Synthesis and Analgesic Activity of Some Side-Chain Modified Anpirtoline Derivatives

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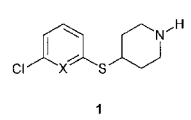
Key Words: *Anpirtoline analogs; [(hetero)arylsulfanyl]methylpiperidines; [(hetero)arylsulfanyl]methylpyrrolidines; [(hetero)arylmethyl]piperazines; (hetero)aroylpiperazines; analgesic activity; 5-HT$_{1A}$ receptor ligands; 5-HT$_{1B}$ receptor ligands*

Summary

New derivatives of anpirtoline and deazaanpirtoline modified in the side chain have been synthesized. The series includes compounds **3** with side-chains containing piperidine or pyrrolidine rings, compounds **4** containing 8-azabicyclo[3.2.1]octane moiety, and compounds **5** having piperazine ring in their side-chains. Their receptor binding profiles (5-HT$_{1A}$, 5-HT$_{1B}$) and analgesic activity (hot plate, acetic acid induced writhing) have been studied. Optimized structures (PM3-MOPAC, Alchemy 2000, Tripos Inc.) of the synthesized compounds **3–5** were compared with that of anpirtoline.



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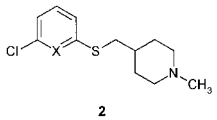
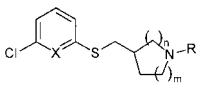
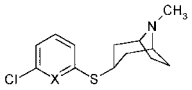


2

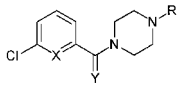
1, 2	X
a	N
b	CH



3	X	m	n	R
a	N	2	1	Me
b	N	2	1	H
c	CH	2	1	Me
d	CH	2	1	H
e	N	3	0	Me
f	N	3	0	H
g	N	1	1	Me
h	N	1	1	H
i	CH	1	1	Me
j	CH	1	1	H



4a, X = N
4b, X = CH



5	X	Y	R
a	CH	O	H
b	CH	H,H	Me
c	CH	H,H	CO_2Et
d	CH	H,H	H
e	N	O	Me
f	N	O	CO_2Et
g	N	H,H	Me

Introduction

In our search for centrally acting analgesics of non-opioid type we used anpirtoline (**1a**), a drug developed by ASTA Medica[1–5], as a lead. In spite of its high analgesic activity, the drug has not become commercially available, probably due to unspecified side effects. Structural modifications of anpirtoline would therefore appear desirable. We have modified the anpirtoline structure in several possible ways. The initial biological evaluation of deazaanpirtoline (**1b**) and several of its derivatives, including homologues **2**, showed these compounds to have weaker analgesic and binding properties than anpirtoline[6,7]. These findings inspired us to prepare a series of similar pyrrolidine and piperidine derivatives **3**, containing 6-chloropyridin-2-yl or 3-chlorophenyl group as an aromatic part and a pyrrolidine or piperidine ring as a cyclic amino group connected to the aromatic part by a methylsulfanyl bridge. We also decided to prepare compounds **4** containing a β-tropanyl (8-azabicyclo[3.2.1]octan-3-yl) moiety representing, in fact, a conformationally-restricted piperidine ring.

There are many piperazine derivatives with profound centrally mediated analgesic activity. Therefore, we also decided to prepare piperazine derivatives **5**.

This paper describes the synthesis of the above mentioned compounds, the binding properties of the prepared compounds to serotonin 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors, as well as their analgesic activity.

Chemistry

Pyridine derivatives **3a**, **3e**, and **3g** were usually prepared by alkylation of sodium 6-chloropyridin-2-ylthiolate with the appropriate *N*-methyl chloromethyl derivatives in boiling *n*-propanol. The corresponding phenyl derivatives **3c** and **3i** were prepared similarly from 3-chlorothiophenole and a suitable base. The thiophenolate anion was generated *in situ* by sodium hydride or potassium carbonate. These *N*-methyl derivatives **3a**, **3c**, **3e**, **3g**, and **3i** were treated with ethyl chloroformate and the intermediate *N*-ethoxycarbonyl derivatives were usually isolated by flash chromatography and then used without further purification to the following hydrolysis yielding the corresponding *N*-unsubstituted compounds **3b**, **3d**, **3f**, **3h**, and **3j**, respectively.

N-Methyl derivatives **4a** and **4b** were prepared by a modified literature procedure[8,9] used for stereospecific synthesis of tropan-3β-yl ethers and thioethers from tropine methanesulfonate[10]. In this reaction, the starting α-mesylate reacts with suitable (thio)phenolate to provide stereospecifically the corresponding β-(thio)phenoxide. In our case, we treated the starting tropine methanesulfonate with sodium 3-chlorothiophenolate and sodium 6-chloropyridin-2-thiolate to obtain compounds **4a** and **4b**, respectively.

