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5-Substituted 3,4-dihydro-3-amino-2*H*-1-benzopyran derivatives: synthesis and interaction with serotoninergic receptors

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Abstract

A new series of 3,4-dihydro-3-amino-2*H*-1-benzopyran derivatives (**1** and **2**) bearing various substituents on the 5-position was successfully prepared via palladium-mediated cross-coupling reactions. Some of the new compounds showed high affinity for 5-HT_{1A} and 5-HT_7 receptors. The best affinity for the 5-HT_{1A} and 5-HT_7 receptors was obtained for **2b** (K_i = 0.3 nM for 5-HT_{1A} and 3.1 nM for 5-HT_7). The anxiolytic activity of compound **2b** was evaluated.

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Introduction

The dysfunction in serotoninergic systems of the brain has been found to be a most probable cause of illnesses such as anxiety and depression (Barrett & Vanover 1993; Blier & de Montigny 1994; Hamon 1994). Physiological actions of serotonin (5-HT) are transduced through receptors located on both pre- and postsynaptic neuronal membranes. Presently, seven receptors divided into different subtypes have been classified (5-HT_{1A-F}, 5-HT_{2A-C}, 5-HT_3, 5-HT_4, 5-HT_{5A-B}, 5-HT_6 and 5-HT_7) (Gerhardt & van Heerikhuizen 1997). The 5-HT_7 receptors, positively coupled to adenylate cyclase, were recently discovered by molecular cloning (Bard et al 1993; Lovenberg et al 1993). Many psychotherapeutic drugs of diverse classes appear to have high affinity for 5-HT_7 receptors. Thus, high 5-HT_7-receptor affinity is observed for 5-HT, 5-carboxyamidotryptamine (5-CT), 5-methoxytryptamine (5-MeOT) and methiothepin, moderate affinity for 8-hydroxy-di-*n*-propylamino-tetralin (8-OH-DPAT), clozapine and ritanserin, and low affinity for pindolol, sumatriptan and buspirone (Roth et al 1994). Recently, three selective 5-HT_7 antagonists SB-258719, SB-269970 and DR-4004 have been reported (Figure 1). The compound SB-258719 has been described as the first 5-HT_7 receptor antagonist with 100-fold selectivity over other 5-HT, α_{1b}-adrenergic and D_2,D_3 dopaminergic receptors (Forbes et al 1998). Compared with SB-258719, the compound SB-269970 shows a high affinity for 5-HT_7 (pK_i = 8.9) with an improved selectivity over 13 other receptors (Lovell et al 2000). The compound DR-4004 acts as a potent 5-HT_7 antagonist (pK_i = 8.7) with selectivity over 5-HT_2 receptors (Kikuchi et al 1999).

The biological function of 5-HT_7 receptors is poorly understood. Nevertheless, receptor localisation studies, combined with some preliminary pharmacological results, suggest that 5-HT_7 receptors could be involved in the pathophysiology of

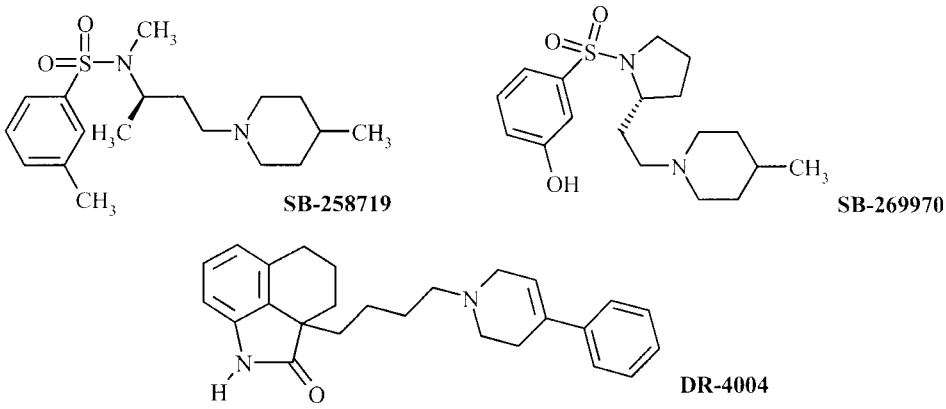


Figure 1 Structures of 5-HT_7 receptor antagonists (Forbes et al 1998; Kikuchi et al 1999; Lovell et al 2000).

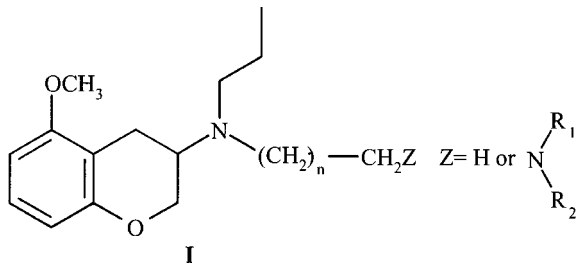


Figure 2 Structures of derivatives **I** (Besson et al 1993; Podona et al 1994; Comoy et al 1996).

sleep disorders, depression and schizophrenia (Eglen et al 1997). Therefore, the discovery of agonists and antagonists of 5-HT_7 receptors may have clinical utility.

A range of *N*-disubstituted (5-methoxy-3,4-dihydro-2*H*-1-benzopyran-3-yl)amino derivatives (**I**; Figure 2), prepared in our laboratory, exhibited high affinity for 5-HT_{1A} receptors (Besson et al 1993; Podona et al 1994; Comoy et al 1996). Moreover, screening of derivatives **I** against the cloned human 5-HT_7 receptor showed, for some of them such as 5-methoxy-3,4-dihydro-3-di-*n*-propylamino-2*H*-1-benzopyran (5-MeO-DPAC) and S20244 (Figure 3), significant affinity for this receptor (Rezaie et al 1998a). On this basis, we planned the further design of potent and selective 5-HT_7 ligands, in particular by varying systematically the nature of the substituent at the 5-position of the 2*H*-1-benzopyran ring to cover further hydrophobic, aromatic ring and H-bond acceptor capacities.

This paper reports the synthesis and the preliminary pharmacological evaluation of a series of 3,4-dihydro-3-amino-2*H*-1-benzopyran derivatives of formulae **1** (Rezaie et al 1998b) and **2** (Figure 4) bearing *N*-substituents already known for their high affinities for 5-HT_{1A} (Podona et al 1994). The substituents on the 5-position were introduced via well-documented palladium-mediated cross-coupling reactions (Carrera & Sheppard 1994; Stille 1986). This chemical strategy has been reported previously for the preparation of 8-substituted 2-(dipropylamino)tetralin (Liu et al 1993).

