8-PIPERAZINYL-2,3-DIHYDRO-1,4-DIOXINO[2,3-*b*] PYRIDINE DERIVATIVES: SYNTHESIS AND INTERACTION WITH 5-HT SEROTONIN BINDING SITES

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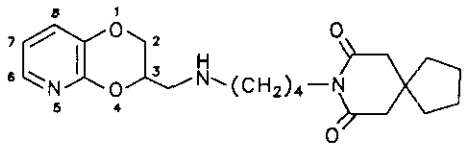
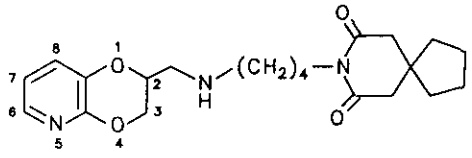
Abstract - The synthesis of 8-piperazinyl-2,3-dihydro-1,4-dioxino[2,3-*b*]pyridine derivatives (**6a-c**) are described. Their affinity and selectivity for 5-HT serotoninergic sites were evaluated.

Eltoprazine, 1-(2,3-dihydro-1,4-benzodioxin-5-yl)piperazine hydrochloride (DU-28853), is a part of a large class of *N*-bicyclic heteroarylpiperazines characterized by strong serenic activity.[1] The drug reduces offensive aggression without affecting defensive behaviour and without causing sedation or muscle relaxation. The mechanism responsible for the behavioral effects of serenics is currently unknown. Behavioural and neurochemical studies have suggested that the anti-aggressive actions may be related to the affinity of eltoprazine for 5-HT_1 receptors.[2] Autoradiographic data indicate that the drug interacts with 5-HT_{1A}, 5-HT_{1B} and, to a lesser extent, with 5-HT_{1C} receptor sites.[3]

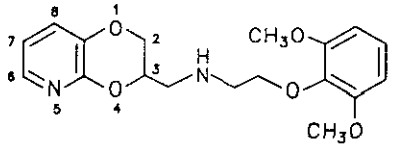
We published the synthesis and the serotoninergic activity of compounds(**A**, **B** and **C**),with a basic 2,3-dihydro-1,4-dioxino[2,3-*b*]pyridine structure.[4-7] The pharmacological study showed that incorporating a nitrogen atom at 5 position increases or decreases the affinity for 5-HT_1 receptors depending on the position of the side chain on the oxygenated ring.



A

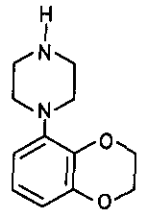


B

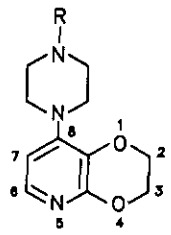


C

Following this work, this paper describes the synthesis and pharmacological characterization of eltoprazine analogues (**6**) having a 2,3-dihydro-1,4-dioxino[2,3-*b*]pyridine skeleton substituted at the 8-position with several piperazines.