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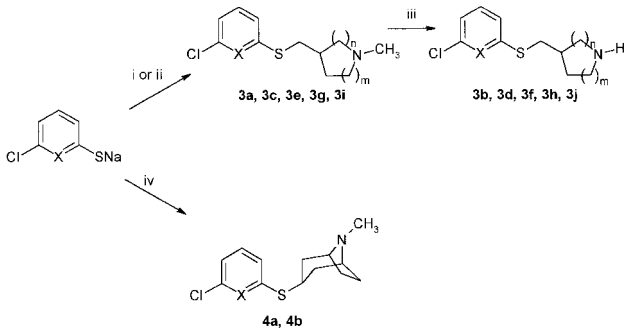
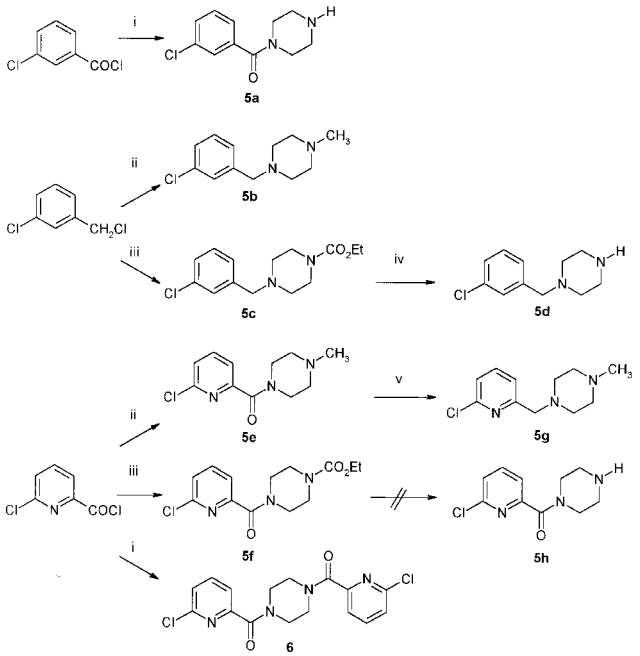


Scheme 1. i, chloro derivative, *n*-propanol, reflux; ii, chloro derivative, DMF-1 iii, 1. $ClCO_2Et$, toluene, 90 °C, 2. AcOH-HCl, reflux; iv, tropine mesylate.

Piperazine-containing derivative **5a** was easily obtained as its hydrochloride by acylation of piperazine with an equivalent of 3-chlorobenzoyl chloride in acetonitrile without using any base. Attempts to prepare similarly compound **5d** using 3-chlorobenzylchloride, provided a complex mixture. However, treatment of 3-chlorobenzylchloride with 1-methylpiperazine or 1-ethoxycarbonylpiperazine in the presence of triethylamine provided **5b** and **5c**, respectively**.** The hydrochloride of **5c** was then hydrolyzed to provide **5d.**

Preparation of the pyridine analogs **5e–5h** started from 2-chloro-6-bromopyridine, which was treated with butyllithium to form the corresponding lithio derivative that, upon treatment with solid carbon dioxide, afforded moderate yields of 6-chloropyridine-2-carboxylic acid. The acid was then converted with thionyl chloride to the corresponding acyl chloride. Pyridine derivative **5e** was prepared from the chloride and 1-methylpiperazine. When we tried to prepare the corresponding *N*-unsubstituted derivative using either equivalent amounts of the reagents or high excess of piperazine, corresponding bis-substituted piperazine **6** was the only isolated product. When 1-ethoxycarbonylpiperazine was used, the corresponding compound **5f** was obtained. All attempts to hydrolyze this compound to the required *N*-unsubstituted derivative **5h** failed and only complex mixtures were obtained. Compound **5g** was finally prepared by reduction of **5e** by borane formed *in situ* from sodium borohydride and boron trifluoride etherate.



Scheme 2. i, piperazine, CH3CN; ii, 1-methylpiperazine; iii, 1-ethoxycarbonylpiperazine; iv, HCl, reflux; v, NaBH4, $BF_3.Et_2O$.

Results and Discussion

All the prepared compounds were evaluated for their antinociceptive activity in two basic tests, the hot plate test and the intraperitoneal writhing test, after oral application of 30 mg/kg of the tested compound. The antinociceptive activity of anpirtoline is supposed to be mediated by 5-HT_{1B}

Table 1. 5-HT_{1A} and 5-HT_{1B} receptor binding and analgesic activity of the prepared compounds.

Compound	Binding studies		Analgesic activity (p.o.)	
	5-HT_{1A} (%)	5-HT_{1B} (%)	Hot plate (%)	Writhing (%)
anpirtoline · HCl	22[a]	40[b]	150	90
deazaanpirtoline · HCl	53[c]	51[d]	70	41
2a · maleate	82	61[e]	34	31
2b · HCl	79	85	47	48
3a · Tos	34[f]	66	19	18
3b · Tos	40	60	14	68
3c · HCl	54	n.d.	27	0
3d · HCl	72	n.d.	21	46
3e · HCl	14[g]	n.d.	29	6
3f · HCl	20[h]	n.d.	30	65
3g · HCl	21[i]	67[j]	63	n.d.
3h · HCl	37	n.d.	27	5
3i · HCl	82	n.d.	33	16
3j · HCl	76	n.d.	n.d.	n.d.
4a · HCl	102	100	25	74
4b · HCl	96	101	21	37
5a · HCl	103	99	n.d.[k]	n.d.[k]
5b · 2HCl	92	116	n.d.[k]	n.d.[k]
5c · HCl	76	89	n.d.[k]	n.d.[k]
5d · 2HCl	88	85	38	7
5e · HCl	89	94	12	n.d.
5g · HCl	93	96	22	n.d.

