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Il Farmaco 57 (2002) 917–923

IL FARMACO

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Rigid analogues of buspirone and gepirone, 5-HT_{1A} receptors partial agonists

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Received 21 February 2002; accepted 11 May 2002

Abstract

Rigid analogues of buspirone and gepirone, 5-HT_{1A} receptors partial agonists, were obtained. The compounds exhibited very low affinity to the receptors. Their structural features resembled to a large extent the arrangement of the respective structural elements found in the solid state of buspirone and in the theoretical structure of NAN-190 (5-HT_{1A} postsynaptic antagonist) rigid analogue exhibiting high affinity to the receptor. The obtained results would thus suggest that the bioactive conformation of buspirone might not be the extended one. That would additionally suggest that either both groups of compounds could occupy different areas at the receptor binding sites (or bind to different receptor states) or the constrained structure of **2** does not represent well 5-HT_{1A} receptor binding site requirements.

Keywords: 5-HT_{1A} receptor ligands; Buspirone; Gepirone; Rigid analogues

1. Introduction

Arylpiperazines have been largely investigated as a group of compounds according to their ability to modify serotonergic neurotransmission via 5-HT_{1A} receptors interaction (for a review see Refs. [1–3]). And although it has been suggested that this pharmacological class failed to yield safer, non sedating and more effective anxiolytic and antidepressant agents [3], considerable efforts were exerted to investigate the receptors ligands.

The important problem of any receptor ligand is its bioactive conformation, that is the conformation it adopts at the molecular target site in a cell. It has been recently postulated that NAN-190 (**1**), 5-HT_{1A} receptor postsynaptic antagonist, should adopt *Z*-conformation of the *n*-butyl spacer (Fig. 1). The assumption was based on the fact that rigid NAN-190 analogues **2** (and some others) exhibited high affinity to the receptor ($K_i = 8$ nM, as compared to 0.55 nM of NAN-190) and antagonistic properties at the postsynaptic 5-HT_{1A} sites. It was thus concluded that the constrained structure of **2** represents very well the 5-HT_{1A} receptor binding [4].

In the present paper we report our results concerning rigid analogues **6** and **7** of other well known 5-HT_{1A} ligands—buspirone (**3**) and gepirone (**4**). Buspirone is as an approved anxiolytic drug and gepirone exhibited an interesting anxiolytic and antidepressive profile in preclinical studies. Both compounds are classified as 5-HT_{1A} partial agonists (for a review see Refs. [2,3]). It has been found on the basis of crystallographic data and quantum mechanical calculations that buspirone (and some other related compounds) should appear in the extended conformation [5]. On the other hand it was also observed that the preferred solid state conformation of buspirone analogue **5** ($K_i = 40$ nM) may depend on the counterion in its salts. It was thus concluded that the interaction with the 5-HT_{1A} receptor might force that class of ligands to adopt the bent conformation at the receptor-binding site [6]. Thus we decided to obtain buspirone and gepirone rigid analogues **6** and **7**, as well as some related compounds **8**–**11** which in principle could exhibit some affinity to 5-HT_{1A} receptors (Fig. 2).

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0014-827X/02/$ - see front matter
PII: S0014-827X(02)01279-X

2. Experimental

2.1. Chemistry

Melting points were determined on a Boetius apparatus (Carl Zeiss Jena) and are uncorrected. IR spectra were recorded on a Perkin–Elmer 1725X spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Brucker AMX 500 (500 MHZ) or Varian Gemini 2000 (200 MHz) with TMS as an internal standard. 2D NMR (H–H) COSY, (C–H) HETCOR technique was used to support interpretation of 1D spectra. Chemical shifts are given in δ (ppm) and the coupling constants J in hertz (Hz). GC/MS spectra were recorded on a Hewlett–Packard GC model 5890 with 5970 mass detector by the electron impact (EI) method. Elementary analyses were performed in the Institute of Organic Chemistry, Polish Academy of Sciences (Warsaw, Poland) and were within 0.4% of theoretical value. The radioligand binding studies were performed in the Institute of Pharmacology, Polish Academy of Sciences (Kraków, Poland). Column chromatography separations were carried out on Merck Kieselgel 100 or aluminium oxide 90, neutral (70–230 mesh). Reagents and solvents were purchased from common commercial suppliers. The following reagents were synthesized by published procedures: 4-hydroxycyclohexyl 4-methylbenzenesulfonate [7], *cis*- and *trans*-1,4-dibromo-cyclohexane [8], 1-(2-pyrimidyl)piperazine [9].