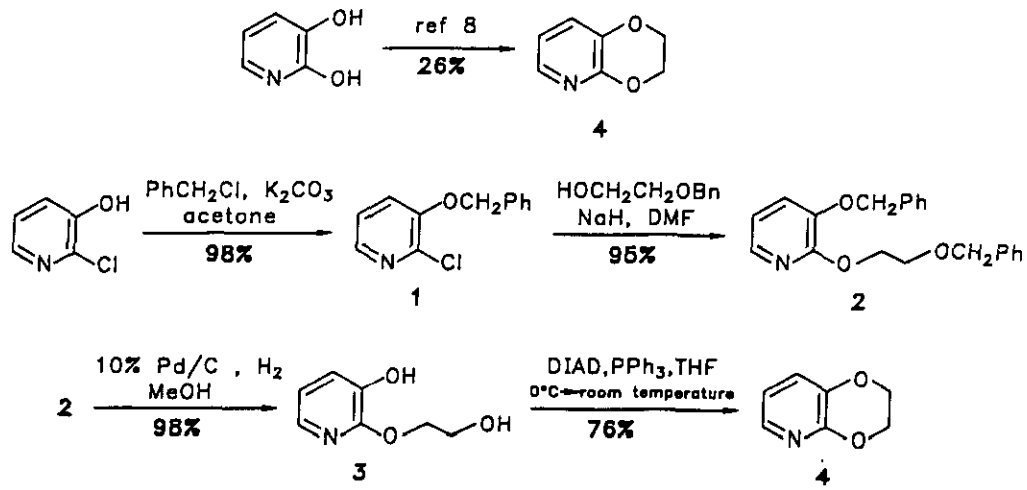
eltoprazine



6a R= H
6b R= $PhCH_2$
6c R= 2-$CH_3OC_6H_4$

The preparation of 2,3-dihydro-1,4-dioxino[2,3-b]pyridine (**4**) has been previously reported by Neunhoffer *et al.*[8] who used the reaction of 3-hydroxy-2-pyridone, 1,2-dibromoethane and sodium hydride in hexamethylphosphorous triamide. Unfortunately, the yield of the reaction is low (26%). To get higher yield of **4**, we developed a new procedure of synthesis as indicated in Scheme 1. We chosed 2-chloro-3-pyridinol as the starting material which was alkylated with benzyl chloride in the presence of potassium carbonate in acetone to give the benzyl ether (**1**) in 98% yield. Substitution of **1** with 2-benzyloxyethanol (95%) and *O*-debenzylation (98%) provided the diol (**3**). Cyclization by the Mitsunobu reaction (triphenylphosphine, diisopropyl azodicarboxylate, tetrahydrofuran) gave the final compound (**4**) in 76% yield. By this method, **4** was obtained in an overall yield of 69%.

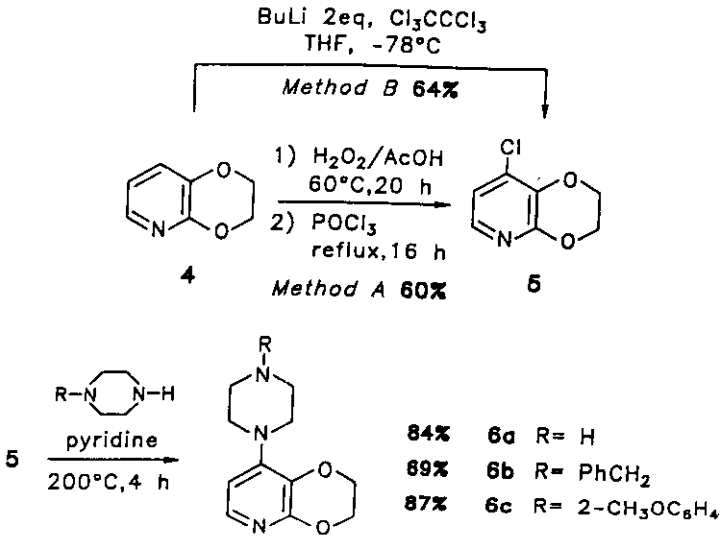
Scheme 1



Scheme 2 illustrates the sequence of reactions for the synthesis of compounds (**6a-c**). Oxidation of **4** with hydrogen peroxide gave the intermediate *N*-oxide which was treated with phosphorus oxychloride to lead to the

8-chloro derivative (**5**) in 60% overall yield. Regioselective halogenation at the 8-position was confirmed by nmr ^{15}N-^{1}H coupling constants study.[9] Compound (**5**)can be also prepared in 64% yield in one step by a lithiation of the 8-position of **4** with *n*-butyllithium (2 eq.) at -78 °C in tetrahydrofuran for 30 min followed by quenching of the carbanion obtained by the appropriate electrophile (Cl_3CCCl_3). Experimentations with 1 or 1.5 equivalents of *n*-butyllithium at -78 °C had furnished **5** in lower yields due to the presence of residual starting material (**4**). When the anionic reaction was performed at higher temperatures (> -78 °C), cleavage of the 2,3-dihydro-1,4-dioxino[2,3-*b*]pyridine ring was observed.[10] Displacement of a chlorine atom with the appropriate piperazines in a sealed tube at 200 °C in pyridine produced the piperazinyl derivatives (**6a-c**) in 69-87% yield.

