebellum ratios of 13.1, 5.3 and 3.7 for the striatum, frontal cortex and hippocampus, respectively (Figure 3). Moreover, reductions of tracer accumulation were observed in the

DOI: 10.1002.jlcr

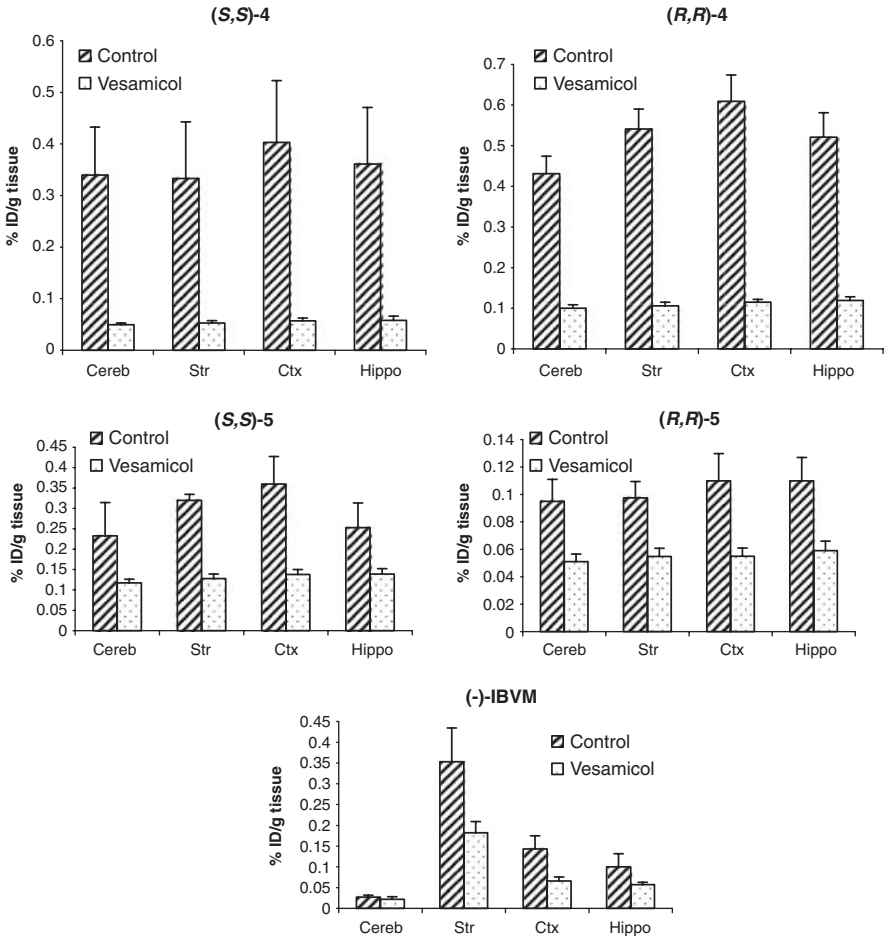


Figure 3 Cerebral biodistribution (% ID/g of tissue ± SEM) of (*S*,*S*)-[^{125}I]-**4**, (*R*,*R*)-[^{125}I]-**4**, (*S*,*S*)-[^{125}I]-**5** and (*R*,*R*)-[^{125}I]-**5** in the rat: competition study. The control rats group received an i.v. injection of the radiotracer (0.74–1.11 MBq; $n = 6$) and the vesamicol rats group received an i.v. 3 min before of the radiotracer with 0.5 μmol/kg of (-)-vesamicol (0.74–1.11 MBq; $n = 6$). Two hours post-injection, regions of interest were collected (Cereb: cerebellum; Str: striatum; Ctx: cortex; hippo: hippocampus), weighed and counted. Accumulations are expressed as percentage of injected dose per gram of tissue.

striatum, frontal cortex and hippocampus (Figure 3) for the vesamicol pre-treated rats group compared to the control group. These results confirmed the specific and selective binding of IBVM to the VAChT.

Conclusion

We have developed two vesamicol analogs as ligands for the VAChT and evaluated them in the rat brain. Biodistribution studies of (*R*,*R*/*S*,*S*)-[^{125}I]-**4** and (*R*,*R*/*S*,*S*)-[^{125}I]-**5** showed an overall homogeneous brain distribution as no significant difference in activity uptake was measured between the cerebellum and other cerebral regions, except for (*R*,*R*)-**4** in the cortex and hippocampus. Pre-injection of vesamicol induced a reduction in the accumulation of all tested compounds throughout the whole brain regions. This could reflect either a general effect of vesamicol on the passage of compounds through the blood–brain barrier or a displacement of tested compounds for sigma receptors (σ-1, σ-2). This potential binding to sigma receptors is currently under investigation.