2.1.1. *cis*-2-[4-(4-Bromocyclohexyl)-piperazin-1-yl]pyrimidine (**16**)

A mixture of *trans*-1,4-dibromocyclohexane (**15**) (20 g, 0.08 mol), 1-(2-pyrimidyl)piperazine (5.4 g, 0.03 mol), anhydrous K_2CO_3 (6.8g, 0.05 mol), KOH (2.2 g, 0.04 mol) and 20 ml toluene was refluxed for 3 h, then cooled to room temperature, washed with water and extracted with aqueous 1 M HCl solution (3×10 ml). The aqueous solution was separated, alkalized to pH 9 by the addition of 5% aq. NaOH solution, extracted with $CHCl_3$ (3×20ml), dried over $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed ($CHCl_3$ as eluent, Kieselgel 100, 70–230 mesh) to give **16** (5 g, 47%). M.p. = 119.5–120.9 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.70–1.73 (m, 2H, H_a7, Ha7′), 1.80–1.94 (m, 4H, H_a8, H_a8', H_e7, He7′), 2.16–2.20 (m, 2H,

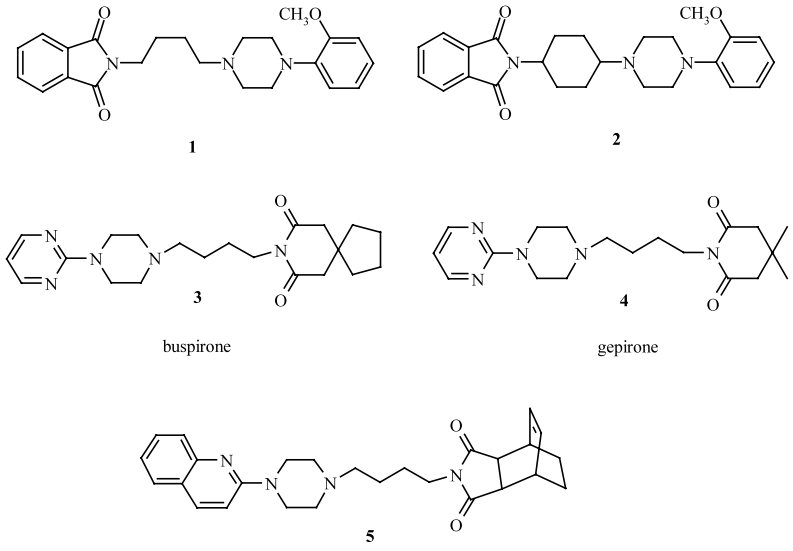


Fig. 1. Structures of NAN 190 (**1**), compound **2**, buspirone (**3**), gepirone (**4**) and compound **5**.

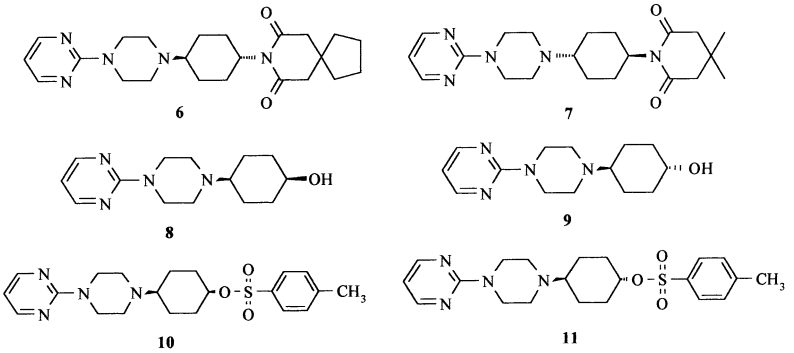


Fig. 2. Structures of compounds **6**–**11**.

H_e8, He8′), 2.37 (tt, $J_{aa} = 10.5$, $J_{ae} = 3.3$, 1H, H_a6), 2.65 (t, $J = 5.1$, 4H, H5, H5′), 3.83 (t, $J = 5.1$, 4H, H4, H4′), 4.57 (m(t), $J = 3.3$, 1H, H_e9), 6.47 (t, $J = 4.7$, 1H, H3), 8.30 (d, $J = 4.7$, 2H, H2); ^{13}C NMR ($CDCl_3$) δ (ppm): 23.82(C7), 34.13(C8), 44.08(C4), 48.92(C5), 53.58(C9), 62.35(C6), 109.73(C3), 157.67(C2), 161.66(C1).

IR ($CHCl_3$) ν (cm^{-1}): 2963, 2937, 2808, 2762, 1584, 1544, 1486, 1440, 1363, 1307, 1260, 1231, 1134, 1021, 982, 952, 800, 692, 637; MS (70 eV); *m/z* (%) 326 (50 M^+), 245(45), 218(60), 204(80), 163(17), 148(25), 121(73), 108(100), 80(48), 56(65); m.p. = 119.5–120.9 °C (hexane).