Materials and Methods

Chemistry

Melting points were determined using a Büchi SMP-20 melting point apparatus and are uncorrected. The IR spectra were recorded on a Perkin Elmer FTIR paragon

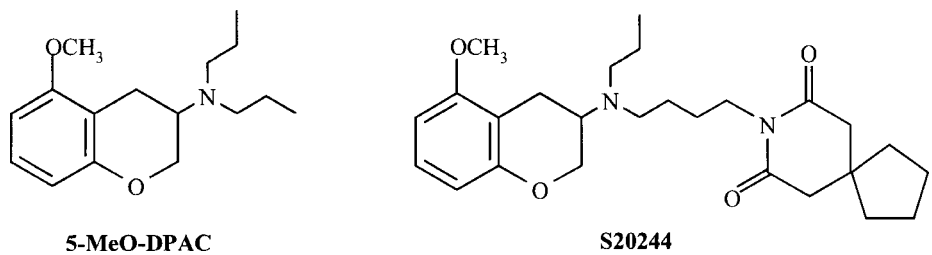
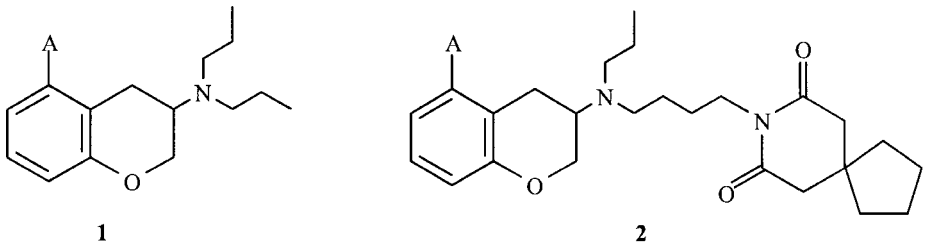


Figure 3 Structures of 5-MeO-DPAC and S20244.



1 2

A: **a**= CH_2CH_3 **b**= $COCH_3$ **c**= Ph

Figure 4 Structures of 5-substituted 3,4-dihydro-3-amino-2*H*-1-benzopyran derivatives **1** and **2**.

1000 spectrophotometer. Proton and carbon NMR were recorded at 300°K in $CDCl_3$ or DMSO-d_6 on a Bruker Avance DPX 250 spectrometer (250.13 MHz for ^{1}H and 62.90 MHz for ^{13}C). Coupling constants are in Hz and chemical shifts are expressed in parts per million and are referenced to tetramethylsilane. MS spectra were recorded on a Perkin-Elmer SCIEX API 300 instrument using ionspray methodology. Analytical TLC was run on pre-coated silica-gel plates (Merck $60F_{254}$) and spots visualised with UV light. Flash chromatography was carried out on column using silica gel 60 Merck (40–63 μm) as the stationary phase. Organic solvents were purified as necessary as described by Armarego & Perrin (1996) or purchased from Sigma-Aldrich or Acros. All reactions requiring anhydrous conditions were conducted in flame-dried apparatus.

*5-Hydroxy-3,4-dihydro-3-(*tert*-butoxycarbonylamino)-2*H*-1-benzopyran* (***4***)

A solution of compound **3** (2.1 g, 12 mmol) in hydrobromic acid 48% in water (13 mL) and glacial acetic acid (20 mL) was heated at 138°C for 6 h. The solution was evaporated to give quantitatively the demethylated hydrobromide salt as crystals. To a suspension of the latter compound (1.9 g, 7.7 mmol) in tetrahydrofuran (20 mL) were added aqueous 1 M sodium hydroxide solution (18 mL) and di-*tert*-butyldicarbonate (2.1 g, 9.6 mmol). The solution was stirred overnight. The two phases were separated and the aqueous phase was acidified with aqueous 1 M hydrochloric acid solution and then extracted with dichloromethane (3 × 20 mL). The combined organic phases were dried over magnesium sulphate and evaporated in-vacuo. The crude residue was purified by flash chromatography (petroleum ether–ethyl acetate, 8:2) to give **4** as crystals (2.0 g, 98% yield); mp 158–160°C (ethyl acetate–petroleum ether); IR (KBr): ν 3374, 1682 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.45 (s, 9H, $C(CH_3)_3$), 2.69 (br d, J = 16.3, 1H, CH_2Ar), 2.89 (dd, J = 5.6, 16.3, 1H, CH_2Ar), 4.02–4.20 (m, 3H, CHN, CH_2O), 4.97 (br d, J = 7.6, 1H, NH), 6.05 (br s, 1H, OH), 6.43 (t, J = 8.3, 2H, H_{Ar}), 6.97 (t, J = 8.3, 1H, H_{Ar}); ^{13}C-NMR ($CDCl_3$): δ 26.0 (CH_2), 28.4 (3 CH_3), 43.0 (CH), 68.0 (CH_2), 80.0 (C), 107.4 (2 CH), 108.9 (CH), 127.4 (CH), 155.0 (2 C), 155.6 (C=O); MS: m/z 266 $(M+1)^+$; Calculated for $C_{14}H_{19}NO_4$: C, 63.38; H, 7.22; C, 5.28; found: C, 63.56; H, 7.07, N, 5.33.

*5-(Trifluoromethylsulfonyloxy)-3,4-dihydro-3-(*tert*-butoxycarbonylamino)-2*H*-1-benzopyran* (***5***)

To a stirred solution of compound **4** (2.2 g, 8.3 mmol) in dichloromethane (50 mL) under argon at −30°C was added pyridine (1.4 mL, 17.3 mmol) followed by trifluoromethanesulfonic anhydride (1.8 mL, 10.7 mmol). The solution was stirred at −20°C for 2 h and then allowed to reach room temperature. The organic solution was then washed with saturated aqueous sodium bicarbonate solution (30 mL) and water (20 mL), dried over magnesium sulphate and evaporated in-vacuo. The residue was purified by flash chromatography (petroleum ether–ethyl acetate, 94:6) to give **5** as crystals (3.0 g, 93% yield); mp 82–84°C (ethyl acetate–petroleum ether); IR (KBr): ν 3374, 1682 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.43 (s, 9H, $C(CH_3)_3$), 2.84 (dd, J = 4.1, 18.4, 1H, CH_2Ar), 3.07 (dd, J = 5.3, 18.4, 1H, CH_2Ar), 4.13–4.26 (m, 3H, CHN, CH_2O), 4.85 (br d, J = 7.6, 1H, NH), 6.89 (d, J = 8.5, 2H, H_{Ar}), 7.19 (t, J = 8.5, 1H, H_{Ar}); ^{13}C-NMR ($CDCl_3$): δ 26.5 (CH_2), 28.3 (3 CH_3), 42.3 (CH), 68.3 (CH_2), 80.1 (C), 113.6 (CH), 113.9 (CH), 117.0 (C), 118.6 (q, J = 320.2 Hz, CF_3), 128.1 (CH), 148.6 (C), 155.0 (C), 155.4 (C=O); MS *m/z* 398 $(M+1)^+$; Calculated for $C_{15}H_{18}F_3NO_6S$: C, 45.34; H, 4.57; C, 3.52; found: C, 44.99; H, 4.38; N, 3.65.

