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Synthesis and characterization of novel benzovesamicol analogs

Thaer ASSAAD
Atomic Energy Commission (AEC), Damascus,
P. O. Box 6091, SYRIAN ARAB REPUBLIC
e-mail: cscientific@aec.org.sy

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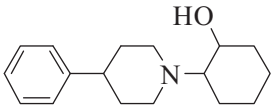
Three new racemic vesamicol analogs were successfully prepared in good yields and high purity. All compounds were characterized by both IR and NMR spectroscopy. Compounds (±)**-5** and (±)**-6** showed a moderate lipophilicity compared with reference compounds IBVM and DRC140, respectively. However, the lipophilicity of DRC140 and (±)**-9** showed very similar behaviors.

Key Words: Benzovesamicol, ethylenedicysteine, vesicular acetylcholine transporter

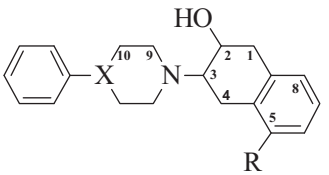
Introduction

Benzovesamicol (BVM), shown in Figure 1, is a (-)-vesamicol analog that is well known to act as a stereoselective inhibitor for acetylcholine uptake into presynaptic cholinergic vesicles. Radiolabeled benzovesamicol analogs have been widely used as imaging probes in single photon emission computer tomography (SPECT) and positron emission tomography (PET) aimed at both in vitro and in vivo studies of Alzheimer's disease (AD).[1–3] For this aim, many efforts have focused on developing vesamicol derivatives as radiotracers using SPECT and PET. Examples include benzovesamicols (-)-5-iodobenzovesamicol, ^{123}I-IBVM,[4] (-)-(^{11}C)-5-*N*-methylamino benzovesamicol (^{11}C)-MABV,[5] (-)-(^{18}F)-fluoroethoxybenzovesamicol (^{18}F)-FEOBV,[6] (E)-(R,R)-2-hydroxy-5-(3-iodoprop-2-en-1-oxy)-3-(4-phenylpiperidino)tetralin, (R,R)-5-AOIB, and fluoropropoxy benzovesamicol (^{18}F)-FPOBV.[7] It has been reported that the piperidine is the main part of the vesamicol that determines the affinity of the vesamicol receptor on the vesicular acetylcholine transporter (VAChT).[8] Bando et al.[9] showed that the replacement of the piperidine ring in the structure of IBVM with a piperazine ring resulted in the formation of a DRC140 derivative (Figure 1), a compound with high affinity and selectivity for the VAChT over σ_1 and σ_2 receptors. To date, however, only a few studies in humans have been reported with these tracers. This may be

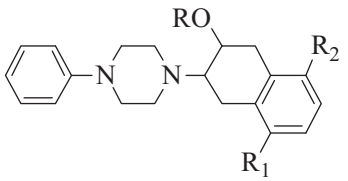
due to the unfavorable pharmacokinetic properties of these highly lipophilic compounds or to their potential toxicity.[10−12]



(-)- Vesamicol



R = I, X = N ; DRC 140
R = NH_2, X = N ; DRC- NH_2
R = I, X= CH ; IBVM
R = $NHCH_3$, X = CH ; MABV
R = OCH_2CH=CHI, X = CH ; AOIBV
R = $O(CH_2)_2F$, X = CH ; FEOBV
R = $O(CH_2)_3F$, X = CH ; FPOBV



New Analogues :
R= EC, R_1 = NH_2, R_2 =H
R= EC, R_1 = H , R_2 =NH_2
R= H, R_1 = CH_2-CH=CH-I, R_2 = H

Figure 1. Vesamicol analogs.

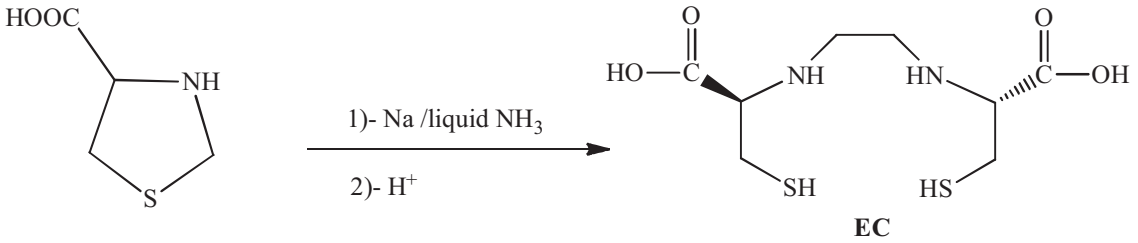
The goal of the present study was to find new analogs with potential use as radiolabeled probes for SPECT, which are useful in both early detection and follow-up of Alzheimer's disease in humans.[13,14] An attempt to control the lipophilicity of racemic compounds was carried out by 2 procedures: elongation of the substituent chain of DRC140 in position (5), or conjugation of the technethium-99m ethylenedicysteine (^{99m}Tc-EC)[15−18] chelate group with DRC-NH_2 in position (2).

Experimental

General remarks: All chemical reagents and solvents were of commercial quality and were used as received. The thin layer chromatographic (TLC) analysis 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. NMR spectra were recorded on a Bruker BioSpin 400 spectrometer (400 MHz for ^{1}H-NMR, 100 MHz for ^{13}C-NMR). Chemical shifts (δ) were expressed in ppm relative to TMS as an internal standard. IR spectra were recorded on an FTIR-JASCO 300E. Melting points were determined using a Stuart SMP3 melting point apparatus.

Synthesis of ethylenedicysteine (EC):

Ethylenedicysteine was synthesized as reported elsewhere[16,17] (Scheme 1). A solution of L-thiazolidine-4-carboxylic acid (22 g, 165 mmol) and liquid ammonia (200 mL) was refluxed for 1 h. Sodium pieces were added to the solution until a blue color persisted. Ammonium chloride (21 g, 400 mmol) was added to the blue solution, and then solvents were removed. The residue was dissolved in water (200 mL), and the pH was adjusted to 2. The precipitate that formed was filtered and washed with water (500 mL). The solid material was dried in a calcium chloride vacuum desiccator to give 8.2 g (37%) of EC (mp 252.7 °C).