2.1.2. 2-(4-Cyclohex-3-enyl-piperazinyl)pyrimidine (*17*)

A mixture of *cis*-1,4-dibromocyclohexane (**14**) (20 g, 0.08 mol), 1-(2-pyrimidyl)piperazine (5.4 g, 0.03 mol), anhydrous K_2CO_3 (6.8 g, 0.05 mol), KOH (2.2 g, 0.04 mol) and 20 ml toluene was refluxed for 3 h, then cooled to room temperature, washed with water and extracted with aqueous 1 M HCl solution (3 × 10 ml). The aqueous solution was separated, alkalized to pH 9 by the addition of 5% aq. NaOH solution, extracted with $CHCl_3$ (3 × 20 ml), dried over $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed ($CHCl_3$ as eluent, Kieselgel 100, 70-230 mesh) to give **17**. m.p. = 93.8–94.8 °C (hexane); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.47 (m, 1H, H_a11), 1.95–2.24 (m, 5H, H7, H10, H_e11), 2.65 (m, 5H, H6, H5, H5′), 3.83 (t, $J = 5.1$, 4H, H4, H4′), 4.57 (m(t), 2H, H8, H9), 6.47 (t, $J = 4.7$, 1H, H3), 8.30 (d, $J = 4.7$, 2H, H2); ^{13}C NMR ($CDCl_3$) δ (ppm): 25.57(C10), 25.86(C11), 27.66(C7), 44.33(C4), 48.90(C5), 60.10(C6), 109.68(C3), 125.53(C8), 126.84(C9), 157.65(C2), 161.62(C1); IR ($CHCl_3$) ν (cm^{-1}): 3016, 2943, 2815, 1585, 1542, 1500, 1436, 1363, 1307, 1262, 1217, 1132, 1014, 981, 795, 778, 725, 666, 637; MS (70 eV); *m/z* (%) 244(79 M^+), 203(10), 190(100), 175(26),149(10), 121(66), 83(30), 56(16).

2.1.3. 4-(4-Pyrimidin-2-yl-piperazin-1-yl)cyclohexan-1-ol (*8*/*9*—mixture of cis and trans)

4-Hydroxycyclohexyl 4-methylbenzenesulfonate (**18**) (114 g, 0.42 mol) was added dropwise during 2 h to a warm (oil bath temperature ca. 120 °C) 1-(2-pyrimidyl)piperazine (140 g, 0.85 mol). After the addition was completed, the reaction mixture was cooled to 80 °C and toluene was added. The resulting mixture was cooled to 0 °C and precipitate was removed by filtration. The filtrate was extracted with aqueous 1 M HCl aq. (3 × 200 ml). The aqueous solution was alkalized to pH 9 with 25% ammonia, extracted with $CHCl_3$, dried over $MgSO_4$, and evaporated under reduced pressure. The resulting oil was dissolved in 500 ml of isopropanol, filtered, 20 ml 48% aq. HBr was added, filtered again and the filtrate evaporated to dryness. The solid material was dissolved in 200 ml water, neutralized with a 5% NaOH aq. solution and concentrated to about 1/3 of initial volume. The precipitate was collected by filtration and dried under reduced pressure (10 mmHg/50 °C) for 10 h to give 9.5 g 4-(4-pyrimidin-2-yl-piperazin-1-yl)cyclohexan-1-ol (*cis*/*trans* mixture). Yield 11.5%.

2.1.4. trans-4-(4-Pyrimidin-2-yl-piperazin-1-yl)-cyclohexan-1-ol (*9*)

The *trans* isomer was separated by crystallization. The mixture of *cis*/*trans* 4-(4-pyrimidin-2-yl-piperazin-1-yl)cyclohexan-1-ol (9 g) was dissolved in 20 ml hot acetone and the precipitate was washed with 5 ml acetone and dried to give 3.7 g (4.2%) *trans* isomer **9**. m.p. = 122.3–123.6 °C (acetone); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.18–1.45 (m, 4H, H7, H7′), 1.70 (s, 1H, –OH), 1.86–2.08 (m, 4H, H8, H8′), 2.24–2.40 (m, 1H, H_a6), 2.59 (t, $J = 5.1$, 4H, H5, H5′), 3.50–3.66 (m, 1H, H_a9), 3.79 (t, $J = 5.1$, 4H, H4, H4′), 6.45 (t, $J = 4.7$, 1H, H3), 8.29 (d, $J = 4.7$, 2H, H2); ^{13}C NMR ($CDCl_3$) δ (ppm): 161.78(C1), 157.87(C2), 109.91(C3), 70.53(C9), 62.75(C6), 49.21(C5, C5′), 44.06(C4, C4′), 34.58(C8, C8′), 26.46(C7, C7′); IR (KBr) ν (cm^{-1}): 3391, 3186, 3021, 2937, 2806, 1670, 1582, 1542, 1493, 1455, 1359, 1306, 1264, 1125, 1070, 982, 963, 811, 797; MS (70 eV); *m/z* (%) 262(41 M^+), 203(100), 154(57), 148(14), 142(63), 134(7), 122(21),108(21), 80(4), 56(9); *Anal*. ($C_{14}H_{22}N_4O \cdot H_2O$) C, H, N. **9·HCl:** white powder, m.p. = 228.5–230.5 °C (EtOH); *Anal*. ($C_{14}H_{22}N_4O \cdot 1.4HCl \cdot H_2O$) C, H, N, Cl.

2.1.5. cis-4-(4-Pyrimidin-2-ylpiperazin-1-yl)cyclohexan-1-ol (*8*)

The filtrate (after crystallization *trans*-isomer **9**) was concentrated under reduced pressure to give a crude residue, which was three times chromatographed (THF/hexane—1:1 as eluent, Kieselgel 100, 70–230 mesh). Yield 3.2 g of **8** (3.8%). m.p. = 138.5–140.4 °C (acetone).

