MOLECULAR MODIFICATION OF ANPIRTOLINE, A NON-OPIOID CENTRALLY ACTING ANALGESIC

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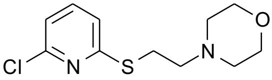
Dedicated to the memory of Miroslav Protiva, our colleague and an outstanding medicinal chemist.

Molecular modification of anpirtoline (**2a**) is described. Several methods of preparation of 4-[(3-chlorophenyl)sulfanyl]-1-methylpiperidine (**3a**) and its demethylation led to the deazaanpirtoline (**3c**). Nucleophilic substitution of piperidine-4-thiole with 2-chloro-4-nitropyridine, 2,4-dichloro-6-methylpyridine, and 3,6-dichloropyridazine led to 2-chloro-4-(piperidin-4-ylsulfanyl)pyridine (**6**), 4-chloro-6-methyl-2-(piperidin-4-ylsulfanyl)pyridine (**7**), and 3-chloro-6-(piperidin-4-ylsulfanyl)pyridazine (**8**), respectively. 2-Chloro-6-(pyridin-4-ylsulfanyl)pyridine (**10**) and 4-[(2-chloropyridin-6-yl)sulfanyl]quinoline (**11**) were obtained from sodium 2-chloropyridine-6-thiolate. Homoanpirtoline analogs with methylene group inserted between the pyridine moiety and the sulfur atom (compound **12b**) as well as between the sulfur atom and the piperidine ring (compound **13b**) were also prepared.

Key words: Anpirtoline; 4-Phenylsulfanylpiperidines; Piperidines; Pyridines; Analgesic activity; Analgesics; 5-HT receptor ligands.

Worldwide attention is paid to the development of centrally acting analgesics not involving opiate receptors. A series of 2-chloro-6-[(aminoalkyl)-sulfanyl]pyridines, of which fopirtoline (**1**) was selected as the most promising compound[1], was reported as a novel structural type complying with these requirements. Further modification led to the discovery of compounds with a better profile, of which 2-chloro-6-[(piperidin-4-yl)sulfanyl]pyridine (**2a**), named anpirtoline, was chosen for further development by ASTA Medica[2–4]. Anpirtoline is a very strong analgesic not interfering with opiate receptors and its 5-HT_{1B} agonistic activity is expected to be the principal mode of action of this drug[5,6]. With the exception of several very close analogs of anpirtoline[2], no systematic structure–activity study has been reported and therefore we decided to perform molecular modification of this compound.

All of the highly active compounds of this type contain a secondary amino (anpirtoline) or tertiary amino group (fopirtoline) linked to the pyridine ring *via* a two or three carbon chain with sulfur atom bound to the heteroaromatic ring. We decided to modify both the pyridine part of the molecule and the side chain.



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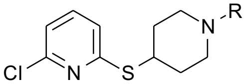
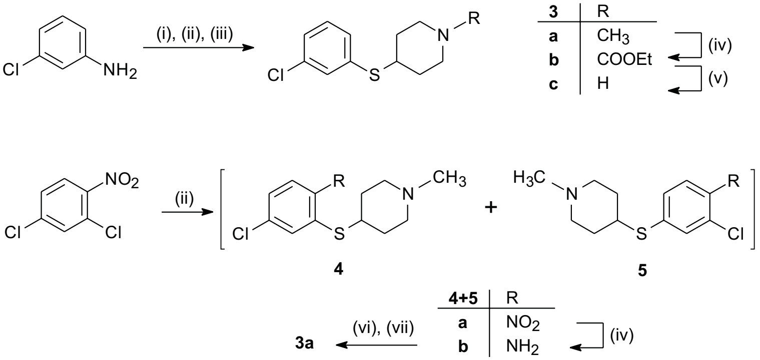


2	R
a	H
b	CH_3

Modification of the pyridine moiety was aimed at the preparation of an anpirtoline deaza analog **3c**, its positional isomers **6** and **7**, as well as aza analogs **8** and **9c**.

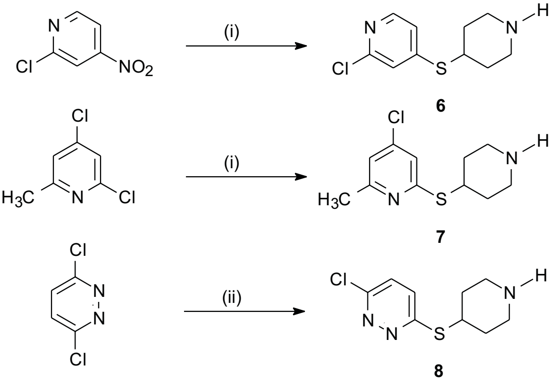
In our attempts to prepare **3a** by alkylation of 3-chlorobenzenethiol with 4-chloro-1-methylpiperidine we failed even when the starting chloro compound was used in a high excess and was added in several portions during the reaction. This failure could be explained by the strong tendency of 4-chloro-1-methylpiperidine to eliminate the chloro atom to give the corresponding piperideine under the used basic conditions. For our purposes, we modified a low-yield method consisting of a treatment of potassium 1-methylpiperidin-4-thiolate with 3-chlorobenzenediazonium chloride and subsequent thermal decomposition of the formed diazotate[7]. The crude reaction mixture after chromatographic purification provided about 30% yield of **3a**, which was treated with ethyl chloroformate to give the corresponding *N*-ethoxycarbonyl derivative **3b**. Acid hydrolysis then provided **3c** as hydrochloride. Clearly, the method was not suitable for preparation of higher amounts of **3c** and, therefore, when this compound was found interestingly active in a primary screen, we had to develop a new approach to this compound to get more material for detailed preclinical testing. We started from 2,4-dichloronitrobenzene which on treatment with 1-methylpiperidine-4-thiol in acetone in the presence of potassium carbonate provided a mixture of isomeric nitro compounds containing 73% of **4a** and 27% of **5a**. The prevailing isomer **4** can be obtained pure as hydrochloride by repeated crystallization from ethanol. For our purposes, we used their mixture which was reduced with iron in dilute hydrochloric acid to give a mixture of the corresponding amino derivatives **4b** and **5b**. The mixture was diazotized to a mixture of corresponding diazonium chlorides, which was then reduced with ethanol to give the required intermediate **3a** (Scheme 1). Though the overall yield is also rather low, the method is suitable for scaling up.