n.d. – not determined; [a] – IC_{50} = 0.57 μM; [b] – IC_{50} = 1.02μM; [c] – IC_{50} = 1.47 μM; [d] – IC_{50} = 2.45 μM; [e] – IC_{50} = 2.25 μM; [f] – IC_{50} = 0.64 μM; [g] – IC_{50} = 0.40 μM; [h] – IC_{50} = 0.42 μM; [i] – IC_{50} = 0.40 μM; [j] – IC_{50} = 1.34 μM; [k] – toxic.

receptors[4,5] and involvement of 5-HT_{1A} receptors in analgesic activity of some active compounds is also anticipated[11] and therefore receptor binding profiles towards these receptor subtypes were also evaluated (Table 1).

The main reason for this work was to check the influence of the structural changes of anpirtoline both on the analgesic activity and on the binding properties toward 5-HT_{1A} and 5-HT_{1B} serotonin receptor subtypes. For this purpose, we have analyzed the optimized structures (PM3-MOPAC, Alchemy 2000, Tripos Inc., 1998) of the synthesized compounds **3–5** and compared them with that of anpirtoline. The series contains mostly compounds derived from anpirtoline and deazaanpirtoline by changing the amino group-containing side chain. However, none of these changes led to compounds with better antinociceptive activity. Some compounds active as analgesics, e.g. **3f** and **3g** also have some levels of binding to the tested serotonin receptors; however, some of them, e.g. **4a**, apparently do not bind to the receptors. On the other hand, compounds **3a** and **3e** with strong binding to the 5-HT_{1A} receptor subtype do not show any analgesic activity at all. This fact can suggest that the analgesic activity of these analogs could be mediated by a quite different mode of action. However, some of the compounds have strong binding to the tested serotonin receptors, especially to the 5-HT_{1A} subtype. It has been shown[13], that both serotonin and all non-indolic serotonergic agents contain both (het)aromatic part and a side chain containing ionisable nitrogen atom. The optimized structures of some of these compounds exhibit an interesting structural similarity. Superimposition of the PM3 optimized conformers of the individual compounds **3–5** and anpirtoline was performed with the RMS fit procedure within Alchemy 2000. All six corresponding ring atoms of the (het)aromatic part and the side-chain nitrogen atom were selected as atom pairs for the fitting procedure. The best fit was found for compounds **3a–3h**; the RMS deviation values of these compounds are in the range of 0.178–0.211 L, with the exception of **3c** with RMS value of 0.305 L. The superimpositions of compounds **3f** (RMS = 0.178 L) and **3g** (RMS = 0.186 L) with anpirtoline are shown in **Figure 1.** Sterically fixed anpirtoline and deazaanpirtoline analogs **4a** and **4b** displayed neither useful levels of analgesic activity nor binding to the tested serotonin receptors. It could be rationalized by the fact that the fit of the optimized structures of anpirtoline with these compounds is not so good as that of most compounds **3** having the RMS deviation values of 0.390 L for **4a** and 0.680 L for **4b**.

1-Aryl- and hetaryl-piperazines are well known serotonin ligands; 1-(3-chlorophenyl)piperazine (mCPP) being one of the best known 5-HT_1 agonists[12–14]. On the other hand, the corresponding 3-chlorobenzoyl (**5a**), 3-chlorobenzyl (**5d**), as well as its *N*-methyl (**5b**) and *N*-ethoxycarbonyl (**5c**) derivatives do not seem to possess a significant affinity for either 5-HT_{1A} or 5-HT_{1B} sites. A similar situation is also observed with their pyridine analogs, namely compounds **5e** and **5g**. This situation is a little difficult to interpret based on the simple molecular energy optimization. It is true that the optimized structures of these compounds cannot fit with the corresponding mCPP model. However, the fit of the pyridine derivatives **5e–5g** with optimized anpirtoline is relatively good, having the RMS values of 0.103, 0.184, and 0.272 L. The fit of compound **5e** with anpirtoline and mCPP is shown in **Figure 2a** and **Figure 2b**, respectively. Evidently, further refinement of the model is required to explain the poor affinities of these compounds. None of the compounds **5** displayed a useful level of analgesic activity; some of them were toxic at the tested doses.

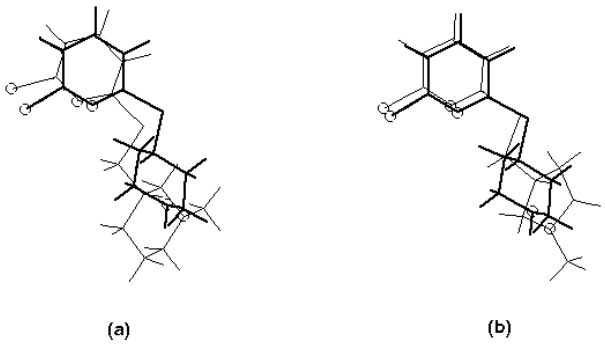


Figure 1. Superimposition of anpirtoline (bold) with compound **3f** (a) and compound **3g** (b).