^{1}H NMR ($CDCl_3$) δ (ppm): 1.48–1.89 (m, 8H, H7, H7′, H8, H8′), 2.24–2.42 (m(tt), $J_{aa} = 9.4$, $J_{ae} = 4.1$, 1H, H_a6), 2.63 (t, $J = 5.1$, 4H, H5, H5′), 3.81 (t, $J = 5.1$, 4H, H4, H4′), 3.94–4.03 (m(t), $J = 3.0$, 1H, H_e9), 6.47 (t, $J = 4.7$, 1H, H3), 8.30 (d, $J = 4.7$, 2H, H2); ^{13}C NMR ($CDCl_3$) δ (ppm): 160.10(C1), 157.87(C2), 109.85(C3), 66.24.7(C9), 62.49(C6), 49.01(C5, C5′), 44.07(C4, C4′), 31.97(C8, C8′), 22.95(C7, C7′); IR (KBr) ν (cm^{-1}): 3386, 3257, 3155, 3039, 2933, 2811, 1690, 1582, 1542, 1497, 1445, 1359, 1306, 1265, 1126, 980, 963, 804; MS (70 eV); *m/z* (%) 262(44 M^+), 203(100), 154(79), 148(19), 142(84), 13411), 122(34),108(41), 96(11), 80(13), 56(38); *Anal*. ($C_{14}H_{22}N_4O \cdot H_2O$) C, H, N. **8·HCl**; white powder, m.p. = 240.0–241.8 °C (EtOH); *Anal*. ($C_{14}H_{22}N_4O \cdot 1.3HCl \cdot 0.9H_2O$) C, H, N, Cl.

*2.1.6. cis-4-(4-pyrimidin-2-yl-piperazin-1-yl)-cyclohexyl 4-methylbenzenesulfonate (**10**)*

A solution of tosyl chloride (320 mg, 0.0016 mol) in 2 ml $CHCl_3$ was added dropwise for 1 h to a cold (0 °C) solution *cis*-4-(4-pyrimidin-2-yl-piperazin-1-yl)cyclohexan-1-ol (**8**) (400 mg, 0.0015 mol) in 2 ml pyridine. After the addition was completed, the reaction mixture was stirred at room temperature for 24 h, diluted with 10 ml $CHCl_3$ and washed with water (3 × 20 ml). The separated organic layer was dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The crude residue was chromatographed ($CHCl_3$ as eluent, Kieselgel 100, 70–230 mesh) and crystallized from cyclohexane to give compound **10** as white needles. Yield 370 mg (60%), m.p. = 137.7–138.3 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.42–1.72 (m, 6H, H7, H7′, H_a8, H_a8′), 1.94–2.00 (m, 2H, H_e8, H_e8′), 2.24–2.42 (m, 1H, H_a6), 2.44 (s, 3H, H14), 2.59 (t, J = 5.1, 4H, H5, H5′), 3.80 (t, J = 5.1, 4H, H4, H4′), 4.68–4.77 (m, 1H, H_e9), 6.47 (t, J = 4.7, 1H, H3), 7.32 (d, J = 8.1, 2H, H12, H12′), 7.79 (d, J = 8.5, 2H, H11, H11′), 8.30 (d, J = 4.7, 2H, H2); ^{13}C NMR ($CDCl_3$) δ (ppm): 161.80(C1), 157.86(C2, C2′), 144.59(C10), 134.69(C13), 129.91(C11, C11′), 127.73(C12, C12′), 109.89(C3), 78.72(C9), 61.81(C6, C6′), 48.95 (C5, C5′), 44.07(C4, C4′), 30.01(C8.C8′), 22.95(C7, C7′), 21.34(C14); IR (KBr) ν (cm^{-1}): 2957, 2835, 1592, 1543, 1510, 1353, 1167, 915, 872, 675, 555; MS (70 eV); *m/z* (%) 416(20 M^+), 308(28), 296(35), 244(74), 203(62), 190(100), 175(31), 149(30), 136(50), 121(85), 108(95), 96(12), 83(29), 56(30); *Anal*. ($C_{21}H_{28}N_4O_3S$) C, H, N, S. **10·HBr**, white powder, m.p. = 149.1–151.1 °C (THF); *Anal*. ($C_{21}H_{28}N_4O_3S·1.1HBr·H_2O$) C, H, N, S, Br.