(i) $NaNO_2$, HCl, 0 °C; (ii) potassium 1-methylpiperidine-4-thiolate; (iii) 80 °C; (iv) $ClCO_2Et$, 90 °C; (v) aq. HCl/AcOH, reflux; (vi) Fe/aq. HCl; (vii) $NaNO_2$, aq. HCl, 0 °C; EtOH, reflux

SCHEME 1

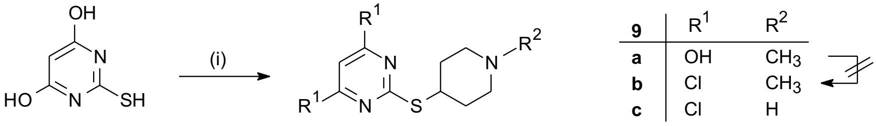
Anpirtoline positional isomer **6** was prepared by a direct reaction of 2-chloro-4-nitropyridine with sodium piperidine-4-thiolate generated *in situ* from piperidine-4-thiol hydrochloride and sodium hydride. A similar preferential nucleophilic displacement of the nitro group in 2-chloro-4-nitropyridine with sodium methoxide is described in the literature[8]. Analogous reactions of 2,4-dichloro-6-methylpyridine and 3,6-dichloropyridazine provided compounds **7** and **8**, respectively (Scheme 2).



(i) Sodium piperidine-4-thiolate, dimethylacetamide, r.t.; (ii) piperidine-4-thiol, Et_3N, $CHCl_3$, reflux

SCHEME 2

In our attempts to prepare the pyrimidine analog **9c**, we alkylated thiobarbituric acid with 4-chloro-1-methylpiperidine to **9a**. However, we failed to prepare the corresponding dichloro derivative **9b** using usual agents like $POCl_3$, PCl_3, PCl_5, and their combinations (Scheme 3).

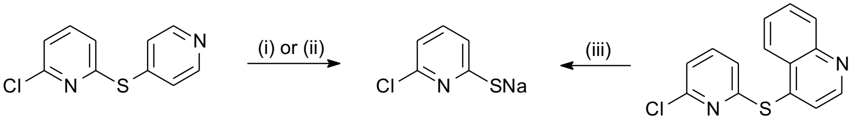


9	R1	R2
a	OH	CH_3
b	Cl	CH_3
c	Cl	H

(i) Sodium 1-methylpiperidine-4-thiolate

SCHEME 3

Aromatic anpirtoline analog **10** was prepared from sodium 2-chloropyridine-6-thiolate and 1-(pyridin-4-yl)pyridinium chloride, the compound known to be useful for nucleophilic introduction of the pyridin-4-yl group[9]. The same product was also obtained using 4-chloropyridine. Compound **11** was prepared similarly using 4-chloroquinoline (Scheme 4).



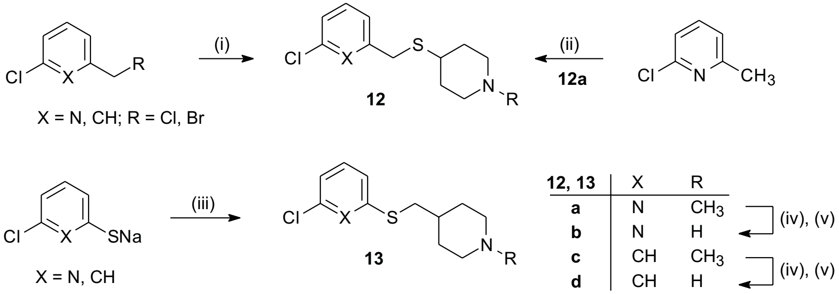
10

11

(i) 1-(Pyridin-4-yl)pyridinium chloride; (ii) 4-chloropyridine; (iii) 4-chloroquinoline

SCHEME 4

Chlorination[10] and bromination[11] of methylpyridines are known to provide mixtures of mono- and dihalomethyl derivatives. 2-Chloro-6-methylpyridine was brominated to a mixture containing both bromomethyl derivatives which, treated with sodium 1-methylpiperidine-4-thiolate, gave the corresponding sulfanyl derivatives. From them *N*-methylhomoanpirtoline **12a**, having inserted a methylene group between the sulfur atom and the pyridine ring, was isolated by chromatography. In attempts to avoid this tedious separation, we tried to prepare 2-bromomethyl-6-chloropyridine by treatment of 2-chloro-6-methylpyridine with butyllithium followed by bromine, but a complex mixture was obtained. Then we decided to use a different strategy based on literature reports that dimethyldisulfane with alkyl or aryllithium compounds gives corresponding alkyl(aryl)sulfanylmethanes[12–14]. For our purposes, we needed bis(1-methylpiperidin-4-yl)disulfane, the compound described as a side product in the preparation of 1-methylpiperidine-4-thiol[15]. We prepared this compound by oxidation of 1-methylpiperidine-4-thiol with iodine. The corresponding lithium species was generated from 2-chloro-6-methylpyridine and lithium diisopropylamide at –78 °C and then the resulting mixture was treated with bis(1-methylpiperidin-4-yl)disulfane. After work up, the required compound **12a** was obtained in good yields. If the lithium species was generated with butyllithium, the required product was contaminated with 4-butylsulfanyl-1-methylpiperidine (^{1}H NMR). Compound **12a** was then demethylated to the *N*-unsubstituted compound **12b** in the same way as described earlier for compound **3a**. 3-Chlorophenyl analogs **12c** and **12d** were obtained in the same way from 3-chlorobenzyl chloride (Scheme 5).