Scheme 1. Synthesis of L,L-ethylenedicysteine.

Spectroscopic data for EC:

^{1}**H-NMR (D$_2$O)**: δ 1.25 (s, 2H, 2SH), 2.61 (s, 2H, 2NH), 2.69-2.98 (m, 8H, 2CH$_2$-N, 2CH$_2$-SH), 3.26-3.29 (m, 2H, 2CH-COOH). ^{13}**C-NMR (D$_2$O)**: δ 26.8 (C-SH), 44.9 (CH$_2$-NH), 65.33 (CH-NH), 162.2 (Na$_2$CO$_3$), 177.6 (COOH). **IR** (KBr, ν cm^{-1}): 3455 (-NH), 1593.1 (-COOH), 2545.3 (-SH). **UV-VIS,** λ_{max} = 234 nm (-COOH).

Synthesis of (±)-trans-5-amino-2-hydroxy-3-(4-phenyl-1-piperazinyl) tetralin (±)-1 and (±)-trans-8-amino-2-hydroxy-3-(4-phenyl-1-piperazinyl) tetralin (±)-2:

Compounds **(±)-1** and **(±)-2** were synthesized as previously reported.[9] 1-Phenylpiperazine (3 g, 19 mmol) was added to a solution of *N*-(trifluoroacetyl)-1-amino-5,8-dihydronaphthalene oxide (1.9 g, 7.4 mmol)[8] in ethanol (25 mL). The solution was allowed to reflux for 16 h, and was then kept for 24 h at room temperature to produce a crystallized solid. The solid was filtered and dried under a vacuum, then dissolved in methanol (25 mL) and treated with 1 N NaOH (15 mL). The mixture was stirred at room temperature for 16 h and then extracted with CH_2Cl_2 (3 × 25 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting regioisomers **(±)-1** and **(±)-2** were separated by silica gel chromatography with Et_2O and Et_3N at a 10:1 ratio to give 665 mg (35%) of **(±)-1**, R_f = 0.55 (mp 231.9 °C), and 684 mg (36%) of **(±)-2**, R_f = 0.26 (mp 171.6 °C).

Spectroscopic data for (±)-1:

^{1}**H-NMR (CDCl$_3$)**: δ 2.49-2.56 (m, 2H, 2H-10), 2.72-3.33 (m, 12H, 2H-1, 2H-4, 2H-10, 4H-9, H-3, OH), 3.60 (s, NH$_2$), 3.91-3.97 (m, 1H, H-2), 6.57-6.63 (m, 2H, H-6, H-8), 6.91 (t, 2H, ^3J = 7.2 Hz, 2H$_{Ar}$), 6.99-7.04 (m, 1H, H-7), 7.28-7.33 (m, 3H, 3H$_{Ar}$). ^{13}**C-NMR (CDCl$_3$)**: δ 20.9 (2C-10), 38.0 (C-1), 48.1 (C-4), 49.9 (C-3), 65.2 (C-2), 66.2 (2C-9), 112.7 (CH$_{Ar}$), 116.3 (2CH$_{Ar}$), 119.3 (C$_{Ar}$), 119.6 (CH$_{Ar}$), 120.0 (CH$_{Ar}$), 127.1 (CH$_{Ar}$), 129.1 (2CH$_{Ar}$), 134.7 (C$_{Ar}$), 144.4 (C- NH$_2$), 151.2 (C$_{Ar}$). **IR** (KBr, ν cm^{-1}): 3200-3350.4 (NH$_2$), 3459.5 (OH), 3050 (CH=CH, Ar), 2918.2-2850.9 (CH$_2$, aliphatic), 2850.9 (CH$_2$-NH$_2$), 1636.6 (C=C), 1137.6 (C-N, piperazine).

Spectroscopic data for (±)-2:

^{1}H-NMR ($CDCl_3$): δ 2.40-2.50 (m, 1H, H-1), 2.74-2.33 (m, 13H, 4H-10, 2H-4, 4H-9, H-3, H-1, OH), 3.65 (s, NH_2), 3.91-4.00 (m, 1H, H-2), 6.57-6.59 (d, 1H, ^3J = 8Hz, H-5), 6.91 (t, 2H, ^3J= 6.7 Hz, $2H_{Ar}$), 6.97-7.03 (m, 1H, H-6), 7.24-7.33 (m, 4H, H-7, $3H_{Ar}$). **^{13}C-NMR ($CDCl_3$)**: δ 26.5 (C-4), 33.1 (C-1), 48.2 (2C-10), 49.8 (2C-9), 65.6 (C-3), 65.9 (C-2), 112.6 (CH_{Ar}), 116.3 ($2CH_{Ar}$), 118.5 (C_{Ar}), 119.3 (CH_{Ar}), 120.0 (CH_{Ar}), 126.9 (CH_{Ar}), 129.1 ($2CH_{Ar}$), 135.5 (C_{Ar}), 144.4 (C-NH_2), 151.2 (C_{Ar}). **IR** (KBr, ν cm^{-1}): 3190-3357 ($N\underline{H}_2$), 3450 (OH), 3024.3 (<u>CH=</u>CH, Ar), 2945-2905.8 (CH_2, aliphatic), 2821.3 (<u>CH_2</u>-NH_2), 1634.7 (C=C), 1142.6 (C-N, piperazine).