To conclude, compounds **4** and **5** are unsuitable for the *in vivo* imaging of the VAChT using SPECT.

Experimental

General

NMR spectra were recorded on a Bruker DPX Avance 200 spectrometer (200 MHz for ^{1}H, 50.3 MHz for ^{13}C). $CDCl_3$ was used as solvent; chemical shifts are expressed in ppm relative to TMS as an internal standard. The thin-layer chromatographic (TLC) analyses were performed using Merck $60F_{254}$ silica gel plates. Flash chromatography was used for routine purification of reaction products using silica gel (40–63 μm). Visualization was accomplished under UV or in

DOI: 10.1002.jlcr

an iodine chamber. All chemicals and solvents were of commercial quality and were purified following standard procedures. Compounds **1**–**3** were synthesized as we have previously described.[13] (-)-IBVM was synthesized in our laboratory as previously described.[2,16] Results of elemental analyses of new compounds were within ± 0.4% of theoretical values.

Resolution of 4-hydroxy-3-(N-phenylpiperazinyl)piperidine ((±)-3)

To a solution of (±)-**3** (0.522 g, 2.0 mmol), 4-dimethylaminopyridine (161 mg, 1.21 mmol), and triethylamine (1 ml) in dry $CHCl_3$ (10 ml) was added dropwise via a syringe (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) (1.26 g, 5 mmol) at room temperature. The resulting solution was stirred for 24 h and then poured into CH_2Cl_2 (30 ml). The solution was washed with saturated $NaHCO_3$ solution (20 ml) and the aqueous layer was extracted with CH_2Cl_2 (2 × 30 ml). The combined extracts were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The two diastereomeric MTPA esters were separated by migrations on preparative thin layer chromatography on silica gel performed after several eluent migrations (3–5) using hexane/EtOAc (95/5). The less polar compound, as for other analogs of trozamicol, is the (*S*, *S*)-MTPA diastereomer and the more polar compound is the (*R*, *R*)-MTPA diastereomer, as confirmed by X-ray crystallography study of both target compound (*S*, *S*)-**4** (Figure 2) and (*R*, *R*)-**4**. Both isomers were isolated in 46% yield. The (*S*, *S*)–MTPA ester (0.568 g, 0.82 mmol) was dissolved in dry toluene and the solution was cooled to –78°C. Diisobutylaluminium hydride (2 ml of 1.0 M solution in cyclohexane, 2.0 mmol) was added dropwise via syringe. The resulting solution was stirred at –78°C for 30 min, and allowed to warm up to room temperature. The reaction was quenched with 2.5 N HCl (25 ml). The aqueous layer was separated, alkalinized (pH = 9) with 3 N NaOH and extracted with CH_2Cl_2 (3 × 30 ml). The organic extracts were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to afford 86% (184 mg, 0.705 mmol) of (*R*, *R*)-**3**. (*S*, *S*)-**3** was obtained in 86% (184 mg) from the (*S*, *S*)–MTPA ester derivative.

General procedure for preparation of N-iodobenzyl substituted (R,R) and (S,S)-4-hydroxy-3-(N-phenylpiperazinyl)piperidine ((R,R)/(S,S)-4 and (R,R)/(S,S)-4)

2-Iodobenzylchloride (for derivative **4**) or 3-iodobenzylbromide (for derivative **5**) (2.5 eq.) was added to a solution of 4-hydroxy-3-(*N*-phenylpiperazinyl)piperidine [(*R*, *R*)-**3** or (*S*,*S*)-**3**] (1 eq.) in absolute EtOH (10 ml/mmol) containing Et_3N (140 µl/mmol) and a catalytic amount of KI. The mixture was refluxed under nitrogen atmosphere for 16 h. The products were concentrated and purified by flash chromatography on silica gel (diethylether/Et_3N; 10/1).