(i) Sodium 1-methylpiperidine-4-thiolate; (ii) 1. LDA, 2. bis(1-methylpiperidin-4-yl)disulfane; (iii) 4-chloromethyl-1-methylpiperidine; (iv) ClCOOEt, 90 °C; (v) aq. HCl/AcOH, reflux

Scheme 5

N-Methylhomoanpirtoline **13a**, with a methylene group inserted between the sulfur atom and the piperidine ring, was prepared by alkylation of sodium 2-chloropyridine-6-thiolate with 4-chloromethyl-1-methylpiperidine, which was prepared in two steps from ethyl 1-methylpiperidine-4-carboxylate by a known procedure. The reaction of **13a** with ethyl chloroformate, however, did not provide the corresponding carbamate which could, after subsequent acidic hydrolysis, provide **13b**. We also failed to demethylate **13a** using vinyl chloroformate as well as bromocyan. Compound **13c** was prepared analogously to **13a** from 3-chlorobenzenethiol. In this case, dealkylation of **13c** with ethyl chloroformate and subsequent acid hydrolysis provided smoothly compound **13d** (Scheme 5).

The prepared compounds were evaluated for their antinociceptive activity in two basic tests, the hot-plate test and intraperitoneal writhing test after oral application. Antinociceptive activity of anpirtoline is assumed to be mediated by 5-HT_{1B} receptors and involvement of 5-HT_{1A} receptors in analgesic activity of some active compounds is also anticipated, therefore, the receptor binding profile towards these receptor subtypes was also evaluated.

Analgesic activity and results of the binding studies are shown in Table I. None of the investigated compounds, including *N*-methyl derivative of anpirtoline **2b**, show analgesic activity comparable to anpirtoline. However, some of them, *e.g.* compounds **3c**, **7**, and **8**, are in doses of 30 mg/kg p.o. significantly active in the used analgesic models. Evidently, in this se-

TABLE I
5-HT_{1A} and 5-HT_{1B} receptor binding affinities and analgesic activity of compounds **1**–**13**

Compound	Binding studies		Activity, 30 mg/kg p.o.	
	5-HT_{1A}, %	5-HT_{1B}, %	hot-plate test, %	writhing test, %
1·HCl	85	84	30	8
2a·HCl	22[a]	40	150	90
2b·HCl	72	78	19	64
3a tosylate	48	82	n.d.[b]	4
3c·HCl	53	51	70	41
6·HCl	107	52	33	15
7·HCl	87	81	55	75
8·HCl	102	97	75	51
9a·HCl	n.d.[b]	n.d.[b]	8	0
10·HCl	97	101	27	–6
11·HCl	92	105	24	–14
12a·HCl	n.d.[b]	n.d.[b]	33	7
12b·HCl	78	56[c]	27	17
12c maleate	79	67[d]	19	28
12d·HCl	71	94	46	67
13a maleate	82	61[e]	34	31
13c·HCl	79	85	47	48
13d·HCl	75	81	toxic	toxic

[a] IC_{50} = 0.57 μM; [b] not determined; [c] IC_{50} = 1.45 μM; [d] IC_{50} = 4.27 μM; [e] IC_{50} = 2.45 μM.

ries of compounds, there is no correlation between the analgesic activity and the binding to the studied receptors and, therefore, at least for some of the compounds, their analgesic activity should be mediated by a different mechanism.

EXPERIMENTAL

Melting points were measured on a Kofler block and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker instrument (250 MHz). Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. Flash and vacuum chromatography were done on silica gel 60 (230–400 mesh) and preparative TLC on pre-coated PLC plates (silica gel 60) from EM Science.

The following starting compounds were prepared by the previously described methods: 1-methylpiperidine-4-thiol[2], 2-chloro-4-nitropyridine[20], 2,4-dichloro-6-methylpyridine[21], 1-(pyridine-4-yl)pyridinium chloride hydrochloride[22], sodium 2-chloropyridine-6-thiolate[2], and 4-chloromethyl-1-methylpiperidine[23]. Fopirtoline[1] (**1**), anpirtoline[2] (**2a**), and 2-chloro-6-[(1-methylpiperidin-4-yl)sulfanyl]pyridine[2] (**2b**), used as standards for biological evaluations, were prepared by methods described in the respective patents.