Synthesis of (±)-trans-*N*-trifluoroacetyl-5-amino-2-hydroxy-3-(4-phenyl-1-piperazinyl) tetralin (±)-3 and (±)-trans-*N*-trifluoroacetyl- 8-amino-2hydroxy-3-(4-phenyl-1-piperazinyl) tetralin (±)-4:

(±)-Trans-8-amino-2-hydroxy-3-(4-phenyl-1-piperazinyl) tetralin **(±)-2** (150 mg, 0.46 mmol) was dissolved in benzene (10 mL) and cooled to 0 °C. Trifluoroacetic anhydride (TFA) (97 mg, 0.46 mmol) was added slowly due to the exothermicity of the reaction. The ammonium salt began to precipitate immediately, but the reaction solution was homogeneous upon complete addition of the TFA. The solution was maintained at 0 °C for 1 h. After that, benzene and trifluoroacetic acid were removed under reduced pressure. More benzene was added, and then evaporated, in order to aid the removal of trifluoroacetic acid. The residue was purified by chromatography on silica gel with Et_2O and Et_3N at a 10:1 ratio to give 190 mg (98%) of **(±)-4** as a white solid (mp 210 °C). The same procedure, starting with (±)-trans-5-amino-2-hydroxy-3-(4-phenyl-1-piperazinyl) tetralin **(±)-1** (500 mg, 1.547 mmol), was used to prepare **(±)-3** as a yellow solid (290 mg, 45%) (mp 211 °C).

Spectroscopic data for (±)-3:

^{1}H-NMR ($CDCl_3$): δ 2.77-3.01 (m, 11H, H-1, 2H-4, 4H-10, 4H-9), 3.27-3.41 (m, 3H, H-3, H-1,OH), 3.92-3.94 (m, 1H, H-2), 6.93 (t, 1H, ^3J = 7.6 Hz, H-7), 6.99 (d, 1H, ^3J = 8 Hz, H-6), 7.13 (d, 1H, ^3J = 7.6 Hz, H-8), 7.24-7.33 (m, 4H, $4H_{Ar}$), 7.52 (d, 1H, ^3J = 7.2 Hz , H_{Ar}), 7.65 (s, NH). **^{13}C-NMR ($CDCl_3$)**: δ 21.2 (2C-10), 37.8 (C-1), 48.2 (C-4), 49.9 (C-3), 64.9 (C-2), 65.7 (2C-9), 116.5 ($2CH_{Ar}$), 116.6 (C-F), 120.3 (C_{Ar}), 122.2 (CH_{Ar}), 127.2 (CH_{Ar}), 128.0 (CH_{Ar}), 128.66 (CH_{Ar}), 129.2 ($2CH_{Ar}$), 132.4 (C_{Ar}), 135.7 (C-NH_2), 151.1 (C_{Ar}), 160.2 (C=O).

IR (KBr, ν cm^{-1}): 3430.4 (OH), 3232.8 (NH), 3061.2 (<u>CH=</u>CH, Ar), 2917.5-2850.6 (CH_2, aliphatic), 1725 (<u>O=C</u>-CF_3).

Spectroscopic data for (±)-4:

^{1}H-NMR (C_6D_6): δ 2.25-2.55 (m, 11H, H-1, 2H-4, 4H-10, 4H-9), 2.95-3.05 (m, 3H, H-3, H-1,OH), 3.68-3.69 (m, 1H, H-2), 6.81 (d, 1H, ^3J = 7.2 Hz, H-6), 6.93 (d, 1H, ^3J = 8 Hz, H-8), 6.99-7.38 (m, 5H, H-5, $4H_{Ar}$), 7.46 (s, NH), 7.58 (d, 1H, ^3J = 7.6 Hz, H_{Ar}). **^{13}C-NMR (C_6D_6)**: δ 22.2 (2C-10), 37.8 (C-1), 47.9 (C-4), 49.6 (C-3), 62.4 (C-2), 65.69 (2C-9), 112.8 (CH_{Ar}), 116.2 (CH_{Ar}), 116.3 (C-F), 120.3 (C_{Ar}), 122.3 (CH_{Ar}),

127.5 (CH_{Ar}), 128.2 (CH_{Ar}), 128.56 (CH_{Ar}), 129.2 ($2CH_{Ar}$), 132.4 (C_{Ar}), 135.7 (C_{Ar}), 151.1 (C- NH), 162.2 (C=O). **IR** (KBr, ν cm^{-1}): 3446 (OH), 3270 (NH), 3050 (CH=CH, Ar), 2950-2850 (CH_2, aliphatic), 1736.2 (O=C-CF_3).

Synthesis of (±2)-trans-5-amino-2-ethylenedicysteine-3-(4-phenyl-1-piperazinyl) tetralin (±)-5 and (±)-trans-8-amino-2-ethylenedicysteine-3-(4-phenyl-1-piperazinyl) tetralin (±)-6:

L,L-ethylenedicysteine (138 mg, 0.5 mmol), carbodiimide (DCC) (106.2 mg, 0.5 mmol), and dimethylaminopyridine (DMAP) (7.6 mg, 0.06 mmol) were added to a solution of **(±)-3** (210 mg, 0.5 mmol) in dry CH_2Cl_2 (10 mL). The resulting solution was stirred at room temperature for 72 h. The solvent was removed by rotary evaporation, leaving a powdered residue. The residue was dissolved in ethanol (20 mL) and treated with 1 N NaOH (20 mL) for deprotection of the amine function. The mixture was stirred at room temperature for 48 h, then extracted with CH_2Cl_2 (3 × 25 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give 280 mg (97%) of **(±)-5** as a yellow solid (mp 219 °C). The same procedure, starting with **(±)-(4)** (100 mg, 0.24 mmol), was carried out to prepare **(±)-(6)** as a yellow solid (270 mg, 94%) (mp 161-164 °C).