*2.1.7. trans-4-(4-Pyrimidin-2-yl-piperazin-1-yl)-cyclohexyl 4-methylbenzenesulfonate (**11**)*

Compound **11** was prepared in the same manner as **10** from *trans*-4-(4-pyrimidin-2-yl-piperazin-1-yl)cyclohexan-1-ol (**9**). The crude product was chromatographed ($CHCl_3$ as eluent, Kieselgel 100, 70–230 mesh) to give compound **11** (170 mg, 30%). m.p. = 119.0–120.0 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.18–2.14 (m, 8H, H7, H7′, H8, H8′), 2.20–2.38 (m, 1H, H_a6), 2.44 (s, 3H, H14), 2.55 (t, J = 5.1, 4H, H5, H5′), 3.78 (t, J = 5.1, 4H, H4, H4′), 4.38 (tt, $J_{9a\text{-}8a,8'a}$ = 10.3, $J_{9a\text{-}8e,8'e}$ = 4.2, 1H, H_a9), 6.46 (t, J = 4.7, 1H, H3), 7.32 (d, J = 8.1, 2H, H12, H12′), 7.79 (d, J = 8.5, 2H, H11, H11′), 8.30 (d, J = 4.7, 2H, H2); ^{13}C NMR ($CDCl_3$) δ (ppm): 161.75(C1), 157.86(C2, C2′), 144.65(C10), 134.13(C13), 129.91(C11, C11′), 127.73(C12, C12′), 109.95(C3), 81.24(C9), 61.64(C6, C6′), 49.14(C5, C5′), 43.98(C4, C4′), 31.18(C8.C8′), 26.07(C7, C7′), 21.63(C14); IR (KBr) ν (cm^{-1}): 2974, 2822, 1583, 1545, 1487, 1461, 1358, 1335, 1306, 1264, 1190, 1168, 924, 891, 853, 803, 690, 560; MS (EI, HR) 416(M^+); *Anal*. ($C_{21}H_{28}N_4O_3S$) C, H, N, S. **11·HBr**, white powder, m.p. = 167.8–169.7 °C (THF); *Anal*. ($C_{21}H_{28}N_4O_3S·2HBr·H_2O$) C, H, N, S, Br.

*2.1.8. trans-8-Aza-8-[4-(4-pyrimidin-2-yl-piperazin-1-yl)cyclohexyl]spiro[4.5]decane-7,9-dione (**6**)*

*2.1.8.1. From cis-2-[4-(4-bromocyclohexyl)-piperazin-1-yl]pyrimidine (**16**).* A mixture of *cis*-2-[4-(4-bromocyclohexyl)-piperazin-1-yl]pyrimidine (**16**) (3.0 g, 0.009 mol), 8-azaspiro[4.5]decane-7,9-dione (4.6 g, 0.005 mol), anhydrous K_2CO_3 (0.8 g, 0.006 mol) and 7 ml xylene was refluxed for 3 h, then cooled to room temperature, washed with water and extracted with 2 M HCl aq. (3 × 10 ml). The aqueous solution was separated, basified to pH 9 by addition 5% NaOH aq. solution, extracted with $CHCl_3$ (3 × 20 ml), dried over $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed ($CHCl_3$ as eluent, Kieselgel 100, 70–230 mesh) and then crystallized from acetone/hexane to give compound **6** (60 mg, 8%). M.p. = 183.8–185.8 °C (acetone/hexane).

*2.1.8.2. From cis/trans mixture 4-(4-pyrimidin-2-yl-piperazin-1-yl)cyclohexan-1-ol (**8**/**9**).* A mixture of 4-(4-pyrimidin-2-yl-piperazin-1-yl)cyclohexan-1-ol (0.4 g, 0.0015 mol), 1,3-dicyclohexylcarbodiimide (DCC) (0.3 g, 0.0016 mol), catalytic amount CuCl (1.5 mg) and 5 ml CH_2Cl_2 was stirred for 72 h at room temperature, then washed with water (2 × 10ml), 25% ammonia aq. (3 × 10 ml), water (4 × 10 ml), dried over $MgSO_4$, and evaporated under reduced pressure. The residue was dissolved in DMF (4 ml), then was added 8-azaspiro[4.5]decane-7,9-dione (4.6 g, 0.0018 mol) and the mixture was heated for 10 h at 110 °C. After 10 h the precipitate was removed by filtration and the filtrate was diluted CH_2Cl_2 (10 ml), washed with water (2 × 10 ml) and extracted with 2 M HCl aq. (3 × 10 ml). The aqueous layer was separated, basified to pH 9 by addition 5% NaOH aq. solution, extracted with $CHCl_3$ (3 × 20 ml), dried over $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed ($CHCl_3$ as eluent, Kieselgel 100, 70–230 mesh) to give (isomer *trans* only) 30 mg of compound **6** (yield 4%). M.p. = 183.8–185.8 °C (acetone/hexane). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.25–1.51 (m, 6H, H13, H13′H_a7, H_a7′), 1.56–176 (m, 6H, H14, H14′, H_e8, H_e8′), 1.93–2.02 (m, 2H, H_e7, H $_e$7′), 2.25–2.52 (m, 3H, H6, H $_a$8, H_a8′), 2.56 (s, 4H, H11, H11′), 2.63 (t, J = 5.1, 4H, H5, H5′), 3.82 (m, J = 5.1, 4H, H4, H4′), 4.53 (tt, J_{aa} = 12.3, J_{ae} = 3.8, 1H, H_a9), 6.46 (t, J = 4.7, 1H, H1), 8.3 (d, J = 4.7, 2H, H2); ^{13}C NMR ($CDCl_3$) δ (ppm): 172.87(C10, C10′), 161.78(C1), 157.89(C2), 109.84(C3), 62.58(C6), 52.35(C9), 48.96(C5, C5′), 45.83(C4, C4′), 44.16(C11, C11′), 39.39(C12), 37.38(C13,C13′), 27.76(C7, C7′), 27.75(C8, C8′), 24.17(C14, C14′); IR (KBr) ν (cm^{-1}): 2941, 2860, 1722, 1662, 1583, 1545, 1480, 1446, 1357, 1257, 1226,