*5-Ethyl-3,4-dihydro-3-(*tert*-butoxycarbonylamino)-2*H*-1-benzopyran* (***6a***)

To a mixture of **5** (2.5 g, 6.3 mmol), lithium chloride (667 mg, 15.7 mmol) and bis(triphenylphosphine)palladium chloride (221 mg, 0.31 mmol) in dry *N*,*N*-dimethylformamide (30 mL) under argon was added tri-

butyl(vinyl)tin (2.2 mL, 7.6 mmol). The stirred solution was heated at 90°C for 5 h. The solvent was then removed in-vacuo and the remaining residue was purified by flash chromatography (petroleum ether–ethyl acetate 9:1) to give the vinyl derivative (1.31 g, 75% yield) used in the next step without further characterisation. A mixture of vinyl compound (532 mg, 1.9 mmol) and palladium on charcoal 10% (50 mg) in ethanol (20 mL) was shaken in a Parr apparatus under 50 psig of hydrogen at room temperature for 6 h. The catalyst was filtered through celite and the solvent was removed in-vacuo. The crude residue was purified by flash chromatography (petroleum ether–ethyl acetate, 9:1) to give **6a** as crystals (365 mg, 65 % overall yield); mp 98–99°C (ethyl acetate–petroleum ether); IR (KBr): ν 3439, 1705 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.19 (t, J = 7.5, 3H, CH_3), 1.44 (s, 9H, $C(CH_3)_3$), 2.54 (q, J = 7.5, 2H, CH_2), 2.72 (br d, J = 16.6, 1H, CH_2Ar), 2.96 (dd, J = 5.6, 16.6, 1H, CH_2Ar), 3.66–3.73 (m, 1H, CHN), 4.08–4.22 (m, 2H, CH_2O), 4.96 (br d, J = 7.0, 1H, NH), 6.72 (d, J = 7.9, 1H, H_{Ar}), 6.80 (d, J = 7.9, 1H, H_{Ar}), 7.09 (t, J = 7.9, 1H, H_{Ar}); ^{13}C-NMR ($CDCl_3$): δ 14.1 (CH_3), 25.3 (CH_2), 28.4 (3 CH_3), 28.5 (CH_2), 43.4 (CH), 67.9 (CH_2), 79.6 (C), 114.5 (CH), 117.7 (C), 120.7 (CH), 127.3 (CH), 144.2 (C), 154.0 (C), 155.3 (C=O); MS: m/z 278 $(M+1)^+$; Calculated for $C_{16}H_{23}NO_3$: C, 69.29; H, 8.36; C, 5.05; found: C, 68.95; H, 8.54; N, 4.93.

*5-Acetyl-3,4-dihydro-3-(*tert*-butoxycarbonylamino)-2*H*-1-benzopyran* (***6b***)

To a mixture of **5** (1.3 g, 3.3 mmol), lithium chloride (350 mg, 8.3 mmol) and bis(triphenylphosphine)palladium chloride (116 mg, 0.16 mmol) in dry *N*,*N*-dimethylformamide (20 mL) under argon was added tributyl(1-ethoxyvinyl)tin (1.34 mL, 4.0 mmol). The stirred solution was heated at 90°C for 3.5 h. The solvent was then removed in-vacuo and the crude residue was purified by flash chromatography (petroleum ether–ethyl acetate, 9:1 then 75:25) to give a pale yellow oil. The oil was then treated with aqueous 1 M hydrochloric acid solution at room temperature for 1 h. Solid potassium carbonate was added to adjust the pH to 9. The basic solution was extracted with ethyl acetate (2 × 15 mL). The organic phase was dried over magnesium sulphate and evaporated in-vacuo to give **6b** as an oil (758 mg, 79% yield); IR (film): ν 3441, 1702, 1688 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.43 (s, 9H, $C(CH_3)_3$), 2.56 (s, 3H, CH_3), 2.96 (dd, J = 5.1, 17.6, 1H, CH_2Ar), 3.31 (dd, J = 5.6, 17.6, 1H, CH_2Ar), 4.01–4.16 (m, 3H, CH_2O, CHN), 4.75 (br s, 1H, NH), 7.01 (dd, J = 1.2, 7.7, 1H, H_{Ar}), 7.20 (t, J = 7.7, 1H, H_{Ar}), 7.36 (dd, J = 1.2, 7.7, 1H, H_{Ar}); ^{13}C-NMR ($CDCl_3$): δ 28.3 (3 CH_3), 29.5 (CH_3), 30.6 (CH_2), 43.2 (CH), 68.0 (CH_2), 79.7 (C), 119.8 (CH), 121.0 (CH), 122.8 (CH), 127.1 (CH), 138.5 (C), 154.7 (C), 155.1 (C=O), 200.9 (C=O); MS: m/z 292 $(M+1)^+$; Calculated for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.27; C, 4.81; found: C, 66.23; H, 7.33; N, 4.96.

*5-Phenyl-3,4-dihydro-3-(*tert*-butoxycarbonylamino)-2*H*-1-benzopyran* (***6c***)

To a stirred solution of **5** (1.5 g, 3.8 mmol) in toluene (30 mL) under argon was added freshly prepared *tetrakis*(triphenylphosphine)palladium (219 mg, 0.2 mmol). The mixture was allowed to stir for 30 min at room temperature. Phenylboronic acid (695 mg, 5.7 mmol) in ethanol (15 mL) was then added, followed immediately by saturated aqueous sodium bicarbonate solution (15 mL). The heterogeneous solution was thereafter refluxed for 5 h. Brine solution was then added, the two layers were separated and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The combined organic extracts were dried over magnesium sulphate and evaporated in-vacuo. The crude residue was purified by flash chromatography (petroleum ether–ethyl acetate, 95:5 then 80:20) to afford **6c** as crystals (990 mg, 80% yield); mp 116–118°C (ethyl acetate–petroleum ether); IR (KBr): ν 3439, 1706 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.40 (s, 9H, $C(CH_3)_3$), 2.54 (dd, J = 4.3, 16.7, 1H, CH_2Ar), 2.96 (br d, J = 16.7, 1H, CH_2Ar), 4.11–4.18 (m, 3H, CH_2O, CHN), 4.77 (br s, 1H, NH), 6.84–6.89 (m, 2H, H_{Ar}), 7.19 (t, J = 7.8, 1H, H_{Ar}), 7.26–7.44 (m, 5H, H_{Ar}); ^{13}C-NMR ($CDCl_3$): δ 28.3 (3 CH_3), 30.4 (CH_2), 43.3 (CH), 68.1 (CH_2), 79.7 (C), 115.9 (CH), 117.2 (C), 122.6 (CH), 127.2 (CH), 127.3 (CH), 128.3 (2 CH), 129.0 (2 CH), 140.5 (C), 143.7 (C), 154.0 (C), 155.1 (C=O); MS: m/z 326 $(M+1)^+$; Calculated for $C_{20}H_{23}NO_3$: C, 73.82; H, 7.12; C, 4.30; found: C, 74.11; H, 7.02; N, 4.47.