Scheme 2



Receptor binding assays were conducted using methods reported in the literature.[11] The affinity of the ligands tested for these receptors was expressed as IC_{50} (concentration inhibiting 50 per cent of the specific binding) and calculated using LUNDON 2 software. Values of IC_{50} for 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} for compounds (**6a-c**) are summarized in Table.

Table. Binding values of compounds (**6a-c**).

		6a	**6b**[a]	**6c**
IC_{50}	5-HT_{1A}	$6.0\ 10^{-7}$	$6.0\ 10^{-7}$	$1.2\ 10^{-5}$
	5-HT_{1B}	$1.2\ 10^{-6}$	$9.3\ 10^{-6}$	$> 10^{-4}$
	5-HT_{1C}	$3.8\ 10^{-6}$	$7.7\ 10^{-6}$	$> 10^{-4}$

[a] Compound (**6b**) was used as oxalate salt.

The results of binding show a relatively low affinity of compounds(**6a-c**)for 5-HT receptors. Substituting of a nitrogen atom in the basic structure (comparing **6a** with eltoprazine) decreases the affinity for 5-HT_{1A} and 5-HT_{1B} receptors. The affinity of compound(**6c**)is still less significant in comparison to compounds(**6a** and **6b**) despite the presence of a *N*-2-methoxyphenylpiperazine moiety which usually confers a good affinity for 5-HT binding sites.[12]

This study represents a convenient synthetic route to obtain derivatives(**6a-c**),analogues of eltoprazine. The basic structure 2,3-dihydro-1,4-dioxino[2,3-*b*]pyridine (**4**) is prepared in high yield by a new procedure. Unfortunately, compounds(**6a-c**)bind with a little affinity at 5-HT serotoninergic sites.

EXPERIMENTAL

Melting points are uncorrected. ^{1}H Nmr (300 MHz) spectra was run on a Bruker AM 300 WB spectrometer. TMS served as an internal standard. Ir spectra of liquid films or KBr pellets were recorded on a Perkin-Elmer 297 instrument. Mass spectra were registered on a Nermag R-10-10-C apparatus. Analytical thin layer chromatography was performed on Merck $60F_{254}$ silica gel plate. Column chromatography was performed using silica gel 60 (0.063-0.200 mm, E. Merck) and flash chromatography was conducted with silica gel (0.040-0.063 mm, E. Merck). All air- and moisture-sensitive reactions were conducted under a prepurified argon atmosphere in flame-dried glassware. Anhydrous solvents or reactifs were transferred via syringe.

3-Benzyloxy 2-chloropyridine (1). To a solution of 2-chloro-3-pyridinol (6 g, 46.3 mmol) and K_2CO_3 (9 g, 65.1 mmol) in acetone (40 ml) was added dropwise benzyl chloride (7 g, 55.3 mmol). After stirring for 16 h, the mixture was filtered and the solvent was evaporated under reduced pressure. H_2O (30 ml) was added to the residue and the oil was extracted with CH_2Cl_2 (3 x 30 ml). The organic layer was dried ($MgSO_4$) and was concentrated, the crude product was purified by flash chromatography (1:1 petroleum ether/Et_2O) to give 9.76 g (98%) of **1** as a white solid: mp 49-51 °C; ^{1}H nmr (300 MHz, $CDCl_3$) δ: 5.18 (s, 2H), 7.11-7.48 (m, 7H), 8.00 (dd, *J* = 1.5 and 4.4 Hz, 1H); *Anal.* Calcd for $C_{12}H_{10}NOCl$: C, 65.61; H, 4.59; N, 6.38. Found: C, 65.50; H, 4.50; N, 6.35.