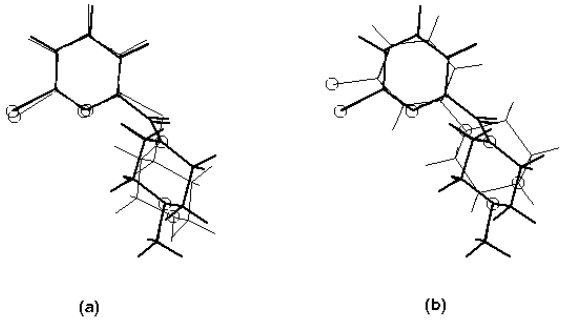


Figure 2. Superimposition of compound **5e** (bold) with anpirtoline (a) and mCPP (b).

Acknowledgment

This study was supported by the Grant Agency of the Ministry of Industry and Trade of the Czech Republic (grant No. PP-Z1/08), and by Léciva Praha.

Experimental Part

General Methods

The melting points were determined on a Kofler block and are not corrected. The ^1H and ^{13}C-NMR spectra were measured on a Bruker DPX 250 spectrometer with tetramethylsilane as an internal standard. The purity of the substances prepared was evaluated by TLC on pre-coated silica gel 60 TLC plates F 254 (Merck). Elemental analyses indicated by the symbols of the elements were within 0.4% of the theoretical values.

The following starting compounds were prepared according to the previously described methods: sodium 6-chloropyridin-2-ylthiolate[1], 2-chloromethyl-1-methylpiperidine[15], 3-chloromethyl-1-methylpiperidine[16], 3-chloromethyl-1-methylpyrrolidine[17], tosylate of 3α-mesyloxy-8-methyl-8-azabicyclo[3.2.1]octane (tosylate of 3α-tropinemethanesulfonate)[18], 2-bromo-6-chloropyridine[19].

Syntheses

*General Procedure for the Preparation of Pyridine Derivatives **3a**, **3e**, and **3g***

A mixture of sodium 6-chloropyridine-2-thiolate (3.35 g, 20 mmol), the appropriate chloro derivative (22 mmol), and *n*-propanol (25 mL) was refluxed for 16 h. The mixture was evaporated under reduced pressure, the residue was treated with water (20 mL) and extracted with chloroform (5 × 20 mL). The extract was dried with magnesium sulfate, evaporated, and the residue was purified by flash chromatography. The pure yellowish oil was converted to its suitable salt and crystallized. According to this method, the following compounds were prepared:

*Tosylate of 2-Chloro-6-(1-methylpiperidin-3-ylmethylsulfanyl)pyridine (**3a**)*

Yield 67%, mp 124–126 °C (ethyl acetate). ^{1}H-NMR of the corresponding base ($CDCl_3$): δ= 1.15–1.50 (m, 8H, H-2′, H-4′, H-5′, H-6′), 2.86 (s, 3H, CH_3), 3.11 (Abq system, 2H, SCH_2), 3.47 (m, 1H, H-3′), 6.98 (dd, *J* = 7.1 Hz, *J* = 1.1 Hz, 1H, H-3), 7.08 (dd, *J* = 7.7 Hz, *J* = 1.0 Hz, 1H, H-5), 7.42 (t, *J* = 7.7 Hz, 1H, H-4). Anal. ($C_{19}H_{25}ClN_2O_3S_2$) C, H, Cl, N, S.

*Hydrochloride of 2-Chloro-6-(1-methylpiperidin-2-ylmethylsulfanyl)-pyridine (**3e**)*

Yield 26%, mp 139–143 °C (ethyl acetate). ^{1}H-NMR ($CDCl_3$): δ = 1.15–1.50 (m, 2H, H-5′), 1.75-2.05 (m, 2H, H-4′), 2.05-2.45 (m, 2H, H-3′), 2.80–3.05 (m, 2H, H-6′), 3.04, 3.06 (ds, 3H, CH_3), 3.24 (dd, *J* = 13.5 Hz, *J* = 9.4 Hz, 1H, SCH_2), 3.52 (m, 1H, CH), 4.09 (dd, *J* = 13.5 Hz, *J* = 3.1Hz, 1H, SCH_2), 7.07 (dd, *J* = 7.5 Hz, *J* = 0.7 Hz, 1H, H-3), 7.13 (dd, *J* = 7.5 Hz, *J* = 0.7 Hz, 1H, H-5), 7.51 (t, *J* = 7.5 Hz, 1H, H-4). Anal. ($C_{12}H_{18}Cl_2N_2S$) C, H, Cl, N, S.