*General method for the preparation of 5-substituted-3,4-dihydro-3-amino-2*H*-1-benzopyrans (7**a–c**)*

To a stirred solution of **6** (4.62 mmol) in dichloromethane (5 mL) was added trifluoroacetic acid (4 mL); stirring continued for 1 h. The solvent was evaporated and the crude residue was extracted with dichloromethane (2 × 20 mL). The organic extract was washed with saturated aqueous sodium bicarbonate solution (2 × 10 mL). The combined aqueous layers were extracted with dichloromethane (30 mL). The combined organic extracts were dried over magnesium sulphate and evaporated to give **7** as an oil.

*5-Ethyl-3,4-dihydro-3-amino-2*H*-1-benzopyran* (***7a***). Oil (90% yield); IR (film): ν 3426, 3361 cm^{-1}; ^{1}H-NMR

($CDCl_3$): δ 1.21 (t, J = 7.6, 3H, CH_3), 1.40 (br s, 2H, NH_2), 2.47 (dd, J = 8.0, 16.3, 1H, CH_2Ar), 2.57 (q, J = 7.6, 2H, CH_2), 3.01 (dd, J = 4.7, 16.3, 1H, CH_2Ar), 3.33–3.39 (m, 1H, CHN), 3.76 (ddd, J = 0.7, 7.4, 10.4, 1H, CH_2O), 4.12 (ddd, J = 1.6, 3.0, 10.4, 1H, CH_2O), 6.71 (d, J = 8.1, 1H, H_{Ar}), 6.79 (d, J = 8.1, 1H, H_{Ar}), 7.07 (t, J = 8.1, 1H, H_{Ar}); ^{13}C-NMR ($CDCl_3$): δ 14.2 (CH_3), 25.4 (CH_2), 32.0 (CH_2), 44.4 (CH), 71.0 (CH_2), 114.3 (CH), 118.5 (C), 120.3 (CH), 127.1 (CH), 143.8 (C), 154.0 (C); MS: m/z 178 $(M+1)^+$; Calculated for $C_{11}H_{15}NO$: C, 74.54; H, 8.53; C, 7.90; found: C, 74.33; H, 8.50; N, 8.05.

*5-Acetyl-3,4-dihydro-3-amino-2*H*-1-benzopyran* (***7b***). Oil (97% yield); IR (film): ν 3426, 1681 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.46 (br s, 2H, NH_2), 2.56 (s, 3H, CH_3), 2.82 (dd, J = 8.8, 18.6, 1H, CH_2Ar), 3.25–3.34 (m, 2H, CH_2Ar, CHN), 3.79–3.86 (m, 1H, CH_2O), 4.11–4.17 (m, 1H, CH_2O), 7.00 (dd, J = 1.3, 8.1, 1H, H_{Ar}), 7.18 (t, J = 8.1, 1H, H_{Ar}), 7.34 (dd, J = 1.3, 8.1, 1H, H_{Ar}); ^{13}C-NMR ($CDCl_3$): δ 29.6 (CH_3), 33.8 (CH_2), 43.8 (CH), 70.8 (CH_2), 120.6 (CH, C), 122.5 (CH), 126.8 (CH), 138.4 (C), 154.7 (C), 201.2 (C═O); MS: m/z 192 $(M+1)^+$; Calculated for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85; C, 7.32; found: C, 68.78; H, 6.97; N, 7.15.

*5-Phenyl-3,4-dihydro-3-amino-2*H*-1-benzopyran* (***7c***). Oil (99% yield); IR (film): ν 3367, 3289 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.43 (br s, 2H, NH_2), 2.44 (dd, J = 6.0, 16.5, 1H, CH_2Ar), 2.86 (br d, J = 16.5, 1H, CH_2Ar), 3.23–3.35 (m, 1H, CHN), 3.82 (dd, J = 7.2, 10.1, 1H, CH_2O), 4.16 (br d, J = 10.1, 1H, CH_2O), 6.81–6.86 (m, 2H, H_{Ar}), 7.16 (t, J = 7.8, 1H, H_{Ar}), 7.26–7.43 (m, 5H, H_{Ar}); ^{13}C-NMR ($CDCl_3$): δ 30.9 (CH_2), 44.1 (CH), 71.2 (CH_2), 115.6 (CH), 118.0 (C), 122.2 (CH), 125.9 (C), 127.1 (CH), 128.1 (2 CH), 129.1 (2 CH), 140.8 (C), 143.5 (C), 154.0 (C); MS: m/z 226 $(M+1)^+$; Calculated for $C_{15}H_{15}NO$: C, 79.97; H, 6.71; C, 6.22; found: C, 80.24; H, 6.88; N, 6.09.

*General method for the preparation of 8-[*N*-(5-substituted-3,4-dihydro-2*H*-1-benzopyran-3-yl)aminobutyl]-8-azaspiro[4.5]decane-7,9-diones* (***8a–c***)

To a stirred solution of **7** (6.8 mmol) in dry acetonitrile (30 mL) under argon was added a solution of 8-(4-bromobutyl)-8-azaspiro[4.5]decane-7,9-dione (10 mmol) in dry acetonitrile (10 mL) and then potassium carbonate (20 mmol) and a catalytic amount of sodium iodide. The mixture was stirred at 90°C overnight, and the solvent was removed in-vacuo. After addition of water, the product was extracted with dichloromethane (30 mL). The organic layer was washed with water (2 × 10 mL), dried over magnesium sulphate, then evaporated in-vacuo. The crude oil was purified by column chromatography (ethyl acetate–MeOH, 95:5 for **8a** and **8b**; petroleum ether–ethyl acetate, 94:6 then ethyl acetate for **8c**) to give **8**.