4-[(3-Chlorophenyl)sulfanyl]-1-methylpiperidine (**3a**)

Method A) A solution of sodium nitrite (3.5 g, 50 mmol) in water (25 ml) was added at 0 °C to a solution of 3-chloroaniline (6.4 g, 50 mmol) in 0.5 M hydrochloric acid (200 ml, 0.1 mol) and the mixture was stirred at this temperature for 30 min. This solution was added dropwise during 1 h to a solution of 1-methylpiperidine-4-thiol (7.2 g, mmol) in 3 M potassium hydroxide (50 ml, 150 mmol) at 0 °C. The temperature was elevated during 30 min to 80 °C and then kept till the nitrogen evolution ceased. The cold mixture was extracted with dichloromethane, the combined extracts were dried with magnesium sulfate and the crude product (6.5 g) was purified by flash chromatography (CH_2Cl_2–MeOH 40 : 1) to give 3.8 g (30%) of an oily product. ^{1}H NMR ($CDCl_3$): 1.75 m, 2 H (H-3, H-5); 1.87 m, 2 H (H-3, H-5); 2.12 m, 2 H (H-2, H-6); 2.26 s, 3 H (CH_3); 2.75 m, 2 H (H-2, H-6); 3.02 m, 1 H (H-4); 7.07–7.19 m, 4 H (H-2′, H-4′, H-5′, H-6′). A part of this oil (1 g) was converted to tosylate (0.8 g), m.p. 89–90 °C. For $C_{19}H_{24}ClNO_3S_2$ (414.0) calculated: 55.13% C, 5.84% H, 8.56% Cl, 3.38% N, 15.49% S; found: 54.75% C, 5.85% H, 8.43% Cl, 3.15% N, 15.16% S.

Method B) A mixture of 2,4-dichloronitrobenzene (19.2 g, 0.1 mol), potassium carbonate (15 g, 0.11 mol), 1-methylpiperidine-4-thiol (15 g, 0.13 mol), and dry acetone (300 ml) was refluxed for 20 h. Then the insoluble portion was filtered off, washed with acetone and the combined filtrates were evaporated. The oily residue (29.2 g) was dissolved in methanol (100 ml) and acidified with saturated ethereal solution of hydrogen chloride. The separated product was filtered off and dried to give a mixture of **4a** and **5a**, yield 30 g (93%). A small sample was alkalinized with aqueous sodium hydroxide, extracted with ether and subjected to GLC using a pure sample of **4a**. The results showed that the mixture contains 73% of **4a** and 27% of **5a**. Pure hydrochloride of **4a** was obtained by repeated crystallization of the mixture from ethanol, m.p. 220–222 °C. For $C_{12}H_{16}Cl_2N_2O_2S$ (323.2) calculated: 44.59% C, 4.99% H, 21.94% Cl, 8.67% N, 9.92% S; found: 44.40% C, 5.05 % H, 21.85% Cl, 8.49% N, 9.93% S. A part of the mixture of **4a** and **5a** (16.2 g, 50 mmol) was dissolved in a mixture of concentrated hydrochloric acid (50 ml) and water (125 ml), then iron powder (7 g, 0.125 mmol) was added and the mixture was stirred under reflux for 4 h. The insoluble portion was filtered through Celite, the filtrate was evaporated and the residue was dissolved in water and alkalinized with sodium hydroxide. Chloroform (500 ml) was added to the cold mixture and after thorough shaking, the mixture was filtered through Celite. The cake was washed with chloroform (500 ml), the filtrate was separated and the water layer was extracted with chloroform (2 l). The organic layers were combined, dried with magnesium sulfate to give after evaporation a dark residue which was purified by vacuum chromatography (silica, CH_2Cl_2–MeOH 9 : 1) to give a mixture of **4b** and **5b** as a yellow oil (6.5 g, 52%). The oil was dissolved in methanol, acidified with methanolic solution of hydrogen chloride and evaporated to give a mixture of dihydrochlorides of the respective compounds (8.2 g, 51%), which was used for the following step without further purification. This mixture was dissolved in a mixture of concentrated hydrochloric acid (6 ml) and water (40 ml) and to the solution cooled below 5 °C, a solution of sodium nitrite (2 g, 29 mmol) was added dropwise. The mixture was stirred at 5 °C for 30 min, the excess of sodium nitrite was removed by addition of urea (0.3 g, 5 mmol), ethanol (25 ml) was added and the mixture was refluxed for 30 min. The cold mixture was alkalinized with sodium hydroxide, extracted with diethyl ether and the extract was dried with magnesium sulfate. The residue was purified by flash chromatography (CH_2Cl_2–MeOH 40 : 1) to give 3.1 g (51% calculated on **4**) of an oily product identical with the sample obtained by method *A* (TLC, ^1H NMR).

Ethyl 4-[(3-Chlorophenyl)sulfanyl]piperidine-1-carboxylate (**3b**)

Ethyl chloroformate (2.2 g, 20 mmol) was added dropwise to a solution of *N*-methyl derivative **3a** (2.4 g, 10 mmol) in toluene (50 ml) at 90 °C and the mixture was stirred at this temperature for 2 h. Then an additional amount of ethyl chloroformate (1.1 g, 10 mmol) was added and the mixture was stirred at 90 °C for further 3 h. Insoluble portion was removed by filtration through Celite, the filtrate was evaporated and purified by flash chromatography (petroleum ether–acetone 9 : 1) to give an oily product (1.8 g, 60%) which was used for the following reaction without further purification. ^1H NMR ($CDCl_3$): 1.25 t, 3 H, $J = 7.0$ (CH_3); 1.60 m, 2 H (H-3, H-5); 1.94 m, 2 H (H-3, H-5); 3.13 m, 4 H (H-2, H-6); 3.95 bt, 1 H (H-4); 4.12 q, 2 H, $J = 7.0$ (CH_2); 7.24 m, 3 H (H-4, H-5, H-6); 7.39 m, 1 H (H-2).

4-[(3-Chlorophenyl)sulfanyl]piperidine (**3c**)

A mixture of **3b** (1.3 g, 4.3 mmol), concentrated hydrochloric acid (10 ml), and acetic acid (10 ml) was refluxed for 24 h. Then the mixture was evaporated to dryness and the crystalline residue was recrystallized twice from propan-2-ol using charcoal to give **3c** (0.6 g, 53%), m.p. 161–165 °C. For $C_{11}H_{15}Cl_2NS$ (264.2) calculated: 50.01% C, 5.72% H, 26.84% Cl, 5.30% N, 12.13% S; found: 49.80% C, 5.71% H, 26.87% Cl, 5.30% N, 12.20% S.