Spectroscopic data for (±)-5:

^{1}H-NMR ($CDCl_3$): δ 1.07-1.43 (m, 7H, $2CH_2$, 2SH, NH), 1.60-1.98 (m, 4H, $2CH_2$), 2.49-2.56 (m, 1H, H-1), 2.72-3.37 (m, 12H, H-1, 4H-10, 2H-4, 4H-9, H-3), 3.46-3.56 (m, 1H, CH), 3.60 (s, NH), 3.88-3.95 (m, 1H, H-2), 4.10 (d, 1H, ^3J = 1.2 Hz, CH), 4.19 (s, NH_2), 6.57-7.33 (m, 8H, H-6, H-7, H-8, $5H_{Ar}$), 8.35 (s, OH). **^{13}C-NMR ($CDCl_3$)**: δ 20.9 (C-4), 24.9 (CH_2), 25.2 (CH_2), 25.6 (CH_2), 33.9 (CH_2), 38.04 (C-1), 48.1 (2C-10), 49.2 (2CH), 49.9 (2C-9), 65.2 (C-3), 66.2 (C-2), 112.7 (CH_{Ar}), 116.3 ($2CH_{Ar}$), 118.6 (C_{Ar}), 119.6 (CH_{Ar}), 120.0 (CH_{Ar}), 127.0 ($2CH_{Ar}$), 129.2 (CH_{Ar}), 134.8 (C_{Ar}), 144.4 (C_{Ar}), 151.2 (C- NH), 156.7 (2C=O). **IR** (KBr, ν cm^{-1}): 3458.7-3348.7 (COOH, EC), 3250 (NH_2, NH (EC)), 3050 (CH=CH, Ar), 2927.4-2851.2 (CH_2, aliphatic), 1627.4 (O=C-O).

Spectroscopic data for (±)-6:

^{1}H-NMR ($CDCl_3$): δ 1.07-1.45 (m, 6H, $2CH_2$, 2SH), 1.28 (s, NH), 1.69-1.98 (m, 4H, $2CH_2$), 2.42-2.48 (m, 1H, H-1), 2.69-3.34 (m, 13H, 2H-1, 4H-10, 2H-4, 4H-9, H-3), 3.45-3.55 (m, 1H, CH), 3.64 (s, NH), 3.94-4.03 (m, 1H, H-2), 4.05 (d, 1H, ^3J = 1.2 Hz, CH), 4.30 (s, NH_2), 6.56-6.59 (m, 1H, H-5), 6.90 (t, 1H, ^3J = 8 Hz, H-6), 6.94-7.02 (m, 2H, $2H_{Ar}$), 7.28-7.32 (m, 4H, H-7, $3H_{Ar}$), 8.26 (s, OH). **^{13}C-NMR ($CDCl_3$)**: δ 24.9 (CH_2), 25.6 (C-4), 26.4 (CH_2), 33.3 (C-1), 39.0 ($2CH_2$), 48.2 (2C-10), 49.2 (2CH), 49.9 (2C-9), 65.6 (C-3), 65.9 (C-2), 112.6 (CH_{Ar}), 116.3 ($2CH_{Ar}$), 118.6 (C_{Ar}), 119.3 (CH_{Ar}), 120.0 (CH_{Ar}), 126.9 (CH_{Ar}), 129.2 ($2CH_{Ar}$), 135.6 (C_{Ar}), 144.3 (C- NH_2), 151.2 (C_{Ar}), 156.7 (2C=O). **IR** (KBr, ν cm^{-1}): 3450-3330.1 (COOH, EC), 3250 (NH_2, NH (EC)), 3050 (CH=CH, Ar), 2927.7 (CH_2, aliphatic), 1627 (O=C-O).

Synthesis of (±)-trans-2, 5-dihydroxy-3-(4-phenyl-1-piperazinyl) tetralin (±)-7:

A 1:2 dilution of concentrated sulfuric acid in water (5.4 mL) was added to a cooled solution of **(±)-1** (508 mg, 1.55 mmol) in THF (25 mL). A solution of sodium nitrite (130 mg, 5.4 mmol) in water (5.4 mL) was added dropwise at 5 °C with stirring for 1 h. The resulting diazonium solution was then added carefully in small aliquots to a second dilution of sulfuric acid (5.4 mL) in water (28 mL) at its boiling point. The mixture was boiled for 15 min after the addition was completed and then allowed to cool to room temperature. The solution was adjusted to pH 9 with 5 N NaOH and extracted with ethyl acetate (3 × 50 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel with EtOAc and n-hexane at a 30:70 ratio to give 340 mg (68%) of **(±)-7** as a pink solid (mp 237.5 °C).

Spectroscopic data for (±)-7:

^{1}H-NMR ($CDCl_3$): δ 2.60-3.34 (m, 15H, 4H-9, 2H-4, H-3, H-11, 2H-1, 4H-10, OH), 3.89-3.93 (m, 1H, H-2), 6.52 (d, 1H, ^3J = 8 Hz, H-6), 6.73 (d, 1H, ^3J = 8 Hz, H-8), 6.91-7.34 (m, 6H, H-7, 5H-Ar). **^{13}C-NMR ($CDCl_3$)**: δ 19.9 (2C-10), 37.8 (C-1), 48.0 (C-4), 50.1 (C-3), 65.3 (C-2), 66.1 (2C-9), 112.1 (CH_{Ar}), 116.5 ($2CH_{Ar}$), 120.2 (C_{Ar}), 121.4 (CH_{Ar}), 121.6 (CH_{Ar}), 126.9 (CH_{Ar}), 129.2 ($2CH_{Ar}$), 135.7 (C_{Ar}), 142.1 (C-OH), 153.5 (C_{Ar}).

Synthesis of (±)-trans-2-hydroxy-5-((E)-3-(tributylstannyl) allyloxy)-3-(4-phenyl-1-piperazinyl) tetralin (±)-8:

Tetrabutylammonium hydroxide (TBAOH) (160 mg) was added to a solution of **(±)-7** (200 mg, 62 mmol) in CH_2Cl_2 (20 mL) under inert atmosphere. The solvent was removed by rotary evaporation at room temperature and the residue was treated twice with dry CH_3CN (10 mL), followed each time by rotary evaporation to azeotropically remove traces of moisture. The mixture was resuspended in dry CH_3CN (10 mL), and then (E)-3-chloro-1-tributylstannylprop-2-ene (293.4 mg, 0.8 mmol) was added. The solution was heated under inert atmosphere at 75 °C for 3 h, and then stirred at room temperature overnight. After removal of the solvent, the crude product was portioned between CH_2Cl_2 and water (20 and 10 mL, respectively) and the organic layer was washed with 0.5 N NaOH, dried over Na_2SO_4, concentrated, and purified by flash chromatography on silica gel (EtOAc and n-hexane, 30:70) to give 100 mg (25%) of **(±)-8**.