*Tosylate of 2-Chloro-6-(1-methylpyrrolidin-3-ylmethylsulfanyl)-pyridine (**3g**)*

Yield 47%, mp 113–115 °C (ethyl acetate). ^{1}H-NMR of the corresponding base ($CDCl_3$): δ = 1.99–2.79 (bm, 7H, H-2′, H-3′, H-4′, H-5′), 2.36 (s, 3H, CH_3), 3.24 (d, *J* = 6.6 Hz, 2H, SCH_2), 6.96 (dd, *J* = 7.8 Hz, *J* = 1.0 Hz, 1H, H-3), 7.05 (dd, *J* = 7.8 Hz, *J* = 1.0 Hz, 1H, H-5), 7.38 (t, *J* = 7.8 Hz, 1H, H-4). Anal. ($C_{18}H_{23}ClN_2O_3S_2$) C, H, Cl, N, S.

*General Procedure for the Preparation of 3-Chlorophenyl Derivatives **3c** and **3i***

Sodium hydride (0.53 g, 50% dispersion in mineral oil, 11 mmol) was added to a solution of 3-chlorothiophenol (1.44 g, 10 mmol) in DMF (15 mL) and the mixture was stirred at room temperature under nitrogen atmosphere for 30 min. Then a solution of the appropriate chloro derivative (10 mmol) in DMF (5 mL) was added and the mixture was stirred for 24 h at room temperature. The insoluble portion was filtered off and washed with DMF (2 mL) and the filtrate was evaporated under reduced pressure. The residue was treated with water (20 mL) and extracted with dichloromethane (5 × 10 mL). The extract was dried with magnesium sulfate, evaporated and the residue was purified by flash chromatography. The pure yellowish oil was converted to its hydrochloride and crystallized. According to this method, the following compounds were prepared:

*Hydrochloride of 3-(3-Chlorophenylsulfanylmethyl)-1-methylpiperidine (**3c**)*

Yield 56%, mp 107–108 °C (ethyl acetate). Anal. ($C_{13}H_{19}Cl_2NS$) C, H, Cl, N, S.

*Hydrochloride of 3-(3-Chlorophenylsulfanylmethyl)-1-methylpyrrolidine (**3i**)*

Yield 68%, mp 98–101 °C (ethyl acetate). Anal. ($C_{12}H_{17}Cl_2NS$) C, H, Cl, N, S.

*General Procedure for Preparation of N-Unsubstituted Derivatives **3b**, **3d**, **3f**, **3h**, and **3j** by N-Demethylation*

Ethyl chloroformate (2 mL, 20 mmol) was added to a solution of the corresponding *N*-methyl derivative **3** (10 mmol) in dry toluene (50 mL) at 90 °C and the mixture was stirred at this temperature for 4 h. The insoluble portion was removed by filtration through Celite and the filtrate was evaporated under reduced pressure. The residue was dissolved in a mixture of concentrated hydrochloric acid (20 mL) and acetic acid (20 mL) and the mixture was refluxed for 24–48 h (TLC). Then the mixture was evaporated, the residue dissolved in water and basified with a 20% solution of sodium hydroxide and extracted with dichloromethane (5 × 20 mL). The extract was dried with magnesium sulfate and the residue after evaporation was either purified by flash chromatography and then converted into a suitable salt, or directly converted into a salt and purified by crystallization.

*Hydrochloride of 2-Chloro-6-(piperidin-3-ylmethylsulfanyl)pyridine (**3b**)*

Yield 61%, mp 123–126 °C. ^{1}H-NMR (*d6*-DMSO): δ = 1.02–2.79 (bm, 8H, H-2′, H-4′, H-5′, H-6′), 3.10–3.75 (bm, 4H, H-2′, H-5′), 2.85–3.39 ?(bm, 1H, H-3′), 3.12 (d, *J* = 6.3 Hz, 2H, SCH_2), 6.97 (d, *J* = 7.7 Hz, 1H, H-3), 7.06 (d, *J* = 7.7 Hz, 1H, H-5), 7.39 (t, *J* = 7.7 Hz, 1H, H-4). Anal. ($C_{11}H_{16}Cl_2N_2S$) C, H, Cl, N, S.

*Hydrochloride of 3-(3-Chlorophenylsulfanylmethyl)piperidine (**3d**)*

Yield 43%, mp 115–118 °C (ethanol – ethyl acetate, 1:1). Anal. ($C_{12}H_{17}Cl_2NS$) C, H, Cl, N, S.

*Hydrochloride of 2-Chloro-6-(piperidin-2-ylmethylsulfanyl)pyridine (**3f**)*

In this case, purification of the base by flash chromatography (dichloromethane – methanol, 9:1) was necessary. Yield 51%, mp 141–143 °C (ethyl acetate). Anal. ($C_{11}H_{16}Cl_2N_2S$) C, H, Cl, N, S.

*Hydrochloride of 2-Chloro-6-(pyrrolidin-3-ylmethylsulfanyl)pyridine (**3h**)*

Yield 57%, mp 202–207 °C. ^{1}H-NMR (*d6*-DMSO): δ = 1.80–1.95 (m, 2H, H-4′), 2.30 (m, 1H, H-3′), 3.10–3.75 (bm, 4H, H-2′, H-5′), 3.78 (dd, *J* = 13.8 Hz, *J* = 9.2 Hz, 1H, SCH_2), 4.09 (dd, *J* = 13.8 Hz, *J* = 4.2 Hz, 1H, SCH_2), 6.95 (dd, *J* = 7.9 Hz, *J* = 0.8 Hz, 1H, H-3), 7.10 (dd, *J* = 7.9 Hz, *J* = 0.8 Hz, 1H, H-5), 7.45 (t, *J* = 7.9 Hz, 1H, H-4). Anal. ($C_{10}H_{14}Cl_2N_2S$) C, H, Cl, N, S.