2-Chloro-4-(piperidin-4-ylsulfanyl)pyridine (**6**)

Sodium hydride (2.55 g, 50% dispersion in mineral oil, 53 mmol) was added under nitrogen to dry *N*,*N*-dimethylacetamide (25 ml) and the stirred mixture was cooled to 0 °C. Piperidine-4-thiol hydrochloride (3.6 g, 24 mmol) was added in one portion, the mixture was stirred for 1 h and then a solution of 2-chloro-4-nitropyridine (3.8 g, 24 mmol) in *N*,*N*-dimethylacetamide (12 ml) was added dropwise during 15 min. The mixture was stirred at room temperature for 2.5 h, then cooled to 0 °C and decomposed by addition of water

(150 ml). The mixture was extracted with dichloromethane, the organic extracts were washed with water and brine and dried with magnesium sulfate. The residue was purified by chromatography on a short column of silica to give a yellowish oil (3.1 g) which was converted to hydrochloride of **6** (2.4 g, 38%), m.p. 215–217 °C (ethanol). For $C_{10}H_{14}Cl_2N_2S$ (265.2) calculated: 45.29% C, 5.32% H, 26.74% Cl, 10.56% N, 12.09% S; found: 45.17% C, 5.32% H, 26.66% Cl, 10.57% N, 12.27% S.

4-Chloro-6-methyl-2-(piperidin-4-ylsulfanyl)pyridine (**7**)

Compound **7** (0.75 g, 77%) was prepared from 2,4-dichloro-6-methylpyridine (0.65 g, 4 mmol) by the same procedure described for the preparation of **6**. ^{1}H NMR ($CDCl_3$): 1.62 m, 2 H (H-3′, H-5′); 2.06 m, 2 H (H-3′, H-5′); 2.45 s, 3 H (CH_3); 2.77 m, 2 H (H-2′, H-6′); 3.15 m, 2 H (H-2′, H-6′); 3.45 bt, 1 H (H-4′); 6.88 s, 1 H and 6.95 s, 1 H (H-3 and H-5). The base (yellowish oil) was converted to hydrochloride (0.8 g, 72%), m.p. 225–228 °C (ethanol). For $C_{11}H_{16}Cl_2N_2S$ (279.2) calculated: 47.32% C, 5.78% H, 25.39% Cl, 10.03% N, 11.48% S; found: 47.07% C, 5.72% H, 25.46% Cl, 10.12% N, 11.25% S.

3-Chloro-6-(piperidin-4-ylsulfanyl)pyridazine (**8**)

Triethylamine (2 ml, 27 mmol) was added to a stirred mixture of 3,6-dichloropyridazine (1.5 g, 10 mmol), piperidine-4-thiol hydrochloride (1.8 g, 12 mmol) and chloroform (50 ml) and the mixture was refluxed for 10 h. The mixture was washed with water, dried with magnesium sulfate and the residue after evaporation was purified by vacuum chromatography (CH_2Cl_2–MeOH 8 : 2). The residue (0.65 g) obtained by evaporation of the respective fractions was converted to hydrochloride (0.45 g, 17%), m.p. 239–241 °C. For $C_9H_{13}Cl_2N_3S$ (266.2) calculated: 40.61% C, 4.92% H, 26.64% Cl, 15.79% N, 12.04% S; found: 40.83% C, 4.99% H, 26.35% Cl, 15.69% N, 11.89% S.

2-[(1-Methylpiperidin-4-yl)sulfanyl]pyrimidine-4,6-diol (**9a**)

4-Chloro-1-methylpiperidine (1.33 g, 10 mmol) was added dropwise to a solution of thiobarbituric acid (1.45 g, 10 mmol) in dry acetone (150 ml) and the formed suspension was stirred at room temperature for 1 h. The insoluble portion was filtered off, washed with acetone and recrystallized from ethanol to give hydrochloride of **9a** (2.6 g, 94%), m.p. 177–180 °C (decomp.). For $C_{10}H_{16}ClN_3O_2S$ (277.8) calculated: 43.24% C, 5.81% H, 12.76% Cl, 15.13% N, 11.54% S; found: 42.79% C, 5.72% H, 12.54% Cl, 15.13% N, 11.61% S.

2-Chloro-6-(pyridin-4-ylsulfanyl)pyridine (**10**)

Method A) A mixture of sodium 2-chloropyridine-6-thiolate (1 g, 6 mmol), 1-(pyridin-4-yl)pyridinium chloride hydrochloride (1.4 g, 6 mmol), sodium hydrogencarbonate (1 g, 12 mmol), and propan-2-ol (10 ml) was stirred under nitrogen at room temperature for 3 h. The mixture was evaporated, the residue was quenched with water, extracted with dichloromethane and the extract was dried with magnesium sulfate. The residue was purified by flash chromatography (silica, petroleum ether–acetone 8 : 2) to give **10**, an oil (0.4 g) which was converted to hydrochloride (0.4 g, 26%), m.p. 148–150 °C (ethanol). For $C_{10}H_8Cl_2N_2S$ (259.2) calculated: 46.35% C, 3.11% H, 27.36% Cl, 10.81% N, 12.37% S; found: 46.11% C, 3.10% H, 26.76% Cl, 10.71% N, 12.73% S. ^{1}H NMR ($(CH_3)_2SO$): 7.04 bs, (HCl); 7.64 d, 1 H, J = 8.0

(H-5); 7.73 d, 1 H, J = 7.8 (H-3); 7.82 d, 2 H, J = 5.4 (H-3′); 8.01 t, 1 H, J = 7.8 (H-4); 8.69 d, 2 H, J = 5.4 (H-2′).