1-Benzyloxy-2-[3-benzyloxy-2-(pyridinyloxy)]ethane (2). To a suspension of NaH (1.38 g of a 50% oil dispersion, 28.9 mmol) in DMF (10 ml) was added dropwise a solution of 2-benzyloxyethanol (4 g, 28.9 mmol) in DMF (20 ml) . After stirring for 30 min, a solution of **1** (3.84 g, 17.5 mmol) in DMF (15 ml) was added. After 3 days at 100 °C, the mixture was filtered and the DMF was removed under pressure. H_2O (30 ml) was added to the residue and the oil was extracted with CH_2Cl_2 (3 x 30 ml). After drying ($MgSO_4$) and concentration, the residue was purified by flash chromatography (Et_2O) to give 5.57 g (95%) of **2** as an amorphous compound; ^{1}H nmr (300 MHz, $CDCl_3$) δ: 3.90 (t, *J* = 6.8 Hz, 2H), 4.61 (t, *J* = 6.8 Hz, 2H), 4.65 (s, 2H), 5.13 (s, 2H), 6.76 (dd, *J* = 5.1 and 8.0 Hz, 1H), 7.06 (d, *J* = 8.0 Hz, 1H), 7.22-7.46 (m, 10H), 7.72 (d, *J* = 5.1 Hz, 1H); *Anal.* Calcd for $C_{21}H_{21}NO_3$: C, 75.20; H, 6.31; N, 4.18. Found: C, 75.00; H, 6.15; N, 4.09.

2-[2-Hydroxyethoxy)-3-pyridinol (3). A mixture of **2** (4.2 g, 12.5 mmol) and 10% Pd/C (400 mg) in methanol (25 ml) was shaken in a Parr apparatus under 45 psi of hydrogen at room temperature for 16 h. The catalyst was filtered through Celite and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (Et_2O) to afford 1.91 g (98%) of **3** as a crystalline compound: mp 48-50 °C; ^{1}H nmr (300 MHz, $CDCl_3$) δ: 3.96-3.99 (m, 2H), 4.44-4.46 (m, 2H), 6.79 (dd, *J* = 5.3 and 7.8 Hz, 1H), 7.12 (d, *J* = 7.8 Hz,

1H), 7.60 (d, J = 5.3 Hz, 1H); *Anal.* Calcd for $C_7H_9NO_3$: C, 54.19; H, 5.85; N, 9.03. Found: C, 54.18; H, 5.83; N, 9.00.

2,3-Dihydro-1,4-dioxino[2,3-*b*]pyridine (4). To a stirred solution of diol **3** (548 mg, 3.66 mmol) and PPh_3 (1.37 g, 5.21 mmol) in anhydrous THF (20 ml) was added dropwise diisopropyl azodicarboxylate (1.13 g, 5.59 mmol) at 0 °C. The reactants were stirred at 0 °C for 15 min and at room temperature for 2 h. The reaction mixture was concentrated and the residue was partitioned between CH_2Cl_2 (30 ml) and aqueous 1N HCl (30 ml). The acidic aqueous extract was made alkaline with aqueous 5 N sodium hydroxyde (30 ml) and extracted with dichloromethane (3 x 30 ml). After drying over $MgSO_4$, the organic layer was evaporated and the residue was purified by column chromatography using petroleum ether/Et_2O (3:7) to give 368 mg (76%) of **4** as an oil; ir (film) 1285 and 1240 (C-O-C) cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ: 4.23-4.27 (m, 2H), 4.42-4.45 (m, 2H), 6.85 (dd, J = 5.1 and 7.5 Hz, 1H), 7.17 (d, J = 7.5 Hz, 1H), 7.81 (d, J = 5.1 Hz, 1H); *ms: m/z* 138 (M^++1); *Anal.* Calcd for $C_7H_7NO_2$. $C_2H_2O_4$: C, 47.58; H, 3.99; N, 6.17. Found: C, 47.35; H, 3.87; N, 5.98.