*Hydrochloride of 3-(3-Chlorophenylsulfanylmethyl)pyrrolidine (**3j**)*

Yield 55%, mp 185–187 °C. ^{1}H-NMR (*d6*-DMSO): δ = 1.55 = 1.72 (m, 2H, H-4), 2.25 (m, 1H, H-3), 2.65–3.10 (bm, 6H, H-2, H-5, SCH_2), 7.16–7.30 (m, 4H, H-2′, H-4′, H-5′, H-6′). Anal. ($C_{11}H_{15}Cl_2NS$) C, H, Cl, N, S.

*Hydrochloride of 3-(6-Chloropyridin-2-ylsulfanyl)-8-methyl-8-aza-bicyclo[3.2.1]octane (**4a**)*

A mixture of sodium 6-chloropyridine-2-thiolate (2 g, 12 mmol), tosylate of 3α-tropinemethanesulfonate (2 g, 5.1 mmol), and ethanol (100 mL) was refluxed under argon atmosphere for 6 h. The mixture was evaporated to dryness, the residue was mixed with water (25 mL), extracted with dichloromethane (5 × 20 mL) and the extract was dried with magnesium sulfate. The residue after evaporation was purified by flash chromatography (dichloromethane, dichloromethane – methanol, 20:1). The combined fractions were evaporated, dissolved in methanol and acidified with an ethereal solution of hydrogen chloride. The formed solid was filtered off and crystallized from ethanol to give **4a** as white crystals (0.65 g, 42%), mp 256–258 °C. Mass spectrum, *m/z* (%): 268 (0.5), 124 (100), 94 (9), 82 (9), 67 (17). ^{1}H-NMR (DMSO-d6): δ = 1.92–2.52 (m, 8H, CH_2), 2.68 (s, 3H, CH_3), 3.86 (??bs, 2H, NCH), 4.09 (m, 1H, SCH), 7.20 (d, *J* = 7.8 Hz, 1H, H-3′), 7.31 (d, *J* = 7.88 Hz, 1H, H-5′), 7. (t, *J* = 7.8 Hz, 1H, H-4′). ^{13}C-NMR (*d6*-DMSO) δ = 24.80 (C-6, C-7), 33.38 (CH_3), 35.90 (C-2, C-4), 39.10 (C-3), 63.87 (C-1, C-5), 120.95 (C-5′), 122.18 (C-3′), 140.89 (C-4′), 150.74 (C-6′), 159.13 (C-2′). Anal. ($C_{13}H_{18}Cl_2N_2S$) C, H, Cl, N, S.

*Hydrochloride of 3-(3-Chlorophenylsulfanyl)-8-methyl-8-azabicyclo-[3.2.1]octane (**4b**)*

Sodium hydride (1 g, 55% dispersion in mineral oil, 23 mmol) was added to a stirred solution of 3-chlorothiophenole (3.3 g, 22.8 mmol) in DMF (40 mL) and the mixture was stirred under nitrogen atmosphere for 1 h. Then a solution of 3α-tropinemethanesulfonate (2.5 g, 5.1 mmol), freshly prepared from its tosylate, in DMF (15 mL) was added and the mixture was stirred at 100 °C under nitrogen for 5 h and then left to stand overnight. The insoluble sodium methanesulfonate was filtered off, washed with DMF (5 mL), the filtrate was acidified with concentrated hydrochloric acid to pH 2–3 and evaporated under reduced pressure. The residue was mixed with water (40 mL), extracted with diethyl ether (4 × 25 mL) and then basified with saturated potassium carbonate. The mixture was extracted with chloroform (4 × 10 mL) and the extract was dried with magnesium sulfate. The residue after evaporation was dissolved in methanol and acidified with an ethereal solution of hydrogen chloride. The formed solid was filtered off and crystallized from ethanol to give **4b** as white crystals (1.75 g, 50%), mp 218–222 °C. ^{1}H-NMR (*d6*-DMSO): δ = 1.90–2.44 (m, 8H, CH_2), 2.75 (s, 3H, CH_3), 3.36 (m, 1H, SCH), 3.84 (bs, 2H, NCH), 7.18–7.35 (m, 4H, H-2′,H-4′, H-5′, H-6′). ^{13}C-NMR (DMSO-d6) δ = 24.86 (C-6, C-7), 31.00 (C-2, C-4), 31.66 (CH_3), 36.24 (C-3), 39.83 (C-1, C-5), 128.11 (C-6′), 130.31 (C-4′), 130.98 (C-2′), 133.08 (C-5′), 134.22 (C-3′), 135.16 (C-1′). Anal. ($C_{14}H_{19}Cl_2NS$) C, H, Cl, N, S.