(R,R)/(S,S)-N-(2-iodobenzyl)-4-hydroxy-3-(N-phenylpiperazinyl)piperidine (S,S)/(R,R)-4. (*S*, *S*)-**4** Yield = 62% (45 mg, 0.094 mmol), white solid. (*R*, *R*)-**4** Yield = 55% (40 mg, 0.084 mmol), white solid. ^{1}H-NMR: δ 1.49–1.69 (m, 1 H, H-5), 2.08–2.21 (m, 2H, H-5, H-2), 2.57–2.69 (m, 3H, 2H-7, H-3), 2.95–3.22 (m, 9H, 2H-6, 2H-7, H-2, 4H-8), 3.49–3.66 (m, 4H, 2H-9, H-4, OH), 6.88–7.00 (m, 4H_{Ar}), 7.27–7.45 (m, 5H_{Ar}), 7.88 (d, 1H, *J*= 7.8 Hz, H_{Ar}). ^{13}C-NMR: δ = 32.0 (C-5), 48.5 (2C-8), 49.8 (2C-7), 50.0 (C-2), 51.4 (C-6), 66.3 (C-9), 67.2, 67.5 (C-4, C-3), 100.4 (C-5), 116.1 (2CH_{Ar}), 119.8 (CH_{Ar}), 128.0 (CH_{Ar}), 128.6 (CH_{Ar}), 129.0 (2CH_{Ar}), 129.9 (CH_{Ar}), 139.4 (CH_{Ar}), 140.4 (C_{Ar}), 151.1 (C_{Ar}).

(R,R)/(S,S)-N-(3-iodobenzyl)-4-hydroxy-3-(N-phenylpiperazinyl)piperidine (S,S)/(R,R)-5. (*S*, *S*)-**5** Yield = 67% (48 mg, 0.100 mmol), white solid. (*R*, *R*)-**5** Yield = 68% (50 mg, 0.105 mmol), white solid. ^{1}H NMR: δ = 1.55–1.71 (m, 1H, H-5), 1.94–2.07 (m, 2H, H-5, H-2), 2.64–2.71 (m, 3H, 2H-7, H-3), 2.91–3.01 (m, 4H, 2H-6, 2H-7), 3.18–3.25 (m, 5H, H-2, 4H-8), 3.51–3.53 (m, 3H, 2H-9, H-4), 3.62 (s, 1H, OH), 6.90–7.05 (m, 3 H_{Ar}), 7.13 (t, 1H, *J*= 7.6 Hz, H_{Ar}), 7.26–7.34 (m, 3H_{Ar}), 7.63 (d, 1H, *J*= 7.8 Hz, H_{Ar}), 7.71 (s, 1H_{Ar}). ^{13}C NMR: δ = 32.1 (C-5), 48.5 (2C-7), 49.8 (2C-8), 50.0 (C-2), 51.5 (C-6), 62.1 (C-9), 67.2, 67.4 (C-4, C-3), 94.3(C-5), 116.1 (2CH_{Ar}), 119.8 (CH_{Ar}), 127.9 (CH_{Ar}), 129.0 (2CH_{Ar}), 129.9 (CH_{Ar}), 136.1 (CH_{Ar}), 137.6 (CH_{Ar}), 140.6 (C_{Ar}), 151.1 (C_{Ar}).

General procedure for preparation of (±)-6 and (±)-7

A solution of hexa *n*-butylditin (5 eq.) in dry toluene (10 ml/mmol) was added dropwise under N_2 to a stirred solution of bromide derivative **1** or **2** (600 mg, 1.40 mmol) in dry toluene (10 ml/mmol) and tetrakistriphenylphosphine palladium(0) (70 mg/mmol). The mixture was stirred at 100°C for 24 h. The crude product was concentrated to an oil and purified by flash chromatography on silica gel (petroleum ether/Et_2O; 10/1).

(±)-4-Hydroxy-3-(N-phenylpiperazinyl)-N-(2-tri-n-butylstannylbenzyl)piperidine (±)-6. Yellow oil. Yield 31% (278 mg, 0.43 mmol). ^{1}H NMR: δ = 0.93 (t, 9H, ^{3}*J*= 7 Hz, 3CH_3), 1.05–1.66 [m, 18H, ($CH_3C\underline{H_2}C\underline{H_2}C\underline{H_2})_3Sn$],