*8-[*N*-(5-Ethyl-3,4-dihydro-2*H*-1-benzopyran-3-yl)-aminobutyl]-8-azaspiro[4.5]decane-7,9-dione* (***8a***). Oil (70% yield); IR (film): ν 3376, 1724, 1667 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.20 (t, J = 7.3, 3H, CH_3), 1.46–1.74 (m, 13H, NH, CH_2), 2.48–2.61 (m, 6H, CH_2CH_3, CH_2CO), 2.65–2.80 (m, 3H, CH_2Ar, CH_2N), 2.94 (dd, J = 5.2, 15.6, 1H, CH_2Ar), 3.07–3.17 (m, 1H, CHN), 3.73–3.85 (m, 3H, CH_2NCO, CH_2O), 4.15–4.22 (m, 1H, CH_2O), 6.69 (d, J = 7.8, 1H, H_{Ar}), 6.77 (d, J = 7.8, 1H, H_{Ar}), 7.05 (t, J = 7.8, 1H, H_{Ar});^{13}C-NMR ($CDCl_3$): δ 14.1 (CH_3), 24.2 (2 CH_2), 25.4 (CH_2), 25.7 (CH_2), 27.8 (CH_2), 29.5 (CH_2), 37.6 (2 CH_2), 39.3 (CH_2), 39.5 (C), 44.9 (2 CH_2), 46.7 (CH_2), 50.4 (CH_2), 68.4 (CH_2), 114.2 (CH), 118.7 (C), 120.2 (CH), 126.9 (CH), 143.7 (C), 154.4 (C), 172.2 (2 C═O); MS: m/z 399 $(M+1)^+$; Calculated for $C_{24}H_{34}N_2O_3$: C, 72.33; H, 8.60; C, 7.03 ; found: C, 72.54; H, 8.52; N, 6.88.

*8-[*N*-(5-Acetyl-3,4-dihydro-2*H*-1-benzopyran-3-yl)-aminobutyl]-8-azaspiro[4.5]decane-7,9-dione* (***8b***). Solid (60% yield); mp 82°C (ethyl acetate–petroleum ether); IR (KBr): ν 3376, 1724, 1669 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.46–1.74 (m, 13H, NH, CH_2), 2.56 (s, 3H, $COCH_3$), 2.57 (s, 4H, CH_2CO), 2.72 (t, J = 7.0, 2H, CH_2N), 2.82 (dd, J = 7.3, 17.3, 1H, CH_2Ar), 3.00–3.10 (m, 1H, CHN), 3.27 (dd, J = 4.8, 17.3, 1H, CH_2Ar), 3.76 (t, J = 7.3, 2H, CH_2NCO), 3.88 (dd, J = 7.3, 10.6, 1H, CH_2O), 4.20 (br d, J = 10.6, 1H, CH_2O), 6.98 (d, J = 7.8, 1H, H_{Ar}), 7.18 (t, J = 7.8, 1H, H_{Ar}), 7.34 (d, J = 7.8, 1H, H_{Ar}). ^{13}C-NMR ($CDCl_3$): δ 24.2 (2 CH_2), 25.7 (CH_2), 27.7 (CH_2), 29.7 (CH_2), 31.2 (CH_2), 37.6 (2 CH_2), 39.3 (CH_2), 39.5 (C), 44.9 (2 CH_2), 46.8 (CH_2), 49.9 (CH), 68.6 (CH_2), 120.7 (CH), 120.9 (C), 122.4 (CH), 126.8 (CH), 138.5 (C), 155.1 (C), 172.3 (2 C═O), 201.3 (C═O); MS: m/z 413 $(M+1)^+$; Calculated for $C_{24}H_{32}N_2O_4$: C, 69.88; H, 7.82; C, 6.79; found: C, 69.67; H, 8.01; N, 6.85.

*8-[*N*-(5-Phenyl-3,4-dihydro-2*H*-1-benzopyran-3-yl)-aminobutyl]-8-azaspiro[4.5]decane-7,9-dione* (***8c***). Oil (57% yield); IR (film): ν 3376, 1723, 1673 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.40–1.71 (m, 13H, NH, CH_2), 2.47–2.67 (m, 7H, CH_2CO, CH_2N, CH_2Ar), 2.77 (dd, J = 6.3,

17.2, 1H, CH_2Ar), 2.99–3.02 (m, 1H, CHN), 3.64–3.89 (m, 3H, CH_2NCO, CH_2O), 4.20–4.27 (m, 1H, CH_2O), 6.80–6.85 (m, 2H, H_{Ar}), 7.14 (t, J = 7.6, 1H, H_{Ar}), 7.27–7.46 (m, 5H, H_{Ar});^{13}C-NMR ($CDCl_3$): δ 24.1 (2 CH_2), 25.7 (CH_2), 27.7 (CH_2), 31.4 (CH_2), 37.5 (2 CH_2), 39.2 (CH_2), 39.5 (C), 44.9 (2 CH_2), 46.8 (CH_2), 50.4 (CH), 68.7 (CH_2), 115.6 (CH), 118.3 (C), 122.1 (CH), 127.0 (2 CH), 128.1 (2 CH), 129.0 (2 CH), 140.8 (C), 143.3 (C), 154.4 (C), 172.2 (2 C═O); MS: *m/z* 447 $(M+1)^+$; Calculated for $C_{28}H_{34}N_2O_3$: C, 75.31; H, 7.67; C, 6.27; found: C, 75.60; H, 7.82; N, 6.11.

*General method for the preparation of 8-[4-*N*-propyl-*N*-(5-substituted-3,4-dihydro-2*H*-1-benzopyran-3-yl)aminobutyl]-8-azaspiro[4.5]decane-7,9-diones (**2a–c**)*

To a stirred solution of **8** (2.7 mmol) in dry acetonitrile (25 mL) under argon were added potassium carbonate (2.7 mmol) and then iodopropane (5.1 mmol). The mixture was stirred at 90°C for 20 h. The reaction was diluted with water, then acetonitrile was evaporated and the mixture was extracted with dichloromethane (40 mL). The organic layer was washed with water (2 × 15 mL), dried over magnesium sulphate and evaporated in-vacuo. The crude residue was purified by column chromatography (petroleum ether–ethyl acetate, 1:1 then ethyl acetate) to afford **2** as an oil.