*Hydrochloride of 1-(3-Chlorobenzoyl)piperazine (**5a**)*

A solution of 3-chlorobenzoyl chloride (1.75 g, 10 mmol) in acetonitrile (5 mL) was added dropwise to a solution of piperazine (0.86 g, 10 mmol) in acetonitrile (10 mL) at 15–20 °C. The solution was stirred for 2 h and then the mixture was left to stand overnight in a refrigerator. The insoluble portion was filtered off and washed with diethyl ether to give **5a** (2.35 g, 89%), mp 194–196 °C. ^{1}H-NMR (*d6*-DMSO): δ = 3.33 (bs, 1H, HCl), 3.41 (bs, 4H, CH_2 of piperazine), 3.64 (bs, 4H, CH_2 of piperazine), 7.40–7.59 (m, 4H, H-2′, H-4′, H-5′ H-6′). Anal. ($C_{11}H_{14}Cl_2N_2O$) C, H, Cl, N.

*Dihydrochloride of 1-(3-Chlorobenzyl)-4-methylpiperazine (**5b**)*

3-Chlorobenzyl chloride (3.2 g, 20 mmol) was added to a stirred solution of 1-methylpiperazine (2.0 g, 20 mmol) and triethylamine (4.0 g, 40 mmol) in chloroform (10 mL) and the mixture was stirred at room temperature for 6 h. Water (10 mL) was added and the mixture was extracted with chloroform (4 × 20 mL), the combined extracts were washed with water (20 mL) and dried with magnesium sulfate. The oily residue after evaporation was dissolved in diethyl ether (20 mL), a small amount of insoluble material was filtered off and the filtrate was acidified with a saturated ethereal solution of hydrogen chloride. The precipitate formed was filtered and crystallized from ethanol to give dihydrochloride of **5b** (3.4 g, 57%), mp 222–225 °C. ^{1}H-NMR (*d6*-DMSO): δ = 2.77 (s, 3H, CH_3), ?3.32 (m, 8H, CH_2 of piperazine), 4.10 (s, 2H, CH_2), 7.42? (m, 2H, H-4′, H-6′), 7.52 (ddd, *J* = 8.7 Hz, *J* = 4.4 Hz, *J* = 1.5 Hz, 1H, H-5′), 7.67 (bm, 1H, H-2′). Anal. ($C_{12}H_{19}Cl_3N_2$) C, H, Cl, N.

*Hydrochloride of 1-(3-Chlorobenzyl)-4-ethoxycarbonylpiperazine (**5c**)*

Using the same procedure described for the preparation of **5b** with 1-ethoxycarbonylpiperazine, compound **5c** was prepared as hydrochloride in 66% yield, mp 188–191 °C. ^{1}H-NMR (*d6*-DMSO): δ = 1.21 (t, *J* = 7.1 Hz, 3H, CH_3), 3.12 (m, 4H, H-3, H-5), 3.75 (m, 4H, H-2. H-6), 4.10 (q, *J* = 7.1 Hz, CH_2), 4.31 (bs, 2H, CH_2), 7.41–7.52 (m, 2H, H-4′, H-6′), 7.65 (m, 1H, H-5′), 7.82 (m, 1H, H-2′). Anal. ($C_{14}H_{20}Cl_2N_2O_2$) C, H, Cl, N.

*Dihydrochloride of 1-(3-Chlorobenzyl)piperazine (**5d**)*

A mixture of hydrochloride of **5c** (2 g, 6.3 mmol) and concentrated hydrochloric acid (10 mL) was refluxed for 18 h. The mixture was evaporated to dryness, the residue was crystallized from ethanol to give dihydrochloride of **5d** (1.2 g, 67%), mp 214–217 °C. ^{1}H-NMR (*d6*-DMSO): δ = 3.34 (m, 4H, H-3, H-5), 3.45 (m, 4H, H-2. H-6), 4.30 (bs, 2H, CH_2), 7.47 (m, 2H, H-4′, H-6′), 7.63 (m, 1H, H-5′), 7.78 (m, 1H, H-2′). Anal. ($C_{11}H_{17}Cl_3N_2$) C, H, Cl, N.

6-Chloropyridine-2-carboxylic Acid

n-Butyllithium (10 mL of a 2.5 M solution in hexanes, 25 mmol) was added to diethyl ether (40 mL) under argon at –78 °C and then a solution of 2-bromo-6-chloropyridine (3.84 g, 20 mmoles) in diethyl ether (25 mL) was added dropwise. The mixture was stirred at –78 °C for 1 h before a large excess of solid carbon dioxide (ca. 10 g) was added. The reaction mixture was stirred at this temperature for additional 1 h, poured into cold water and extracted with diethyl ether. The water layer was acidified with concentrated hydrochloric acid and then extracted with chloroform. The extract was dried with magnesium sulfate, evaporated, and the residue was crystallized from ethyl acetate to give 2.1 g (67%) of colorless crystals, mp 185–188 °C (Ref. [20] gives mp 190 °C). ^{1}H-NMR ($CDCl_3$): δ = 3.40 (bs, 1H, COOH), 7.60 (dd, *J* = 0.8 Hz, *J* = 7.8 Hz, 1H, H-5), 8.05 (m, 2H, H-3, H-4).