8-Chloro-2,3-dihydro-1,4-dioxino[2,3-*b*]pyridine (5). *Method A*: To a stirred solution of **4** (1 g, 7.30 mmol) in AcOH (25 ml) was added 30% hydrogen peroxide (10 ml). The mixture was heated to 60 °C for 20 h. After cooling to room temperature, the solvents were evaporated and the residue was filtrated on a silica gel column (1:9 then 2:8; methanol/dichloromethane) to afford 1.1 g of the *N*-oxide. Without another characterization, the *N*-oxide (1.1 g, 7.2 mmol) was added by small portions to a solution of $POCl_3$ (8 ml) at 0 °C. The reaction mixture was heated under reflux for 16 h. After cooling, the solvent was evaporated. The residue was partitioned between CH_2Cl_2 (30 ml) and saturated aqueous sodium hydrogen carbonate (30 ml). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (eluent 1:3, petroleum ether/Et_2O) to give 754 mg (60%) of **5** as a crystalline product.

Method B: To a stirred solution of 2,3-dihydro-1,4-dioxino[2,3-*b*]pyridine (**4**) (250 mg, 1.82 mmol) in THF (5 ml) was added slowly a solution of BuLi (1,6 M in hexane, 2.3 ml, 3.68 mmol) under argon atmosphere at -78 °C. The mixture was then kept at -78 °C for 30 min A solution of Cl_3CCCl_3 (646 mg, 2.72 mmol) in THF (5 ml) was added dropwise. The mixture was kept 10 min at -78 °C. Water (10 ml) was added to the mixture at -78 °C and the solution was brought to room temperature. THF was evaporated, CH_2Cl_2 (10 ml) was then added and the mixture was extracted. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (eluent 1:3; petroleum ether/Et_2O) to give 200 mg (64%) of **5** as a crystalline product; mp 82 °C; ir (KBr) 1260 and 1230 (C-O-C) cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ: 4.34-4.37 (m, 2H), 4.45-4.48 (m, 2H), 6.95 (d, J = 5.1 Hz, 1H), 7.22 (d, J = 5.1 Hz, 1H); ms: *m/z* 172 (M^++1); *Anal.* Calcd for $C_7H_6NO_2Cl$: C, 49.00; H, 3.52; N, 8.16. Found: C, 48.90; H, 3.46; N, 8.00.

8-Piperazinyl-2,3-dihydro-1,4-dioxino[2,3-*b*]pyridine (6a). A solution of **5** (300 mg, 1.75 mmol), piperazine (753 mg, 8.75 mmol) and pyridine (3 ml) was heated at 200 °C for 4 h in a sealed tube. The reaction mixture was concentrated and piperazine and pyridine were azeotropically evaporated with toluene several times. The residue was purified by flash chromatography (eluent 8:2 CH_2Cl_2/MeOH) to give 325 mg (84%) of **6a** as crystalline compound: mp 124-125 °C; ir (KBr) 3600-3200 (NH), 1265 (C-O-C) cm^{-1}; ^1H nmr ($CDCl_3$) δ 2.23 (bs, 1H), 3.07-3.11 (m, 4H), 3.21-3.25 (m, 4H), 4.26-4.29 (m, 2H), 4.39-4.42 (m, 2H), 6.45 (d, J = 5.9 Hz, 1H), 7.69 (d, J = 5.9 Hz, 1H); ms: *m/z* 222 (M^++1); *Anal.* Calcd for $C_{11}H_{15}N_3O_2$: C, 59.71; H, 6.83; N, 18.99.

Found: C, 59.47; H, 6.87; N, 18.52.