Spectroscopic data for (±)-8:

^{1}H-NMR ($CDCl_3$): δ 0.95 (t, 9H, ^3J = 7.0 Hz, $3CH_3$), 1.26-1.57 (m, 18H, $(CH_3C\underline{H}_2C\underline{H}_2C\underline{H}_2)_3Sn$), 2.35-2.62 (m, 2H, 2H-10), 2.44-3.19 (m, 12H, H-11, 2H-1, 2H-4, H-3, 4H-9, 2H-10), 3.91-3.93 (m, 1H, H-2), 4.58 (d, 2H , ^3J = 4.3 Hz, OCH_2), 6.21-6.38 (m, 2H, -CH=CH-), 6.67-6.54 (m, 8H, $8H_{Ar}$). **^{13}C-NMR ($CDCl_3$)**: δ 9.4 [$(CH_2)_3Sn$], 13.7 ($3CH_3$), 27.2 [$(CH_3\underline{C}H_2CH_2CH_2)_3Sn$], 29.0 [$(CH_3CH_2\ \underline{C}H_2CH_2)_3Sn$], 34.4 (2C-10), 38.0 (C-1), 42.9 (C-4), 52.5 (C-3), 65.3 (C-2), 66.1 (2C-9), 71.3 (OCH_2), 112.1 (CH_{Ar}), 116.5 ($2CH_{Ar}$), 120.2 (C_{Ar}), 121.4 (CH_{Ar}), 126.9 (CH_{Ar}), 129.2 ($2CH_{Ar}$), 131.1 (SnCH=), 135.7 (C_{Ar}), 153.5 (C_{Ar}), 143.1 (CH=), 146.1 (C_{Ar}), 156.5 (C_{Ar}).

Synthesis of (±)-trans-2-hydroxy-5-((E)-3-(iodo) allyloxy)-3-(4-phenyl-1-piperazinyl) tetralin (±)-9:

Stannyl derivative (±)-**8** (50 mg, 76.5 mmol) was dissolved in $CHCl_3$ (5 mL) and cooled to -5 °C. A solution of iodine in CH_2Cl_2 (0.1 N) was then added in aliquots, under constant stirring, until a colored solution resulted. The reaction mixture was washed with 12% $NaHSO_3$ (5 mL), the organic layer was separated, and the aqueous was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by flash chromatography on silica gel (EtOAc and n-hexane, 30:70) to give 30 mg (80%) of (±)-**9**.

Spectroscopic data for (±)-9:

^{1}H-NMR ($CDCl_3$): δ 2.72-2.91 (m, 2H, 2H-10), 2.65-3.32 (m, 12H, H-11, 2H-1, 2H-4, H-3, 4H-9, 2H-10), 4.11-4.30 (m, 1H, H-2), 4.5 (d, 2H, ^3J = 4.3 Hz, OCH_2), 6.44-6.58 (m, 2H, -CH=CH-), 6.77-6.87 (m, 8H, $8H_{Ar}$). **^{13}C-NMR ($CDCl_3$)**: δ 34.2 (2C-10), 38.3 (C-1), 42.5 (C-4), 52.1 (C-3), 65.0 (C-2), 65.9 (2C-9), 71.8 (OCH_2), 79.2 (I-CH), 112.2 (CH_{Ar}), 116.3 ($2CH_{Ar}$), 120.5 (C_{Ar}), 121.2 (CH_{Ar}), 126.3 (CH_{Ar}), 129.4 ($2CH_{Ar}$), 135.7 (C_{Ar}), 140.8 (CH=), 153.5 (C_{Ar}), 146.1 (C_{Ar}), 156.5 (C_{Ar}).