*8-[4-*N*-Propyl-*N*-(5-ethyl-3,4-dihydro-2*H*-1-benzopyran-3-yl)aminobutyl]-8-azaspiro[4.5] decane-7,9-dione (**2a**)*. Oil (49% yield); IR (film): ν 1725, 1673 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.91 (t, J = 7.4, 3H, CH_3), 1.25 (t, J = 7.6, 3H, CH_3), 1.44–1.75 (m, 14H, CH_2), 2.51–2.71 (m, 11H, CH_2CO, CH_2N, CH_2Ar, CH_3CH_2), 2.90 (dd, J = 5.2, 15.6 Hz, 1H, CH_2Ar), 3.10–3.22 (m, 1H, CHN), 3.63–3.73 (m, 3H, CH_2NCO, CH_2O), 4.27–4.31 (m, 1H, CH_2O), 6.70 (d, J = 8.1, 1H, H_{Ar}), 6.79 (d, J = 8.1, 1H, H_{Ar}), 7.07 (t, J = 8.1, 1H, H_{Ar});^{13}C-NMR ($CDCl_3$): δ 11.8 (CH_3), 14.1 (CH_3), 22.0 (CH_2), 24.2 (2 CH_2), 25.3 (CH_2), 25.5 (CH_2), 25.8 (CH_2), 26.3 (CH_2), 37.6 (2 CH_2), 39.4 (CH_2), 39.5 (C), 44.9 (2 CH_2), 50.3 (CH), 52.6 (CH_2), 53.5 (CH_2), 67.2 (CH_2), 114.2 (CH), 119.9 (C), 120.0 (CH), 126.8 (CH), 143.7 (C), 154.5 (C), 172.2 (2 C═O); MS: m/z 441 $(M+1)^+$; Calculated for $C_{27}H_{40}N_2O_3$: C, 73.60; H, 9.15; C, 6.36; found: C, 73.56; H, 9.06; N, 6.49.

*8-[4-N-Propyl-N-(5-acetyl-3,4-dihydro-2H-1-benzopyran-3-yl)aminobutyl]-8-azaspiro[4.5]decane-7,9-dione (**2b**)*. Oil (44% yield); IR (film): ν 1724, 1671 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.85 (t, J = 7.3, 3H, CH_3), 1.37–1.72 (m, 14 H, CH_2), 2.43–2.57 (m, 11H, CH_2CO, CH_2N, CH_3), 2.99–3.10 (m, 3H, CH_2Ar, CHN), 3.72–3.85 (m, 3H, CH_2NCO, CH_2O), 4.21–4.27 (m, 1H, CH_2O), 6.93 (dd, J = 1.2, 7.9, 1H, H_{Ar}), 7.14 (t, J = 7.9, 1H, H_{Ar}), 7.28 (dd, J = 1.2, 7.9, 1H, H_{Ar});^{13}C-NMR ($CDCl_3$): δ 11.7 (CH_3), 21.8 (CH_2), 24.1 (2 CH_2), 25.7 (CH_2), 26.1 (CH_2), 26.8 (CH_2), 29.7 (CH_2), 37.5 (2 CH_2), 39.3 (CH_2), 39.4 (C), 44.8 (2 CH_2), 50.3 (CH_2), 52.5 (CH), 53.0 (CH_2), 67.7 (CH_2), 120.4 (CH), 121.9 (C), 122.0 (CH), 126.5 (CH), 138.6 (C), 155.2 (C), 172.1 (2 C═O), 201.3 (C═O); MS: *m/z* 455 $(M+1)^+$; Calculated for $C_{27}H_{38}N_2O_4$: C, 71.34; H, 8.43; C, 6.16; found: C, 71.15; H, 8.27; N, 6.23.

*8-[4-N-Propyl-N-(5-phenyl-3,4-dihydro-2H-1-benzopyran-3-yl)aminobutyl]-8-azaspiro[4.5]decane-7,9-dione (**2c**)*. Oil (53% yield); IR (film): ν 1724, 1671 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.80 (t, J = 7.3, 3H, CH_3), 1.26–1.73 (m, 14H, CH_2), 2.37–2.75 (m, 10H, CH_2N, CH_2CO, CH_2Ar), 2.99–3.10 (m, 1H, CHN), 3.72 (t, J = 7.2, 2H, CH_2NCO), 3.85 (t, J = 10.2, 1H, CH_2O), 4.23–4.30 (m, 1H, CH_2O), 6.79–6.87 (m, 2H, H_{Ar}), 7.14 (t, J = 7.8, 1H, H_{Ar}), 7.30–7.45 (m, 5H, H_{Ar});^{13}C-NMR ($CDCl_3$): δ 11.7 (CH_3), 21.8 (CH_2), 24.2 (2 CH_2), 25.7 (CH_2), 26.1 (CH_2), 27.3 (CH_2), 37.5 (2 CH_2), 39.3 (CH_2), 39.5 (C), 44.9 (2 CH_2), 50.2 (CH_2), 52.6 (CH), 53.5 (CH_2), 67.7 (CH_2), 115.6 (CH), 119.6 (C), 122.0 (CH), 126.9 (CH), 127.0 (CH), 128.1 (2 CH), 129.0 (2 CH), 141.0 (C), 143.4 (C), 154.5 (C), 172.2 (2 C═O); MS: m/z 489 $(M+1)^+$; Calculated for $C_{31}H_{40}N_2O_3$: C, 76.19; H, 8.25; C, 5.73; found: C, 76.47; H, 8.33; N, 5.87.

Pharmacology

5-HT$_{1A}$ receptor binding

5-HT_{1A} receptor binding was determined on bovine frontal cortex and hippocampus membranes. Membranes were incubated at 23°C for 40 min with 0.5 nM [^{3}H]-8-OH-DPAT in 50 mM Tris-HCl buffer, pH 7.4, supplemented with 4 mM $CaCl_2$ and 10 μM pargyline. Non-specific binding was determined in the presence of 10 μM buspirone. Competition experiments were analysed using the iterative non-linear least-square fitting with GRAPHPAD software. Results were expressed as $K_i \pm$ s.e.m. and K_i were determined using the method of Cheng & Prusoff (1973).

5-HT$_7$ receptor binding

5-HT_7 receptor binding was determined on the human recombinant receptor. Membranes were incubated at 27°C for 90 min with 3.7 nM [^{3}H]-LSD (D-lysergic acid diethylamide) in 50 mM Tris-HCl buffer, pH 7.4, sup-

plemented with 4 mM $MgCl_2$. Non-specific binding was determined in the presence of 10 μM of methiothepin. Competition experiments were analysed using the iterative non-linear least-square fitting with GRAPHPAD software. Results were expressed as $K_i \pm$ s.e.m. and K_i were determined using the method of Cheng & Prusoff (1973).