1147, 1135, 983, 808, 637; MS (70 eV); *m/z* (%) 411(20 M^+), 316(12), 303(67), 248(45), 244(25), 203(100), 168(41), 163(5), 148(14), 134(10), 122(23), 108(28), 96(8), 81(17), 67(6), 56(12). **6·HCl**, white powder, m.p. > 278 °C (dec.) (EtOH/THF); *Anal*. ($C_{23}H_{33}N_5O_2$·HCl) C, H, N, Cl.

2.1.9. *trans-4,4-Dimethyl-1-[4-(4-pyrimidin-2-yl-piperazin-1-yl)cyclohexyl]piperidine-2,6-dione (7)*

A mixture of *cis*-2-[4-(4-bromocyclohexyl)-piperazin-1-yl]pyrimidine (**16**) (4.0 g, 0.012 mol), 4,4-dimethylpiperidine-2,6-dione (5.2 g, 0.037 mol), anhydrous K_2CO_3 (1.7 g, 0.011 mol) and 8 ml xylene was refluxed for 3 h, then cooled to room temperature, washed with water and extracted with 2 M HCl aq. (3 × 10 ml). The aqueous solution was separated, basified to pH 9 by addition 5% NaOH aq. solution, extracted with $CHCl_3$ (3 × 20 ml), dried over $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed ($CHCl_3$ as eluent, Kieselgel 100, 70–230 mesh) and then crystallized from acetone/hexane to give compound **7** 100 mg (2.1%). M.p. = 192.9–194.3 °C (AcOEt/hexane); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.05 (s, 6H, H13, H13′), 1.39 (dq, $J_{a7\text{-}e8} = 3.6$, $J_{a7\text{-}a8} = 12.6$, $J_{a7\text{-}a6} = 12.4$, $J_{a7\text{-}e7} = 12.4$, 2H, H_a7, Ha7′), 1.60 (m, 2H, H_e8, H_e8′), 1.95 (m, 2H, H_e7, H $_e$7′), 2.36 (dq, $J_{a8\text{-}e7} = 3.5$, $J_{a8\text{-}a7} = 12.7$, $J_{a8\text{-}a9} = 12.7$, $J_{a8\text{-}e8} = 12.7$, 2H, H $_a$8, H_a8′), 2.43–2.49 (m, 5H, H6, H11, H11′), 2.62 (t, $J = 5.1$, 4H, H5, H5′), 3.81 (m, $J = 5.1$, 4H, H4, H4′), 4.54 (tt, $J_{aa} = 12.3$, $J_{ae} = 3.9$, 1H, H_a9), 6.45 (t, $J = 4.7$, 1H, H1), 8.3 (d, $J = 4.7$, 2H, H2); ^{13}C NMR ($CDCl_3$) δ (ppm): 172.28(C10, C10′), 161.62(C1), 157.64(C2), 109.63(C3), 62.47(C6), 52.22(C9), 48.88(C5, C5′), 47.30(C11, C11), 44.11(C4, C4′), 28.89(C12), 27.93(C7, C7′), 27.68(C8, C8′), 24.44(C13, C13′); IR (KBr) ν (cm^{-1}): 2983, 2871, 1722, 1673, 1581, 1547, 1479, 1451, 1360, 1328, 1260, 1230, 1147, 1131, 978, 805, 637, 601; MS (70 eV); *m/z* (%) 385(10 M^+), 277(66), 265(99), 244(22), 222(46), 203(100), 175(7), 149(12), 122(24), 108(31), 96(8), 83(18), 81(11), 67(6), 56(17); *Anal*. ($C_{21}H_{31}N_5O_2$) C, H, N. **7·HCl** white powder, m.p. > 270 °C (dec.) (MeOH); *Anal*. ($C_{21}H_{31}N_5O_2$·HBr) C, H, N, Br.

2.2. Molecular modelling

The calculations were carried out with the aid of molecular mechanics implemented in the Chem Plus 2.0 package (HyperChem 5.1., 1998). The internal MM+ force field was used molecular geometry optimization and for the conformational analysis the conjugate gradient (Polak–Ribier) method was used. The four isomeric structures were constructed, corresponding to different conformations of the piperazine ring, with substituents located at the axial or equatorial positions. Each of the structures was optimized in the course of the minimal internal energy search. In the next step the three bonds were rotated (scanned with the increment of 30° under the Conformational Search option). The rotamers were rejected if their relative energy exceeded by 4 kcal mol^{-1} the energy of the optimal structure.