6-Chloropyridine-2-carbonyl Chloride

A mixture of 6-chloropyridine-2-carboxylic acid (3.1 g, 20 mmol) and thionyl chloride (35 mL) was refluxed for 3 h. The reaction mixture was evaporated to give a slightly brown semisolid residue (3.5 g, 99%) that was used for further reaction without purification.

*Hydrochloride of 1-(6-Chloropyridine-2-carbonyl)-4-methylpiperazine (**5e**)*

A solution of 6-chloropyridine-2-carbonyl chloride (1.76 g, 10 mmol) in DMF (10 mL) was added to a stirred solution of 1-methylpiperazine (2.50 g, 25 mmol) in DMF (20 mL) and the mixture was stirred at room temperature for 3 h. The mixture was poured into water (250 mL), extracted with ethyl acetate (4 × 25 mL), the combined extracts were washed with brine (25 mL) and dried with magnesium sulfate. The residue after evaporation was dissolved in ethanol, acidified with ethanolic hydrogen chloride and evaporated. The residue was twice crystallized from ethanol to give compound **5e** as white crystals (1.6 g, 58%), mp 192–197 °C. ^{1}H-NMR (*d6*-DMSO): δ = 2.78 (s, 3H, CH_3), 3.05–3.95 (bm, 8H, CH_2), 7.68 (d, *J* = 7.6 Hz, 2H, H-3′, H-5′), 8.05 (, *J* = 7.6 Hz t, 1H, H-4′). Anal. ($C_{11}H_{15}Cl_2N_3O$) C, H, Cl, N.

*Dihydrochloride of 1-(6-Chloropyridine-2-ylmethyl)-4-methylpiperazine (**5g**)*

Sodium borohydride (0.58 g, 15.3 mmol) was slowly added to a solution of a base of compound **5e** (1.7 g, 7.1 mmol) in THF (18 mL) and then boron trifluoride etherate (2.6 g, 1.8 mmol) was added dropwise. The mixture was stirred at room temperature for 30 min and then refluxed for 3 h. The mixture was cooled to 10 °C, further portions of sodium borohydride (0.29 g, 7.6 mmol) and boron trifluoride etherate (1.3 g, 0.9 mmol) were added, the mixture was stirred at room temperature for 30 min and then refluxed for 4 h and then left to stand overnight at room temperature. 5M hydrochloric acid was slowly added to the cooled mixture that was then refluxed for 2 h. The cold mixture was basified with a 5M solution of sodium hydroxide to pH 10–11 and extracted with ethyl acetate. The combined extracts were washed with brine and dried with magnesium sulfate to give an oily residue, which was converted into the corresponding dihydrochloride. Crystallization with isopropyl alcohol gave dihydrochloride of compound **5g** (1.1 g, 49%), mp 178–181 °C. ^{1}H-NMR (*d6*-DMSO): δ = 2.28 (s, 3H, CH_3), 2.25–2.75 (bm, 8H, CH_2 of piperazine), 3.65 (m, 2H, CH_2), 7.25 (d, *J* = 7.6 Hz, 1H, H-3′), 7.45 (d, *J* = 7.6 Hz, 1H, H-5′), 7.68 (t, *J* = 7.6 Hz, 1H, H-4′). Anal. ($C_{11}H_{18}Cl_3N_3O$) C, H, Cl, N.

Biological Experiments

Acetic Acid-Induced Writhing

Writhing was induced by intraperitoneal injection of 0.2 mL of 0.7% solution of acetic acid to male NMRI mice 30 min after the administration of 30 mg/kg of the tested compound[21]. Writhings were counted for 20 min, compared with the control and expressed as decrease of the stretching movements (%). The data were statistically analyzed using the paired t-test ($p < 0.05$).

Hot Plate Test

The hot-plate test was used to measure the response latencies according to the method described earlier[22], with minor modifications. All animals (male NMRI mice) were selected on the basis of their reactivity in the model. The selected animals were placed into a glass cylinder and the plate temperature was maintained at 54 °C. The time necessary to induce the licking reflex of the forepaws or jumping was recorded. The measurement was done 30 and 60 min after oral administration of 30 mg/kg of the tested compound and the results were expressed as prolongation of the licking latencies (%). The data were statistically analyzed using the paired t-test ($p < 0.05$).

Serotonin Receptor Binding Studies

Serotonin radioligand displacement receptor binding assays were conducted in the hippocampus of the rat brain for 5-HT_{1A} receptors, and in the rat striatum for 5-HT_{1B} receptors, according to the published procedures[23, 24]. Adult male rats were killed by cervical dislocation and decapitation. Their brains were dissected, immediately frozen, and stored at –80 °C until needed. [^{3}H]-8-OH-DPAT (c = 0.25 nM) and [^{3}H]-5-HT (c = 2.00 nM) were used for labeling of 5-HT_{1A} and 5-HT_{1B} receptors, respectively. The tested compounds were used in 10^{-6} M concentrations. The incubation was terminated by filtration through Whatman GF/B glass fiber filters and the bound radioactivity retained on filters was determined by liquid scintillation spectrometry. Generally, the binding is expressed as the retained bound radioligand (%) after the displacement period.

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Received: September 27, 1999 [FP428a]