Method B) The same procedure as method *A*) using hydrochloride of 4-chloropyridine provided 25% yield of **10**.

4-[(2-Chloropyridin-6-yl)sulfanyl]quinoline (**11**)

A mixture of sodium 2-chloropyridine-6-thiolate (1 g, 6 mmol), 4-chloroquinoline (1 g, 6 mmol), and propan-2-ol (10 ml) was refluxed under nitrogen for 2 h. The mixture was evaporated, the residue was quenched with water, extracted with dichloromethane and the extract was dried with magnesium sulfate. The residue was purified by flash chromatography (silica, petroleum ether–acetone 9 : 1) to give **11** as white crystals (0.5 g, 31%), m.p. 98–100 °C (ethanol). For $C_{14}H_9ClN_2S$ (272.7) calculated: 61.65% C, 3.33% H, 13.00% Cl, 10.27% N, 11.75% S; found: 61.35% C, 3.37% H, 13.10% Cl, 10.17% N, 11.99% S. ^{1}H NMR ($CDCl_3$): 6.83 dd, 1 H, J = 7.8, 0.8 (H-3′); 7.14 dd, 1 H, J = 7.8, 0.8 (H-5′); 7.44 t, 1 H, J = 7.8 (H-4′); 7.60 ddd, 1 H, J = 1.5, 6.9, 8.4 (H-6); 7.65 d, 1 H, J = 4.6 (H-3); 7.79 ddd, 1 H, J = 1.2, 6.9, 8.4 (H-7); 8.19 ddd, 1 H, J = 0.6, 1.2, 8.4 (H-5); 8.26 ddd, 1 H, J = 0.6, 1.5, 8.4 (H-8); 8.9 d, 1 H, J = 4.6 (H-2). MS, *m/z* (%): 272 (69), 241 (30), 239 (100), 237 (91), 210 (61), 112 (14), 101 (45), 89 (40), 75 (75). Hydrochloride, m.p. 177–179 °C (ethanol). For $C_{14}H_{10}Cl_2N_2S$ (309.2) calculated: 54.38% C, 3.26% H, 22.93% Cl, 9.06% N, 10.37% S; found: 54.06% C, 3.42% H, 22.60% Cl, 9.07% N, 10.76% S.

Bis(1-methylpiperidin-4-yl)disulfane

A solution of iodine (2.7 g, 10.5 mmol) in ethanol (20 ml) was added dropwise to a solution of 1-methylpiperidine-4-thiol (2.6 g, 10 mmol) and potassium hydroxide (1.1 g, 20 mmol) in ethanol (10 ml) and the mixture was stirred for 15 min. Several drops of concentrated solution of sodium thiosulfate were added untill the solution contained iodine, the mixture was evaporated to dryness, the residue was treated with water (20 ml) and extracted with diethyl ether (5 × 20 ml). The extract was dried with magnesium sulfate, the residue (2.2 g) was crystallized from hexane to give cream crystals (2.1 g, 81%), m.p. 54–55 °C. For $C_{12}H_{24}N_2S_2$ (260.5) calculated: 55.34% C, 9.29% H, 10.76% N, 24.62% S; found: 55.28% C, 9.06% H, 10.74% N, 25.11% S. ^{1}H NMR ($CDCl_3$): 1.67 m, 4 H (H-3, H-5); 1.99 m, 8 H (H-2, H-3, H-5, H-6); 2.26 s, 6 H (CH_3); 2.66 m, 2 H (H-4); 2.82 m, 4 H (H-2, H-6).

2-Chloro-6-{[(1-methylpiperidin-4-yl)sulfanyl]methyl}pyridine (**12a**)

Method A) A mixture of 2-chloro-6-methylpyridine (1.27 g, 10 mmol), *N*-bromosuccinimide (1.8 g, 10 mmol), dibenzoyl peroxide (0.1 g, 0.4 mmol), and tetrachloromethane (20 ml) was stirred at room temperature for 24 h. Then the mixture was washed with water (10 ml) and the water layer was extracted with tetrachloromethane (2 × 10 ml) and the combined organic extracts were dried with magnesium sulfate. According to GC/MS, the mixture contained 33% of the starting 2-chloro-6-methylpyridine, 57% of 2-bromomethyl-6-chloropyridine, and 10% of 2-chloro-6-dibromomethylpyridine. The residue after evaporation (1.7 g) was dissolved in dry acetone (20 ml) and, after addition of 1-methylpiperidine-4-thiol (1.3 g, 10 mmol) and potassium carbonate (3 g, 22 mmol), the mixture was refluxed for 4 h. The insoluble portion was filtered off, washed with acetone and the filtrate was evaporated. The residue was dissolved in chloroform (20 ml), the solution was washed twice with 5 M NaOH (5 ml) and water (5 ml) and then twice with 2 M HCl (5 ml). The acid extract was washed with chloroform (2 × 5 ml) and then alkalinized with 5 M NaOH. The mixture was extracted with chlororform (4 × 5 ml) and the organic extract was dried with magnesium sulfate. The residue after evaporation (1.7 g) was then purified by flash chromatography (silica, CH_2Cl_2–MeOH 25 : 1) to give **12a** as colorless oil (1.1 g, 43%). ^{1}H NMR ($CDCl_3$): 1.35–1.65 m, 2 H (H-3′, H-5′); 2.22 s, 3 H (CH_3); 2.64 m, 3 H (H-3′, H-4′, H-5′); 2.83 m, 4 H (H-2′, H-6′); 3.92 s, 2 H (CH_2); 7.12 d, 1 H, *J* = 7.2 (H-3 or H-5); 7.34 d, 1 H, *J* = 7.2 (H-3 or H-5); 7.66 t, 1 H, *J* = 7.7 (H-4). Maleate, m.p. 98–100 °C (ethyl acetate). For $C_{16}H_{21}ClN_2O_4S$ (372.9) calculated: 51.54% C, 5.68% H, 9.51% Cl, 7.51% N, 8.60% S; found: 51.22% C, 5.89% H, 9.04% Cl, 7.32% N, 8.26% S.