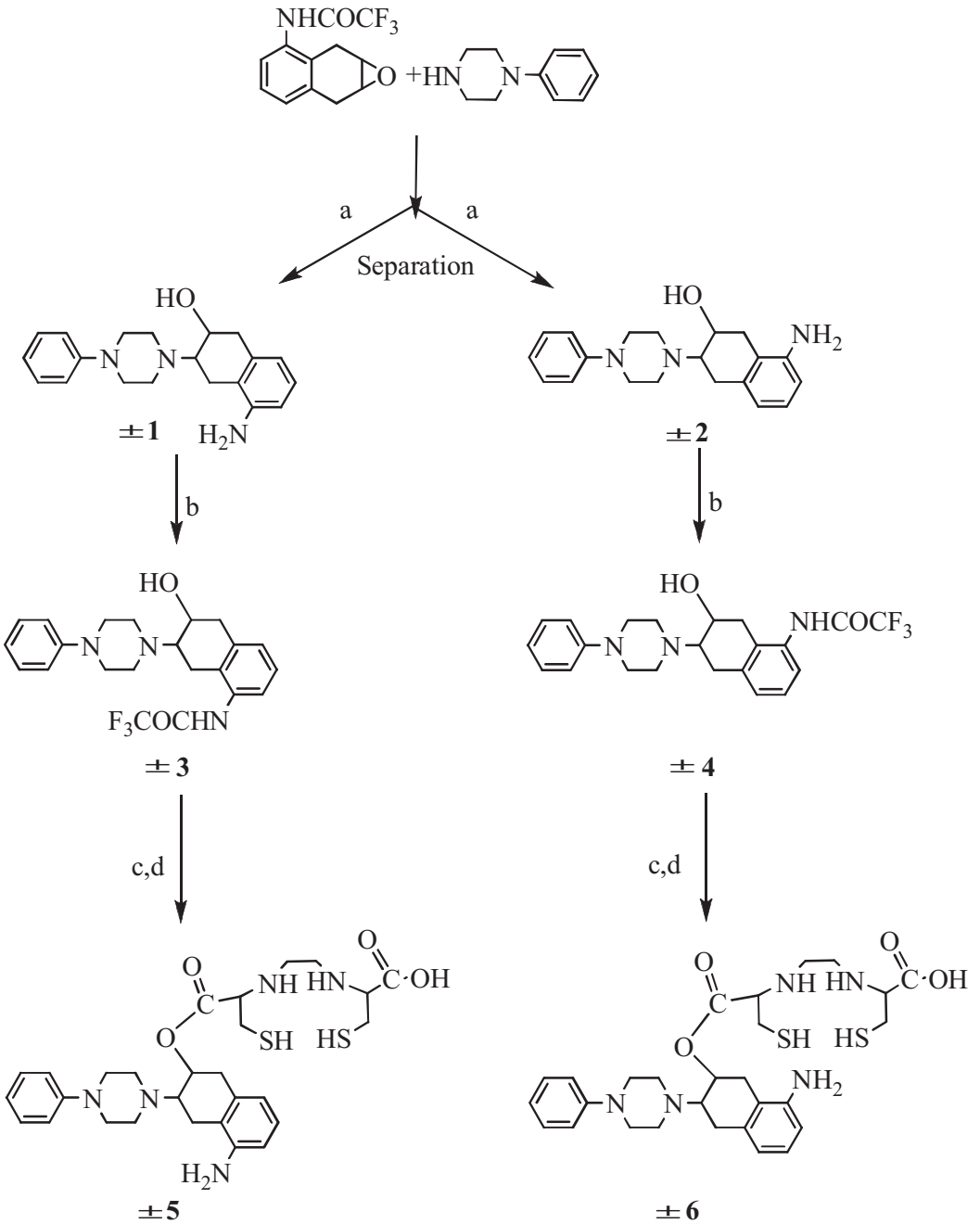
Results and discussion

New products (±)-**5**, (±)-**6**, and (±)-**9** were synthesized according to the reactions shown in Schemes 2 and 3. First, the synthesis was started with the addition of phenylpiperazine to the *N*-(trifluoroacetyl)-1-amino-5,8-dihydronaphthalene oxide to obtain 2 regioisomers, (±)-**1** and (±)-**2**, which were separated by flash chromatography (silica gel; Et_2O and Et_3N, 10:1). The amine function was then protected by the reaction with trifluoroacetic anhydride in benzene to give (±)-**3** and (±)-**4** in 62% and 98% yields, respectively. Conjugation of (±)-**3** and (±)-**4** was performed with the appropriate EC in dry CH_2Cl_2 in the presence of DCC and DMAP, which gave the corresponding (±)-trans-N-trifluoroacetyl-5-amino-2-hydroxy-3-(4-phenyl-1-piperazinyl) tetralin and (±)-trans-N-trifluoroacetyl-8-amino-2-hydroxy-3-(4-phenyl-1-piperazinyl) tetralin. The amine function was then deprotected by reduction with a solution of 1 N NaOH in methanol to give (±)-**5** and (±)-**6** in 97% and 94% yields, respectively.

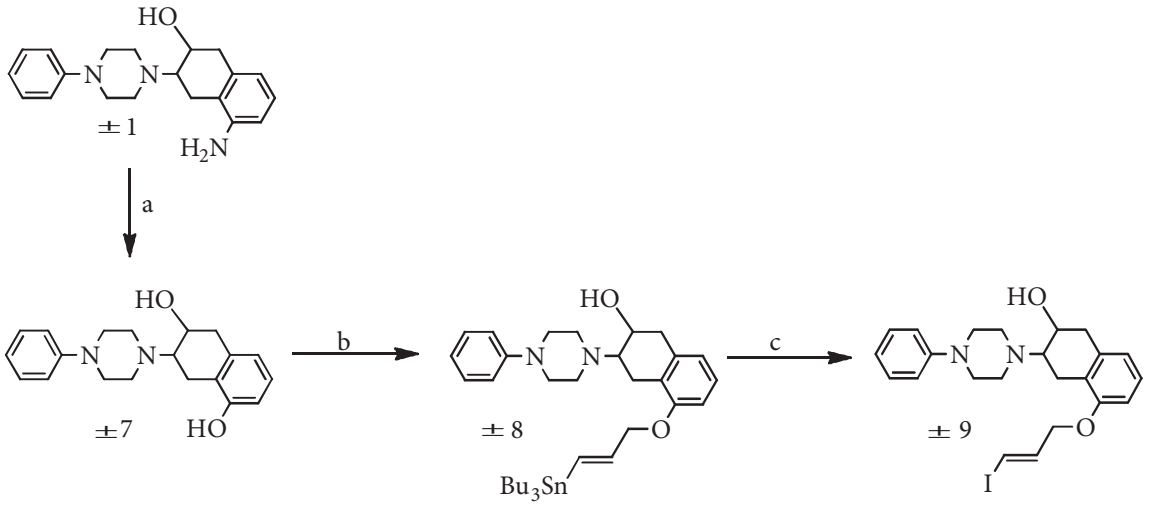
Compound (±)-**9** was obtained in 3 steps. First, the Sandmeyer reaction on racemic (±)-**1** produced the corresponding (±)-trans-2, 5-dihydroxy-3-(4-phenyl-1-piperazinyl) tetralin (±)-**7** (DRC-OH). Stannyl analog (±)-**8** was prepared by O-alkylation of (±)-**7** with (E)-3-chloro-1-tributylstannylprop-2-ene in ethanol.[15] Treatment of (±)-**8** with iodine solution gave compound (±)-**9**.

All compounds were characterized by ^{13}C- and ^{1}H-NMR and IR spectroscopic techniques. The IR, ^{1}H-NMR, and ^{13}C-NMR spectra of (±)-**2** were very similar to the corresponding spectra of (±)-**1**. The COSY-NMR spectrum of (±)-**1** (Figure 2) showed the following correlations: the H-2 proton was coupled with the 3 protons H-3 and 2H-1, while the H-3 proton was also correlated with 3 protons corresponding to 2H-4 and H-2. The 2 protons 2H-1 were correlated with H-2. The 4 protons 4H-10 were correlated with the 4 protons 4H-9. Proton H-6 was correlated with H-7, which was coupled with the 2 protons H-8 and H-6, respectively. The HSQC-NMR spectrum of (±)-**1** (Figure 3) showed the following main correlations: H-2 was coupled with

C-2 at 65.2 and H-3 was coupled with C-3 at 49.9 ppm. Each of the remaining protons was coupled with its bonded carbon center.