2.3. Binding studies

The affinity of the compounds for central 5-HT_{1A} and 5-HT_{2A} receptors in vitro was assessed on the basis of their ability to displace [^{3}H]8-OH-DPAT and [^{3}H]ketanserin, respectively. Radioligand binding studies were performed in the rat brain using the following structures: hippocampus (5-HT_{1A}) and cortex (5-HT_{2A}) according to the published procedures [10,11]. Buspirone and ritanserin were employed as reference compounds. Radioligands used were [^{3}H]8-OH-DPAT (190 Ci/mmol, Amersham) and [^{3}H]ketanserin (60 Ci/mmol, NEN Chemicals) for 5-HT_{1A} and 5-HT_{2A}, respectively. K_i values were determined from three competition binding experiments in which several drug concentrations run in triplicates were used.

3. Results and discussion

Compounds **6** and **7** were obtained in a reaction sequence as shown in Fig. 3. In brief, 1,4-cyclohexanediol (**12**) was converted to 1,4-dibromocyclohexane (**13**) followed by the separation of *cis* and *trans* isomers **14** and **15**. *trans* derivative **15** was subjected to the reaction with 1-(2-pyrimidyl)piperazine to give compound **16** which upon the reaction with 8-azaspiro[4,5]decane-7,9-dione gave compound **6**. Similarly compound **7** was obtained by the reaction of derivative **16** with 4,4-dimethylpiperidine-2,6-dione. In another reaction sequence compound **6** was obtained from the mixture of *cis* and *trans* 4-(4-pyrimidin-2-yl-piperazin-1-yl)cyclohexan-1-ol (**8/9**, Fig. 4). *cis*- (**8**) and *trans*- (**9**) 4-(4-pyrimidin-2-yl-piperazin-1-yl)cyclohexan-1-ol were obtained from isomeric mixture and compounds **10** and **11** by tosylation of the respective isomers **8** and **9**.

The compounds' affinities to 5-HT_{1A} and 5-HT_{2A} receptors were measured in radioligand displacement experiments. Unexpectedly the affinities of rigid analogues **6** and **7** to 5-HT_{1A} (as well as to 5-HT_{2A}) receptors were very low. The affinities of the buspirone (compound **6**) and gepirone (compound **7**) analogues to 5-HT_{1A} receptors were 1607 and 472 nM, respectively. Their affinity to 5-HT_{2A} receptors was negligible. Compounds **8**–**11** did not exhibit an affinity (K_i > 10 000) to any of the receptors (Table 1) although *n*-hexyl and *n*-heptyl *N*-4-derivatives of 1-(2-pyrimidinyl)piperazine were shown to be relatively good 5-HT_{1A} receptor ligands (K_i = 84 and 60 nM, respectively) [12].

^{1}H NMR spectra showed that compounds **6** and **7**, similarly to compound **2**, existed in a diequatorial 1e,4e

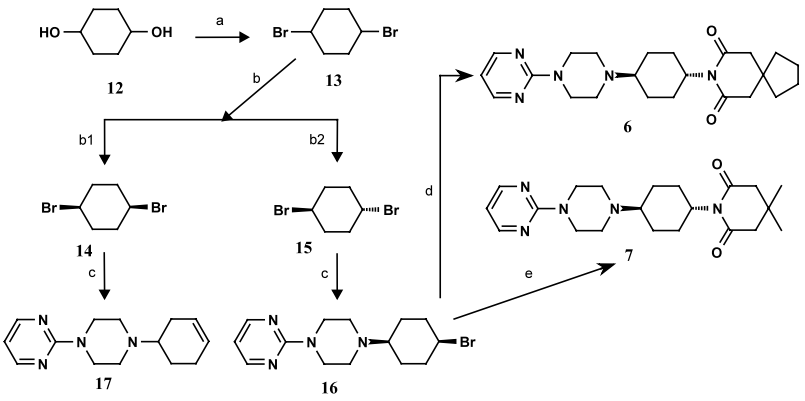


Fig. 3. Synthesis of compounds **6** and **7**. Reagents and conditions: (a) HBr, reflux; (b) separation; (b1) chromatography, (b2) crystallization; (c) 1-(2-pyrimidyl)piperazine, K_2CO_3, KOH, xylene, reflux; (d) 8-azaspiro[4,5]decane-7,9-dione, K_2CO_3, xylene, reflux; (e) 4,4-dimethylpiperazine-2,6-dione, K_2CO_3, xylene, reflux.