Light–dark test

The anxiolytic-like activity of the compound was tested using an unconditioned conflict test, the light–dark test behaviorally validated for detecting anxiolytic agents in mice. In brief, the apparatus consisted of two poly(vinyl chloride) boxes covered by plexiglass. One of these boxes was darkened, and the other was lightened by a lamp. Swiss mice (n = 15) were placed in the lit box to start the test session. The amount of time spent by mice in the lit box (TLB) and the number of transitions through the tunnel were recorded over a 5-min period, after the first entry in the dark box. A mouse with all four paws in the new box was considered as having changed boxes. Compound **2b** was tested at 0.25, 0.5 and 1 mg kg^{-1} intraperitoneally. The lack of sedative or excitatory effects of the compounds at the tested doses was previously measured in a free exploratory test. The statistical significance of differences between control and treated groups was ascertained by a combined analysis of variance and a Bonferroni's posteriori *t*-test.

Results and Discussion

Chemistry

The 3,4-dihydro-3-amino-2*H*-l-benzopyran derivatives, **2**, were all prepared from the precursor triflate, **5**. This key intermediate in turn was elaborated in excellent yield from 5-methoxy-3,4-dihydro-3-amino-2*H*-1-benzopyran **3** (Figure 5). Demethylation of **3** followed by Boc-protection of the primary amine afforded **4** in 98 % overall yield. The triflate **5** was then obtained in 93 % yield by reaction of the hydroxy compound **4** with triflic anhydride in methylene chloride in the presence of pyridine.

Coupling of **5** with tributyl(vinyl)tin in the presence of bis(triphenylphosphine)palladium (II) chloride, followed by hydrogenation, afforded the ethyl compound **6a** in 65 % yield. The palladium coupling reaction of **5** with tributyl(1-ethoxyvinyl)tin afforded, after a subsequent hydrolysis, the desired acetyl product **6b** in 79 % yield. Finally, a Suzuki coupling reaction of **5** and phenylboronic acid in the presence of freshly prepared *tetrakis*(triphenylphosphine)palladium (0) in ethanol and saturated aqueous sodium bicarbonate solution gave the phenyl compound **6c** in 80 % yield (Figure 6).

Using the same methodologies, the compounds **1a–c** were prepared from 5-[(trifluoromethyl)sulfonyl]oxy-3,4-dihydro-3-di-*n*-propylamino-2*H*-1-benzopyran in good yields (Rezaie et al 1998b); the compound **1b** has already been described in the literature (Larsson et al 1991).

The products **6a–c** were deprotected in high yields (90–99 %, Figure 7) using trifluoroacetic acid in dry dichloromethane. *N*-Alkylation of **7** was then carried out using 8-(4-bromobutyl)-8-azaspiro[4.5]decane-7,9-dione in the presence of potassium carbonate and sodium iodide in acetonitrile to afford compounds **8a–c** in moderate-to-good yields. Final *N*-alkylation of **8** was performed with iodopropane to give **2** in 44–53 % yields.

Pharmacology

Six 5-substituted 3,4-dihydro-3-amino-2*H*-1-benzopyran derivatives (**1**, **2**) were designed, prepared and first evaluated for their affinity for both 5-HT_{1A} and 5-HT_7 receptors. Binding values of compounds **1a–c**, **2a–c**, 5-MeO-DPAC, S20244, 5-CT and buspirone are reported in Table 1.

Whatever the substituent at the 5-position of the

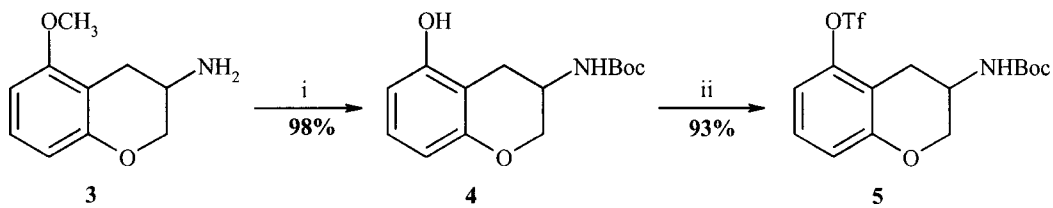


Figure 5 Synthesis of 5-(trifluoromethylsulfonyloxy)-3,4-dihydro-3-(*tert*-butoxycarbonylamino)-2*H*-1-benzopyran **5**. Reagents and conditions: i, HBr 48 % in CH_3COOH, then i M NaOH, Boc_2O; ii, Tf_2O, pyridine, CH_2Cl_2.

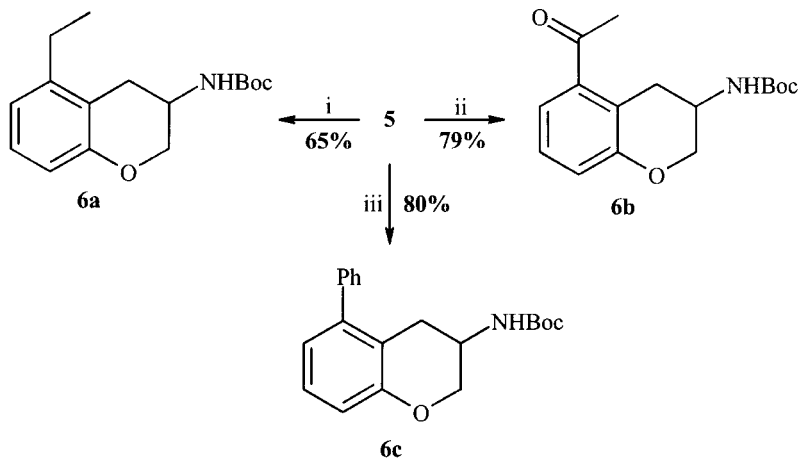


Figure 6 Synthesis of 5-substituted derivatives **6**. Reagents and conditions: i, $CH_2=CHSnBu_3$, $Pd(PPh_3)_2Cl_2$, DMF, 90°C, then H_2, Pd/C 10%, EtOH, rt, 50 psig; ii, $CH_2=C(OEt)SnBu_3$, $Pd(PPh_3)_2Cl_2$, DMF, 90°C, then 1M HCl, H_2O; iii, $PhB(OH)_2$, $Pd(PPh_3)_4$, $NaHCO_3$, toluene–EtOH reflux.