Scheme 2. Synthesis of (±)-5 and (±)-6. Reagents and conditions: a) EtOH, reflux, 24 h, separation; b) TFA, benzene, 0 °C,1 h; c) dry CH_2Cl_2, EC, DCC, DMAP, RT; d) EtOH, NaOH.



Scheme 3. Synthesis of (±)-9. Reagents and conditions: a) THF, H_2SO_4, $NaNO_2$, 0 °C; b) TBAOH, CH_3CN, (*E*)-Bu_3SnCH=CH-CH_2-Cl, 80 °C; c) I_2, $CHCl_3$, RT.

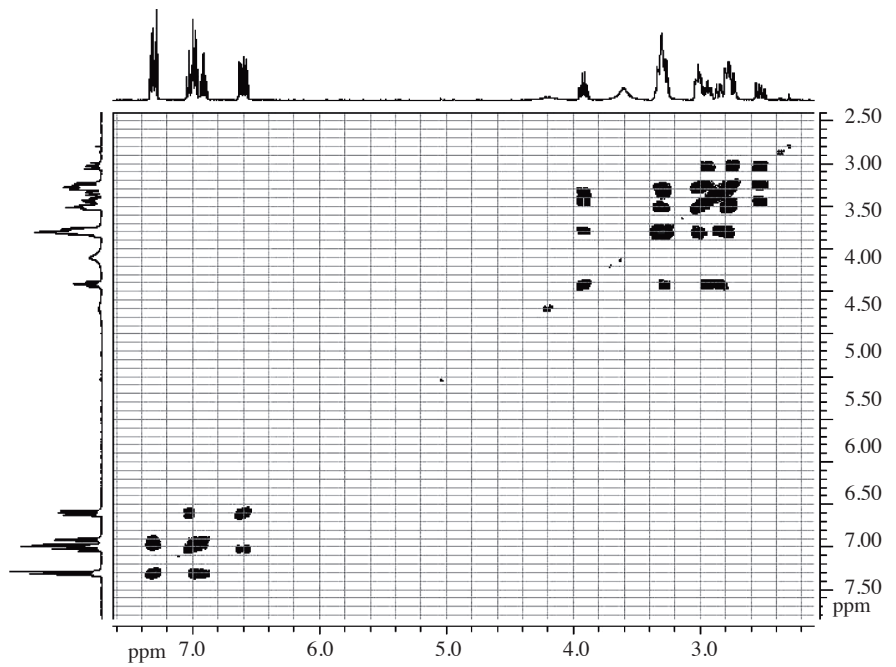


Figure 2. ^{1}H-^{1}H COSY-NMR of (±)-1.

The IR spectrum of new compound **(±)-5** showed the characteristic absorption bands of the hydroxyl and ester groups of EC at 3250 and 1627.4 cm^{-1}, respectively. The ^{1}H-NMR spectrum of **(±)-5** revealed 3 extra multiplets in comparison with the ^{1}H-NMR spectrum of **(±)-1**, at 1.25, 1.79, and 3.51, which corresponded with the $4CH_2$, 2SH, NH, and CH groups of EC. The free OH and the remaining NH groups of EC gave 2 singlets at 8.35 and 3.60, respectively. The ^{13}C-NMR spectrum of **(±)-5** also showed 6 new singlets in comparison

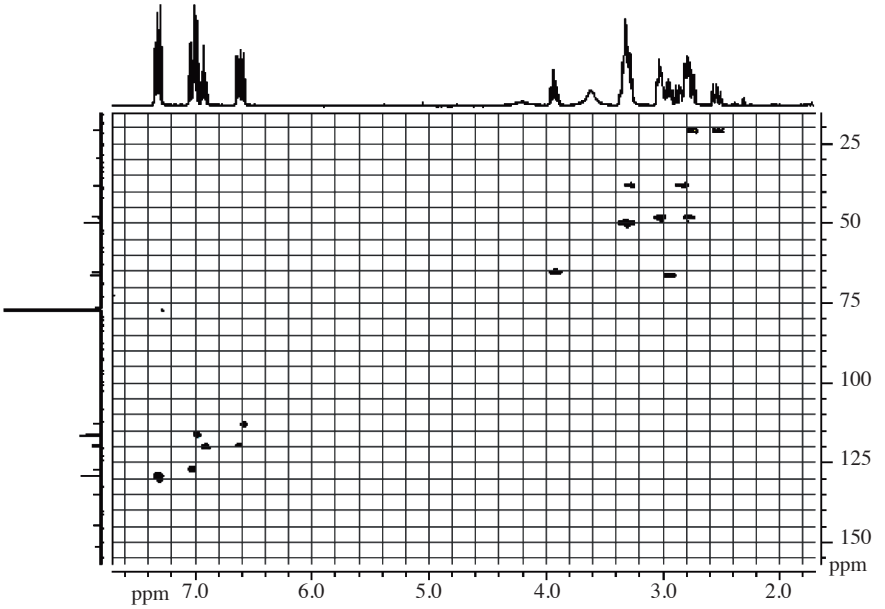


Figure 3. ^{1}H-^{13}C HSQC-NMR of (±)-1.