8-(1-Benzylpiperazinyl)-2,3-dihydro-1,4-dioxino[2,3-*b*]pyridine (6b). Following the procedure used for **6a** but substituting piperazine by 1-benzylpiperazine (1.52 ml, 8.75 mmol), purification of the residue by flash chromatography (eluent: 97:3 Et_2O/MeOH) yielded 375 mg (69%) of **6b** as an oil; ir (film) 1265 (C-O-C) cm^{-1}; ^1H nmr ($CDCl_3$): δ 2.59-2.63 (m, 4H), 3.21-3.25 (m, 4H), 3.57 (s, 2H), 4.24-4.27 (m, 2H), 4.37-4.40 (m, 2H), 6.43 (d, *J* = 5.6 Hz, 1H), 7.10-7.33 (m, 5H), 7.66 (d, *J* = 5.6 Hz, 1H); ms: *m/z* 312 (M^++1); *Anal.* Calcd for $C_{18}H_{21}N_3O_2$, $C_2H_2O_4$: C, 58.52; H, 5.89; N, 10.23. Found: C, 58.45; H, 5.88; N, 10.18.

8-[1-(2-Methoxyphenyl)piperazinyl]-2,3-dihydro-1,4-dioxino[2,3-*b*]pyridine (6c). Following the procedure used for **6a** but substituting piperazine by 1-(2-methoxyphenyl)piperazine (1.68 g, 8.75 mmol), purification of the residue by flash chromatography (eluent 95:5 Et_2O/MeOH) yielded 498 mg (87%) of **6c** as a crystalline compound: mp 145-146 °C; ir (KBr) 1265 and 1240 (C-O-C) cm^{-1}; ^1H nmr ($CDCl_3$): δ 3.21-3.25 (m, 4H), 3.39-3.43 (m, 4H), 3.89 (s, 3H), 4.28-4.32 (m, 2H), 4.40-4.44 (m, 2H), 6.52 (d, *J* = 5.1 Hz, 1H), 6.88-7.06 (m, 4H), 7.71 (d, *J* = 5.1 Hz, 1H); ms: *m/z* 328 (M^++1); *Anal.* Calcd for $C_{18}H_{21}N_3O_3$: C, 66.01; H, 6.46; N, 12.83. Found: C, 65.71; H, 6.40; N, 12.79.

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REFERENCES

1 Duphar International Research B.V., *Jpn. Kokai Tokkyo Koho JP 61,152,655*, July 1986 (*Chem. Abs.*, 1987, **106**, 5080t).

2 J. Hartog and B. Olivier, *Drugs of the Future*, **1988**, *13*, 222.

3 H. Sijbesma, J. Schipper, and E.R. De Kloet, *Eur. J. Pharmacol.*, **1990**, *177*, 55.

4 A. Benarab, P. Poirot, and G. Guillaumet, *Heterocycles*, **1993**, *36*, 1589.

5 A. Benarab and G. Guillaumet, *Heterocycles*, **1993**, *36*, 2327.

6 G. Guillaumet, A. Benarab, and P. Poirot, *Eur. Patent n°92 400 603.4*, March 1992.

7 A. Benarab, B. Joseph, and G. Guillaumet, *XXIXèmes Rencontres Internationales de Chimie Thérapeutique*, Dijon, 26-28 Septembre 1993.

8 H. Neunhoffer and O. Sponheimer, *Chem. Ber.*, **1990**, *123*, 2453.

9 H. Jacobsen, P.I. Yang, and W.S. Brey, *J. Org. Magn. Reson.*, **1981**, *17*, 290.

10 (a) G. Guillaumet, M. Hretani, and G. Coudert, *Tetrahedron Lett.*, **1988**, *29*, 475. (b) A.C. Ranade and S. Jayakshimi, *Chem. Ind.*, **1978**, 234.

11 E. Zifa and G. Fillion, *Pharmacol. Reviews*, **1992**, *44*, 401.

12 R.A. Glennon, N.A. Naiman, R.A. Lyon, and M. Titeler, *J. Med. Chem.*, **1988**, *31*, 1968.

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