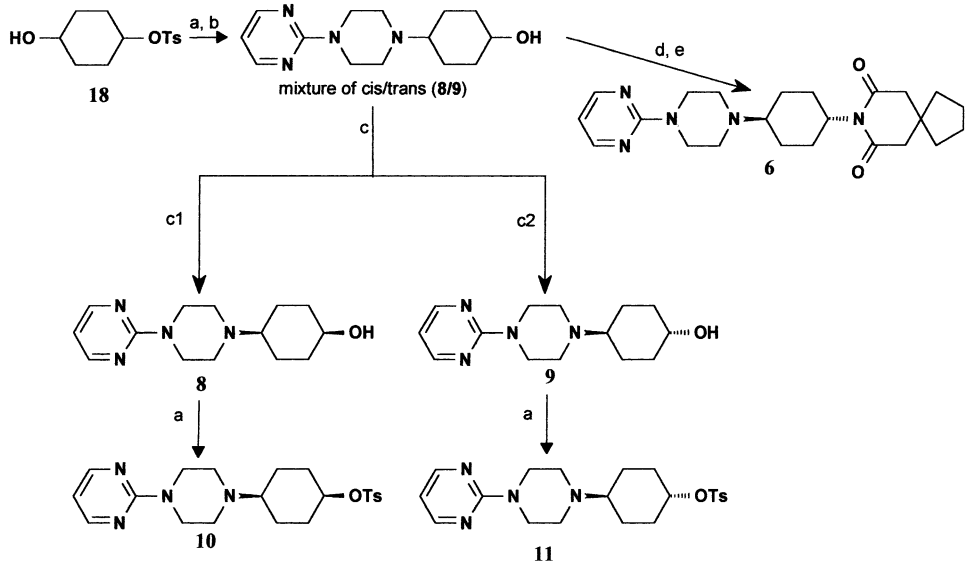


Fig. 4. Synthesis of compounds **8**–**11**. Reagents and conditions: (a) TsCl, pyridine, r.t., 24 h; (b) 1-(2-pyrimidyl)piperazine, 115 °C; (c) separation; (c1) chromatography, (c2) crystallization; (d) DCC, CuCl; (e) 8-zaspiro[4.5]decane-7,9-dione, DMF.

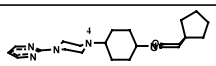
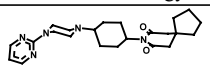
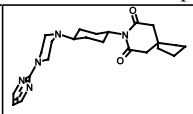
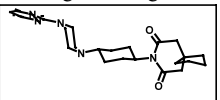
chair form with respect to the cyclohexane ring. In order to recognize the flexibility of the investigated structures conformational analysis with the aid of MM+ force field implemented in the Chem Plus 2.0 package (HyperChem 5.1., 1998) was performed. It was found that the energetically most stable conformations of compounds **6** and **7**, differing in the orientation of substituents at the piperazine ring, were very similar to those found in buspirone and compound **5** hydrochlorides solid states (Table 2). Those conformations of buspirone and gepirone analogues **6** and **7** also resembled the most stable theoretical conformations of NAN-190 rigid analogue **2**, the respective distances between the aromatic and imide ring centres being 12.52–12.67 Å for different conformers of **2** [4] and 11.08–12.73 Å for **6**. They, however, differed from the bent conformation found in the solid state of compound **5** perchlorate, where the heteroaromatic ring centre was much more near the imide carbonyl group (7.93 Å) than found in the extended structures (11.59–11.96 Å).

Table 1
Affinities of compounds **6**–**11** to 5-HT_{1A} and 5-HT_{2A} receptors

Comp.	K_i (nM)	
	5-HT_{1A}	5-HT_{2A}
Buspirone (**3**)	14 [a]	794 [b]
Gepirone (**4**)	32 [c]	3630 [d]
6	1607 ± 501	44 367 ± 3628
7	492 ± 9	117 000 ± 27 000
8	68 903 ± 12 056	74 186 ± 6075
9	75 827 ± 12 855	32 020 ± 5016
10	13 450 ± 3526	27 762 ± 8251
11	55 693 ± 13 293	196 566 ± 31 026

[a,b,c,d] Data taken from Refs. [6,13–15], respectively.

Table 2
Intramolecular distances (Å) between an aromatic ring centroid, N4-nitrogen atom and imide oxygen atom in buspirone hydrochloride (**3**), compound **5** hydrochloride and perchlorate solid state structures and theoretical low energy conformations of buspirone rigid analogue **6***

	3[#]	**5***HCl[#]	**5***$HCLO_4$[#]	 **6a**, ee[$]	 **6b**, ae[$]	 **6c**, ea[$]	 **6d**, aa[$]
Ar – O	11.79	11.96	7.93	11.87	11.17	11.59	11.89
N4 – O	7.16	7.10	6.91	6.75	6.74	6.64	6.68
Relative energy MM+	-	-	-	39.87	43.62	41.27	42.90

*For gepirone analogue **7** very similar results were obtained.
[#]Data taken from Ref. [3].
[$]Conformations of substituents at the piperazine ring.

4. Conclusions

The obtained results suggest that the bioactive conformation of buspirone might not be the extended one. NAN-190 (**1**) and its rigid analogue **2** differ from buspirone (**3**), gepirone (**4**) and their constrained analogues **6** and **7** both in the composition of the imide and aromatic parts of their molecules. Thus the results could suggest that either both groups of compounds occupy different areas at the receptor binding sites, bind to different receptor states [16] or the constrained structure of **2** does not represent well 5-HT_{1A} receptor binding site requirements. Although all three rigid analogues **2**, **6** and **7** exhibit smaller affinity to 5-HT_{1A} receptors than compounds **1**, **3**, and **4** (15, 94 and 15 times, respectively), none of the possibilities can be excluded on the basis of the available data.

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