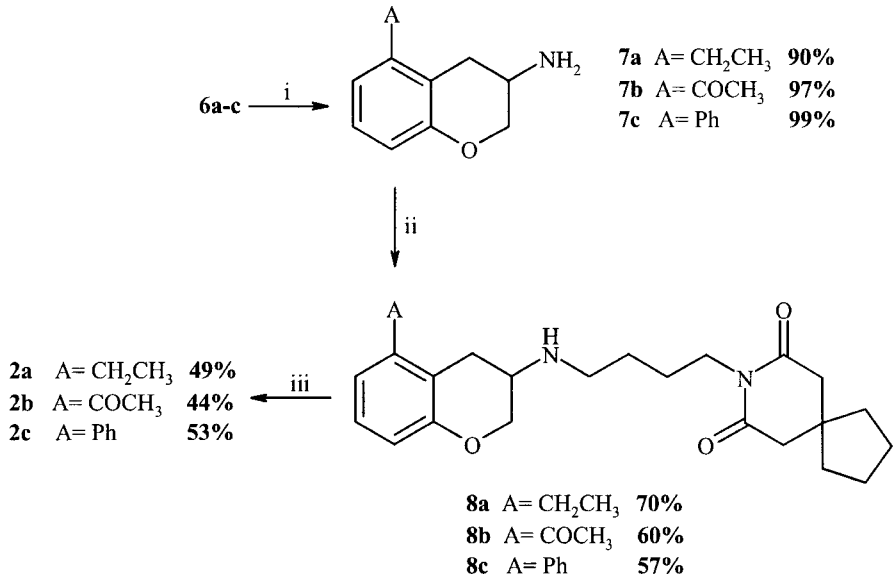


Figure 7 Synthesis of final compounds **2**. Reagents and conditions: I, TFA, CH_2Cl_2; ii, 8-(4-bromobutyl)-8-azaspiro[4.5]decane-7,9-dione, K_2CO_3, NaI, acetonitrile, 90°C; iii, $CH_3CH_2CH_2I$, K_2CO_3, DMF, 90°C.

benzopyranic system, all the compounds bearing an *N*-(4-(8-azaspiro[4.5]decane-7,9-dione)butyl moiety on the basic nitrogen (**2a–c** and S20244) had better affinities for both 5-HT_{1A} and 5-HT_7 receptors than their *N*-propyl substituted counterparts (**1a–c** and 5-MeO-DPAC).

Concerning the substitution of the benzopyran, the best affinities for 5-HT_{1A} and 5-HT_7 receptors in both series were obtained with a 5-acetyl substituent (**2b**: K_i = 0.3 nM for 5-HT_{1A} and 3.1 nM for 5-HT_7). Replacement of the acetyl with ethyl, phenyl or methoxy substituents resulted in a clear decrease in affinity for the 5-HT_{1A} receptor (by a factor of about 10) and the 5-HT_7 receptor (2- to 7-fold). These results suggest perhaps a hydrogen donor site at each receptor binding site interacts with the carbonyl group oxygen. Unfortunately, none of these evaluated compounds showed any selectivity for 5-HT_7 over 5-HT_{1A} receptors. Selectivity over other serotoninergic receptor subtypes or different receptor types has not been investigated as yet.

Table 1 In-vitro binding values of benzopyran derivatives **1** and **2**.

Compound	A[g]	K_i (nM)	
		5-HT$_{1A}$	5-HT$_7$
1a[a]	CH_2CH_3	31 ± 7	157 ± 27
1b[a]	$COCH_3$	0.50 ± 0.07	32 ± 6
1c[b]	Ph	62 ± 4	104 ± 12
2a[c]	CH_2CH_3	2.0 ± 0.4	12 ± 4
2b[c]	$COCH_3$	0.30 ± 0.03	3.1 ± 0.7
2c[c]	Ph	7.8 ± 0.5	13 ± 2
5-MeO-DPAC		6.9 ± 0.8	50 ± 7
S20244		1.3 ± 0.3	5.5 ± 0.7
5-CT		6.9 ± 1.0	0.93 ± 0.20[d]
Buspirone		7.0 ± 1.0[e]	398[f]

Values are means ± s.e.m. K_i was calculated from salt (**1a–b**, **2a–c**) and base (**1c**) forms. All compounds tested were racemic forms and used, respectively, as the hydrochloride[a], free base[b] or oxalate[c] forms. [d]Bard et al 1993. [e]El Ahmad et al 1991. [f]Ruat et al 1993. [g]See Figure 4.

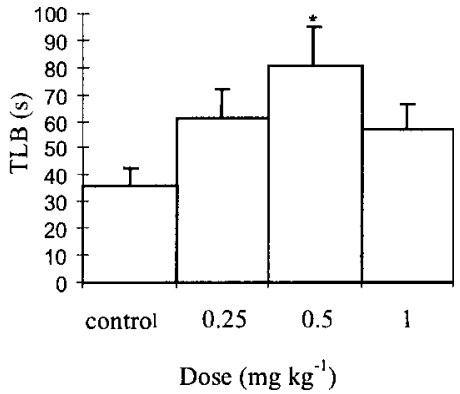


Figure 8 Time spent by mice in the lit box (TLB) after intraperitoneal administration of **2b** (oxalate form). Values are mean ± s.e.m., n = 15. *$P < 0.05$, treated group vs control group (combined analysis of variance and a Bonferroni's posteriori *t*-test).

Considering its very high affinity for both the 5-HT$_{1A}$ and 5-HT$_7$ receptors, the anxiolytic-like activity of compound **2b** was tested using an unconditioned conflict test, the light–dark test, behaviourally validated for detecting anxiolytic agents in mice (Costall et al 1989; Misslin et al 1989). The light–dark test results are shown in Figures 8 and 9. Compound **2b** administered in mice increased the time spent in the aversive compartment as well as the number of transitions between the two compartments. This effect was significant for the two parameters at a dose of 0.5 mg kg^{-1} intraperitoneally but not at 0.25 or 1 mg kg^{-1}. This compound was devoid of any sedative or excitatory effects at the active dose.

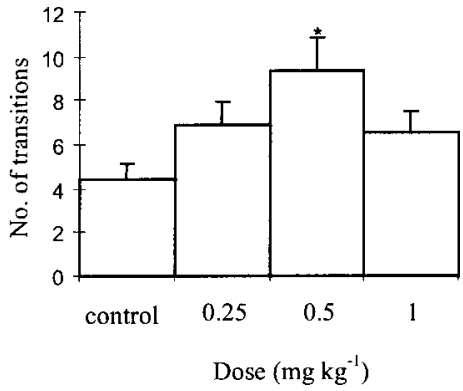


Figure 9 Number of transitions, made by mice, through the tunnel after intraperitoneal administration of **2b** (oxalate form). Values are mean ± s.e.m., n = 15. *$P < 0.05$, treated group vs control group (combined analysis of variance and a Bonferroni's posteriori *t*-test).

Conclusion

The chemical modulations performed within this series led to the generation of very potent ligands for both 5-HT$_{1A}$ and 5-HT$_7$ receptors. Compound **2b**, which exhibits nano and sub-nanomolar affinities (K_i = 0.3 nM for 5-HT$_{1A}$ and 3.1 nM for 5-HT$_7$), was also found to be anxiolytic in mice at a very low dose in the light–dark test. According to the preliminary results, compound **2b** could be a promising candidate for future development.

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