DOI: 10.1002.jlcr

1.86–1.97 (m, 3H, 2H-5, H-2), 2.61–2.66 (m, 3H, 2H-7, H-6), 2.95–3.21 (m, 9H, 2H-6, 2H-7, H-2, 4H-8), 3.47–3.55 (m, 4H, 2H-9, H-4, OH), 6.87–6.97 (m, $4H_{Ar}$), 7.22–7.34 (m, $5H_{Ar}$). ^{13}C NMR: $\delta = 10.5$ [$(C_3H_7\underline{C}H_2)_3Sn$, $^1J^{119}_{Sn-C} = 331$ Hz, $^1J^{117}_{Sn-C} = 328$ Hz], 13.7 (CH_3), 27.5 [$(CH_3\underline{C}H_2C_2H_4)_3Sn$, $^3J_{Sn-C} = 55$ Hz], 29.2 [$(C_2H_5\underline{C}H_2CH_2)_3Sn$, $^2J_{Sn-C} = 10$ Hz], 31.9 (C-5), 48.6 (2C-8), 50.0 (2C-7), 50.4 (C-2), 51.9 (C-6), 66.0 (C-9), 67.4, 67.5 (C-4, C-3), 116.3 ($2CH_{Ar}$), 120.0 (CH_{Ar}), 126.5 (CH_{Ar}), 127.9 (CH_{Ar}), 129.0 ($2CH_{Ar}$), 130.9 (CH_{Ar}), 137.1 (CH_{Ar}), 142.6 (C_{Ar}), 144.9 (C_{Ar}), 151.2 (C_{Ar}).

(±)-4-Hydroxy-3-(N-phenylpiperazinyl)-N-(3-tri-n-butylstannylbenzyl)piperidine (±)-7. Orange oil. Yield 36% (323 mg, 0.50 mmol). ^1H NMR: $\delta = 0.93$ (t, 9H, $^3J = 7$ Hz, $3CH_3$), 1.05–1.66 [m, 18H, $(CH_3C\underline{H}_2C\underline{H}_2C\underline{H}_2)_3Sn$], 1.94–2.05 (m, 3H, 2H-5, H-2), 2.62–2.68 (m, 3H, 2H-7, H-3), 2.93–3.21 (m, 9H, 2H-6, 2H-7, H-2, 4H-8), 3.47–3.58 (m, 3H, 2H-9, H-4), 3.65 (s, OH), 6.90–6.97 (m, $3H_{Ar}$), 7.26–7.40 (m, $6H_{Ar}$). ^{13}C NMR: $\delta = 9.5$ [$(C_3H_7\underline{C}H_2)_3Sn$, $^1J^{119}_{Sn-C} = 331$ Hz, $^1J^{117}_{Sn-C} = 328$ Hz], 14.2 (CH_3), 27.2 [$(CH_3\underline{C}H_2C_2H_4)_3Sn$, $^3J_{Sn-C} = 55$ Hz], 29.0 [$(C_2H_5\underline{C}H_2CH_2)_3Sn$, $^2J_{Sn-C} = 10$ Hz], 32.1 (C-5), 49.1 (2C-8), 49.7 (2C-7, C-2), 51.3 (C-6), 63.0 (C-9), 67.3, 67.6 (C-4, C-3), 116.1 ($2CH_{Ar}$), 119.8 (CH_{Ar}), 127.7 (CH_{Ar}), 128.6 (CH_{Ar}), 129.0 ($2CH_{Ar}$), 135.1 (CH_{Ar}), 136.8 (CH_{Ar}), 137.1 (C_{Ar}), 141.7 (C_{Ar}), 151.2 (C_{Ar}).

Radiolabelling procedure of (±)-6 and (±)-7

To a vial containing 50 μg of one of the tributylstannyl precursors [(±)-**6** or (±)-**7**] were added 100 μl EtOH, 100 μl 0.1 N HCl, 55.5 MBq (1.5 mCi) [^{125}I]NaI ([^{125}I]NaI in 10 μl NaOH 0.1 N, specific activity: 3626 MBq/ml (98 mCi/ml), Amersham Biosciences) and 200 μl of 3% w/v hydrogen peroxide. The reaction mixture was allowed to stand at 50°C for 30 min, quenched with 100 μl $Na_2S_2O_5$ solution (300 mg/ml), basified with saturated $NaHCO_3$ solution (2 ml) and extracted with ethyl acetate (3×1 ml). The combined ethyl acetate extracts were evaporated under a nitrogen stream and the residue was dissolved in 200 μl of the HPLC mobile phase. The radioiodinated solution was purified by HPLC using a C-18 reverse-phase column (Phenominex Ultracarb ODS (30), 2 mm × 30 mm; 5 μm particle, Torrance, CA, USA) and a mixture of EtOH/H_2O/CH_3CN/Et_3N (25/30/45/0.1 v/v) as mobile phase (flow rate = 1 ml/min). The fractions eluting at the retention time of (*R*,*R*/*S*,*S*)-[^{125}I]-**4** ($R_t = 22.5$ min) and (*R*,*R*/*S*,*S*)-[^{125}I]-**5** ($R_t = 17$ min) were collected and passed through a SEP-Pak C18 column. The radioiodinated products were then eluted with (2×1 ml) EtOH and evaporated under nitrogen stream. Each compound was obtained with a radiochemical purity >98%.