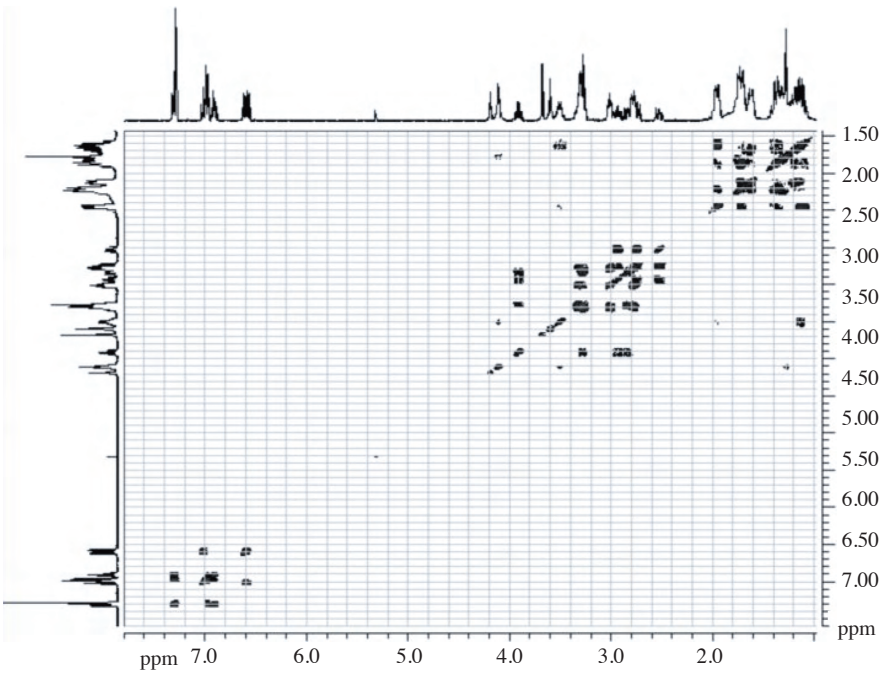


Figure 4. ^{1}H-^{1}H COSY-NMR of (±)-5.

with the ^{13}C-NMR spectrum of **(±)-1**, which corresponded to the 4CH_2, 2CH, and C=O groups of EC. The COSY-NMR spectrum of **(±)-5** (Figure 4) was similar to that of compound **(±)-1.** However, new peaks were found arising from EC protons (NH at 1.23, CH_2 at 1.24 and 1.83, and 2H (SH) at 1.79 ppm). Each peak was coupling with itself. The HSQC-NMR spectrum of **(±)-5** (Figure 5) showed the following correlation: the

$4CH_2$ of EC at 1.24 and 1.83 ppm were coupled with 2 carbons, CH_2, at 24.95 and 33.97 ppm. Each of the remaining protons was coupled with their bonded carbon center. The IR, ^{1}H-NMR, and ^{13}C-NMR spectra of **(±)-6** were very similar to the corresponding spectra of **(±)-5**.

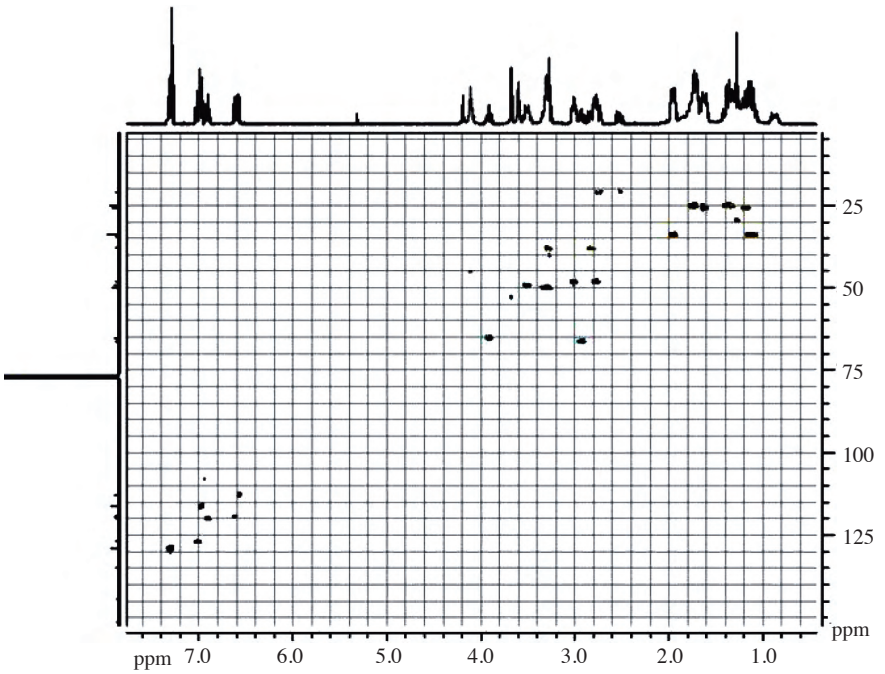


Figure 5. ^{1}H-^{13}C HSQC-NMR of (±)-5

The ^{1}H-NMR spectrum of **(±)-9** was similar to that of compound **(±)-1.** However, new peaks were found arising from (E)-3-chloro-1-tributylstannylprop-2-ene protons (doublet and multiplet at 4.50 and 6.51 ppm corresponding to O-$\underline{CH_2}$ and -$\underline{CH{=}CH}$-, respectively). The ^{13}C-NMR spectrum of **(±)-9** showed 20 singlets, corresponding with 20 different carbon environments in the molecule at their expected chemical shifts.

Compounds **(±)-5** and **(±)-6** showed a moderate lipophilicity (2.0659) (Table) compared with reference compounds IBVM and DRC140 (5.1592 and 4.5292, respectively). However, the lipophilicity of **(±)-9** and DRC140 showed very similar behaviors (4.5292 and 4.8722, respectively). Therefore, the changing of the lipophilicity of compounds **(±)-5** and **(±)-6** may be considered as a potential method for preparing benzovesamicol derivatives with high affinity and selectivity for VAChT. These could be useful in early detection and follow-up of Alzheimer's disease in humans. Attempts were also made to obtain crystals of **(±)-5** and **(±)-9** suitable for single-crystal X-ray analysis, but these were unsuccessful.

Table. Calculated log *p* (clog *p*) (Chem Office 2006 v.10).

	IBVM	DRC140	AOIBV	(±)-5 or (±)-6	(±)-9
Clog*p*	5.1592	4.5292	5.5022	2.0659	4.8722

Conclusion

Three new regioisomer analogs of benzovesamicol, **(±)-5**, **(±)-6**, and **(±)-9**, were synthesized with good yields and purities. They were characterized by both IR and NMR spectroscopy. These compounds will be radiolabeled for further biological evaluation using single photon emission computed tomography (SPECT).

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