Method B) Lithium diisopropylamide (5 ml, 2 M solution in heptane–tetrahydrofuran–ethylbenzene, 10 mmol) was added dropwise to a solution of 2-chloro-6-methylpyridine (1.27 g, 10 mmol) in dry diethyl ether (20 ml) at –78 °C and the mixture was stirred at this temperature under argon for 2 h. Then a solution of bis(1-methylpiperidin-4-yl)disulfane (2.6 g, 10 mmol) in diethyl ether (25 ml) was added dropwise, the mixture was stirred at –78 °C for 2 h. Then the mixture was allowed to warm during 2 h to 0 °C and then stirred at this temperature for 4 h. The mixture was poured into saturated solution of ammonium chloride (50 ml) and extracted with diethyl ether (100 ml). The extract was dried with potassium carbonate, the residue after evaporation (1.7 g) was purified by flash chromatography (silica, CH_2Cl_2–MeOH 25 : 1) to give **12a** as colorless oil (1.4 g, 55%), identical with that prepared by method *A* (TLC, GC, ^{1}H NMR).

2-Chloro-6-[(piperidin-4-ylsulfanyl)methyl]pyridine (**12b**)

Ethyl chloroformate (1.1 g, 10 mmol) was added dropwise to a stirred solution of **12a** (1.0 g, 3.9 mmol) in toluene (10 ml) at 90 °C and then the mixture was stirred at this temperature for 2 h. The insoluble portion was filtered through Celite, the toluene solution was evaporated (0.8 g) and the residue was dissolved in a mixture of acetic acid (10 ml) and concentrated hydrochloric acid (10 ml). The mixture was refluxed for 20 h, filtered through Celite and the filtrate was evaporated. The residue was dissolved in water, alkalinized with 5 M NaOH and extracted with chloroform (5 × 10 ml). The organic extract was dried with magnesium sulfate, the residue was evaporated (0.25 g) and the base was converted to maleate (0.25 g, 17%), m.p. 101–103 °C (ethyl acetate). For $C_{16}H_{19}ClN_2O_4S$ (358.8) calculated: 50.21% C, 5.34% H, 8.93% Cl, 7.81% N, 8.64% S; found: 50.11% C, 5.33% H, 9.27% Cl, 7.52% N, 8.40% S. ^{1}H NMR ($(CD_3)_2SO$): 1.55–1.75 m, 2 H (H-3′, H-5′); 2.09 dd, 2 H, *J* = 14.4, 3.9 (H-2′, H-6′); 2.82 m, 3 H (H-3′, H-4′, H-5′); 3.10 bs, 2 H (CO_2H); 3.29 t, 2 H, *J* = 3.9 (H-2′, H-6′); 3.91 s, 2 H (CH_2); 6.08 s, 2 H (CH, maleic acid); 7.38 d, 1 H, *J* = 7.2 (H-3 or H-5); 7.45 d, 1 H, *J* = 7.2 (H-3 or H-5); 7.84 t, 1 H, *J* = 7.7 (H-4); 8.43 bs, 1 H (NH).

4-[(3-Chlorobenzyl)sulfanyl]-1-methylpiperidine (**12c**)

A mixture of 3-chlorobenzyl chloride (4.8 g, 30 mmol), 1-methylpiperidine-4-thiol (3.9 g, 30 mmol), potassium carbonate (8.3 g, 60 mmol), and acetone (50 ml) was refluxed for 5 h. Then the mixture was evaporated to dryness, the residue was quenched with water and extracted with ether. The extract was dried with potassium carbonate, the residue after evaporation (7.8 g) was used without further purification. Maleate, m.p. 101–106 °C (ethyl acetate). For $C_{17}H_{22}ClNO_4S$ (371.9) calculated: 54.91% C, 5.96% H, 9.53% Cl, 3.77% N, 8.62% S; found: 54.85% C, 6.01% H, 8.93% Cl, 3.60% N, 8.76% S.

4-[(3-Chlorobenzyl)sulfanyl]piperidine (**12d**)

Ethyl chloroformate (4.4 g, 40 mmol) was added dropwise to a stirred solution of **12c** (5.2 g, 20 mmol) in toluene (25 ml) at 90 °C and then the mixture was stirred at this temperature for 2 h. The insoluble portion was filtered through Celite, the toluene solution was evaporated (5.1 g) and the residue was dissolved in a mixture of acetic acid (40 ml) and concentrated hydrochloric acid (40 ml). The mixture was refluxed for 20 h and filtered through Celite. The solution was evaporated and the residue was crystallized from ethyl acetate to give hydrochloride of **12d** (4.2 g, 75%), m.p. 101–102 °C. For $C_{12}H_{17}Cl_2NS$ (278.2) calculated: 51.80% C, 6.16% H, 25.48% Cl, 5.03% N, 11.52% S; found: 51.79% C, 6.26% H, 25.53% Cl, 4.97% N, 11.53% S.