Resolution of (R,R/S,S)-(^{125}I)-4 and (R,R/S,S)-(^{125}I)-5

Resolution of (*R*,*R*/*S*,*S*)-[^{125}I]-**4** and (*R*,*R*/*S*,*S*)-[^{125}I]-**5** was performed with a Beckman 110B (solvent Delivery module) HPLC Pump and a Chiracel OD column (4.6 mm × 250 mm; 10 μm particle, Daicel Chemical Industries, Illkirch, France). Ultraviolet absorption was monitored with a Gilson 112 UV/visible detector at 254 nm. (*R*,*R*/*S*,*S*)-[^{125}I]-**4** and (*R*,*R*/*S*,*S*)-[^{125}I]-**5** were dissolved in *i*-PrOH-hexane (200 μl) (90/10) and the two enantiomers were separated by HPLC with *i*-PrOH/hexane/Et_3N (10/90/0.1 v/v), as mobile phase (flow rate = 1 ml/min) to provide (i) 13.3 MBq (360 μCi) (36% radiochemical yield) of (*S*,*S*)- [^{125}I]-**4** and 12.6 MBq (340 μCi) (34% radiochemical yield) of (*R*,*R*)-[^{125}I]-**4**; (ii) 20.0 MBq (540 μCi) (41% radiochemical yield) of (*S*,*S*)-[^{125}I]-**5** and 17.0 MBq (460 μCi) (36% radiochemical yield) of (*R*,*R*)-[^{125}I]-**5**. Retention times of (*S*,*S*)-[^{125}I]-**4** and (*R*,*R*)-[^{125}I]-**4** were 15 and 18.5 min, respectively, with an enantiomeric purity of 95 and 88%, respectively. Retention times of (*S*,*S*)-[^{125}I]-**5** and (*R*,*R*)-[^{125}I]-**5** were 17.5 and 21 min, respectively, with an enantiomeric purity of 99 and 92%, respectively. After purification by HPLC, each compound was obtained in a no-carrier added form with a specific activity of 74 TBq/mmol.

Cerebral biodistribution studies in rats

Experiments in rats were carried out in compliance with appropriate European Community directive guidelines (86/609/EEC). Male 2-month-old Wistar rats (230–250 g) were purchased from CERJ (Le Genest, France). They received an intravenous injection via the penis vein of 0.74–1.11 MBq of one of the radioiodinated compounds in 0.3 ml of a solution of EtOH with 0.9% of NaCl, under isoflurane gas anesthesia. For each tested compound, a group of six rats received 3 min before the labelled product via the penis vein an intravenous injection of 0.9% NaCl or 0.5 μmol/kg of (-)-vesamicol (Sigma-Aldrich, France) in 0.2 ml 0.9% NaCl. [^{125}I]-IBVM was used in the same experimental conditions as the reference compound. All animals were sacrificed 2 h after the second injection by decapitation. The brain was isolated and fractions of the cerebellum, striatum, frontal cortex and hippocampus were dissected and weighed. The radioactivity of each sample was measured in a LKB gamma counter. Results were expressed as mean percentage of injected dose/g tissue ± SD.

DOI: 10.1002.jlcr

X-ray diffraction analysis

Colourless crystals of (*R*,*R*)-**2** (same procedure with (*S*,*S*)-**2** derivative) were grown by slow evaporation of ethanol/dichloromethane solutions at 298 K and crystallizes in monoclinic system, space group $P2_1$. Diffraction data were collected with a CAD-4 Enraf-Nonius diffractometer (Delft, Nederland) equipped with a graphite monochromator MoKα radiation ($\lambda = 0.71073$ Å).[19] Intensities were collected using the ω–2θ scan mode. The reflections were measured in the range: $0 \leqslant h \leqslant 9$, $-10 \leqslant k \leqslant 10$, $-29 \leqslant l \leqslant 29$ ($\theta_{max} = 30°$). The crystal absorption correction was performed using the psi scan technique.[20] The crystal structure was solved by the direct method using the program SHELXS97.[21]

Acknowledgements

This work was supported by a grant (82/2001 and 582/2003) in aid scientific research from the AECS (Atomic Energy Commission of Syria). This study was funded in part by the EC-FP6-project DiMI, LSHB-CT-2005-512146. We thank SAVIT (Tours, France) for chemical analyses.

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