2-Chloro-6-{[(1-methylpiperidin-4-yl)methyl]sulfanyl}pyridine (**13a**)

A mixture of sodium 2-chloropyridine-6-thiolate (6.6 g, 39 mmol), 4-chloromethyl-1-methylpiperidine (5.8 g, 39 mmol), and propan-1-ol (55 ml) was refluxed under nitrogen for 10 h, then the mixture was evaporated, the residue treated with water and extracted with chloroform. The extract was washed with water, dried with magnesium sulfate and the residue after evaporation was purified by flash chromatography (silica, CH_2Cl_2–MeOH 9 : 1) to give **13a** as yellowish oil (2.1 g, 21%). ^{1}H NMR spectrum ($CDCl_3$): 1.50–1.56 m, 2 H (H-3′, H-5′); 1.66–1.74 m, 5 H (H-2′, H-4′, H-6′); 1.86 s, 2 H (H-3′, H-5′); 2.83 s, 3 H (CH_3); 3.33 dd, 1 H, *J* = 13.5, 3.0 (CH_2); 3.65 dd, 1 H, *J* = 13.5, 9.5 (CH_2); 6.97 dd, 1 H, *J* = 7.9, 0.9 (H-3 or H-5); 7.05 dd, 1 H, *J* = 7.9, 0.9 (H-3 or H-5); 7.39 t, 1 H, *J* = 7.9 (H-4). Maleate, m.p. 98–110 °C (ethyl acetate). For $C_{16}H_{21}ClN_2O_4S$ (372.9) calculated: 51.54% C, 5.68% H, 9.51% Cl, 7.51% N, 8.60% S; found: 51.77% C, 5.21% H, 9.33% Cl, 7.47% N, 8.21 % S.

4-{[(3-Chlorophenyl)sulfanyl]methyl}-1-methylpiperidine (**13c**)

A mixture of 3-chlorobenzenethiol (2.8 g, 19 mmol), 4-chloromethyl-1-methylpiperidine (2.8 g, 19 mmol), potassium carbonate (3.3 g, 3.8 mmol), and dry acetone (50 ml) was refluxed for 10 h, then the mixture was evaporated, the residue treated with water and extracted with chloroform. The extract was washed with water, dried with magnesium sulfate and the residue after evaporation was purified by flash chromatography (silica, CH_2Cl_2–MeOH 9 : 1) to give **13c** as yellowish oil (3 g, 61%). ^{1}H NMR ($CDCl_3$): 1.34 m, 2 H (H-3, H-5); 1.49 m, 1 H (H-4); 1.78 m, 2 H (H-2, H-6); 1.86 s, 2 H (H-3, H-5); 2.25 s, 3 H (CH_3); 2.83 m, 4 H (CH_2, H-2, H-6); 7.13 m, 3 H (H-4′, H-5′, H-6′); 7.26 m, 1 H (H-2′). ^{13}C NMR ($CDCl_3$): 31.63 (C-3, C-5); 34.99 (C-4); 39.75 (CH_2); 46.24 (CH_3); 55.46 (C-2, C-6); 127.88 (C-2′); 125.56, 126.35, 129.72 (C-4′, C-5′, C-6′); 164.55 (C-3′); 139.42 (C-1′). Hydrochloride, m.p. 143–145 °C (ethanol). For $C_{13}H_{19}Cl_2NS$ (297.3) calculated: 53.42% C, 6.55% H, 24.26% Cl, 4.79% N, 10.97% S; found: 53.20% C, 6.59% H, 23.98% Cl, 4.85% N, 10.77% S.

4-{[(3-Chlorophenyl)sulfanyl]methyl}piperidine (**13d**)

Hydrochloride of **13d** (1.7 g, 51%), m.p. 129–132 °C, was prepared from compound **13c** (3.0 g, 12 mmol) by analogous procedure described for the preparation of **12d**. For $C_{12}H_{17}Cl_2NS$ (278.2) calculated: 51.80% C, 6.16% H, 25.48% Cl, 5.03% N, 11.52% S; found: 52.05% C, 6.20% H, 25.11% Cl, 4.90% N, 11.37% S. ^{1}H NMR ($(CD_3)_2SO$): 1.75 bs, 3 H (H-3, H-4, H-5);

2.08 m, 2 H (H-3, H-5); 2.85 m, 4 H, (CH_2, H-2, H-6); 3.51 m, 2 H (H-2, H-6); 7.17 m, 3 H (H-4′, H-5′, H-6′); 7.29 m, 1 H (H-2′); 9.40 bs, 1 H and 9.58 bs, 1 H (NH and HCl).

Biological Evaluation

Hot-plate test. The hot-plate test was used to measure the response latencies according to the method described earlier[20] 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 the tested compound and the results were expressed as prolongation of the licking latencies (%). Compounds were considered to have interesting antinociceptive properties if they produced a significant increase in threshold ($p < 0.05$).

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 the tested compound[21]. Writhings were counted for 20 min, compared with the control and expressed as decrease in the stretching movements (%).

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[5], according to the published procedures[22,23]. Adult male rats were killed by cervical dislocation and decapitation. Their brains were dissected, immediately frozen, and stored at –80 °C until needed. The heart atria were homogenized in a medium consisting of 100 mM NaCl and 20 mM sodium 4-(2-hydroxyethyl)piperazine-1-ethanesulfonate (pH 7.4), and stored at –80 °C. [^{3}H]-8-OH-DPAT (c = 0.25 nmol l^{-1}) and [^{3}H]-5-HT (c = 2.00 nmol l^{-1}) were used for labelling 5-HT_{1A} and 5-HT_{1B} receptors, respectively. The tested compounds were used in 10^{-6} mol l^{-1} 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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