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Original article

Derivatives of 3-cyano-6-phenyl-4-(3′-pyridyl)-pyridine-2(1H)-thione and their neurotropic activity

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Abstract – 3-Cyano-6-phenyl-4-(3′-pyridyl)pyridine-2(1H)-thione, the related 2,2′-bis-pyridyldisulfide, 2-alkylthiopyridines and 2-amino-thieno[2,3-b]pyridines were synthesised and their neurotropic activities were examined. Bispyridyldisulfide exhibited low toxicity (LD_{50} > 5 000 mg/kg, ICR mice, i.p.) and selective antiamesic activity at the doses of 0.05–0.5 mg/kg p.o. This effect was significantly higher than that induced by Piracetam at 50 mg/kg. © Elsevier, Paris

1,2-dihydro-2-thioxopyridine-3-carbonitrile / thieno[2,3-b]pyridine / bispyridyldisulfide / antiamnesic agent

1. Introduction

3-Cyanopyridine-2(1H)-thione, 3-amino-2-carbofunctionally substituted thieno[2,3-b]pyridine and related compounds are found to be very reactive substances for the synthesis of many different heterocyclic systems. They have diverse biological activities.

3-Cyanopyridine-2(1H)-thione and their derivatives, 2-alkylthiopyridines, thienopyridines and isothiazolopyridines exhibited antibacterial [1–5], pesticidal [6], antifungal [3, 7] and acaricidal [3] properties. Pyridine-2(1H)-selenones and 2-alkylthio(seleno)pyridines revealed antiviral activity [8], and pyridothiophenes and their derivatives (pyridothienopyrimidines, pyridothienopyridazines and pyridothienotriazines) have diuretic [2], analgesic [2, 9, 10], anti-inflammatory [2, 10] and anti-allergenic (antianaphylactic) activities [11–20]. Thienopyridines have also been examined as cholesterol biosynthesis inhibitors [21]. Cardiovascular activity of 3-cyanopyridine-2(1H)-thiones was profoundly investigated and vasodilating [22], antihypertensive [22, 23] and cardiotonic [24–28] activities were revealed.

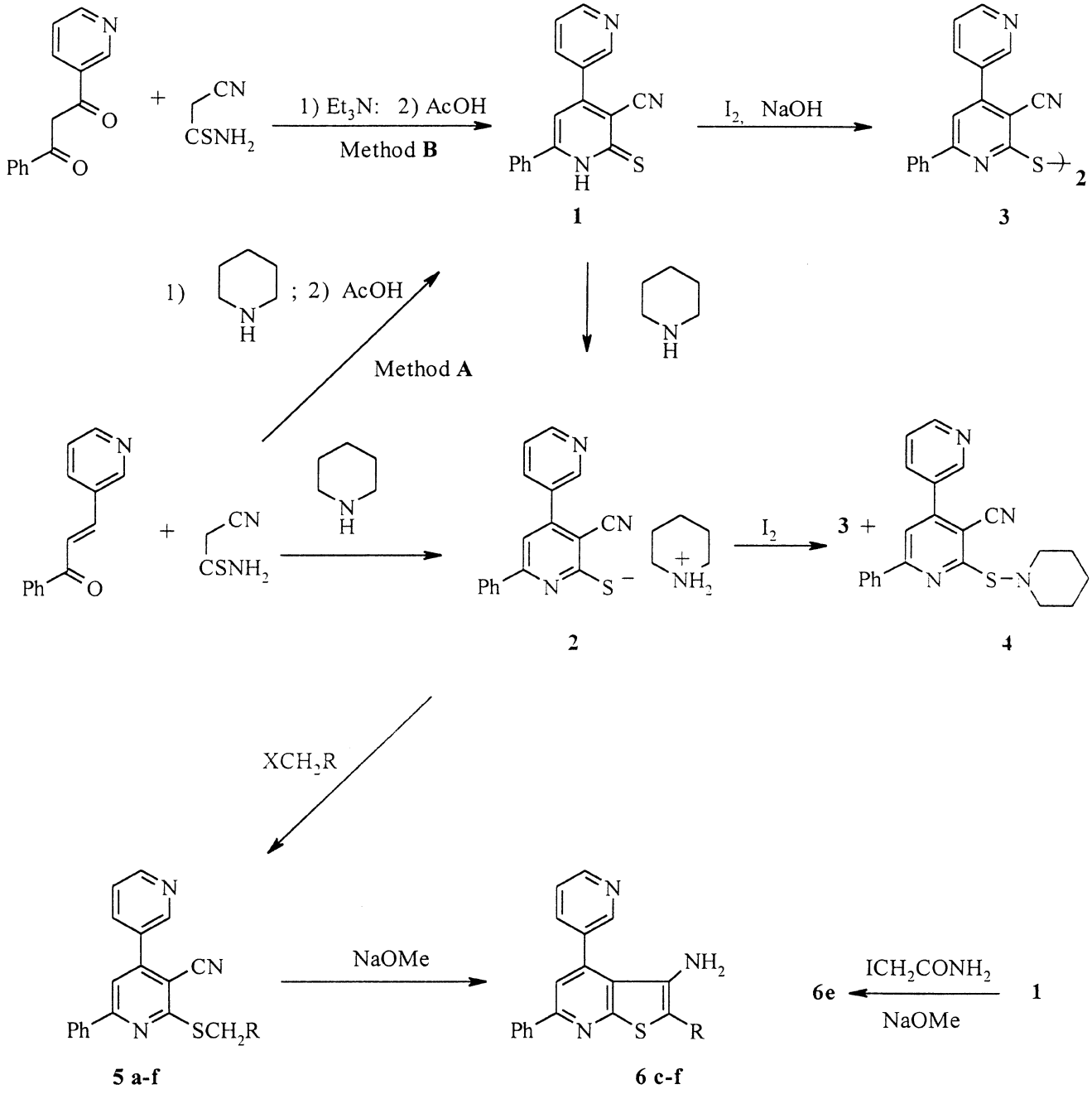
There are only a few reports (preliminary data) of the neurotropic activity of 3-cyano(carbamoyl, carboxy) pyridine-2(1H)-thione related compounds: the corresponding 2-alkylthio derivatives [29, 30] and pyrido[3,2-c]1,3-thiazin-4-ones [31] are central nervous system depressants, and 1-piperazinopropylisothiazolo[5,4-b] pyridines have antipsychotic activity [32].

Our concept was to introduce an additional pyridyl moiety in pyridine-2(1H)-thiones and related compounds (2-alkylthiopyridines, 2,2′-bispyridyldisulfides and 2-aminothieno[2,3-b]-pyridines) and to study these compounds as neurotropic agents. These compounds were examined in routine neuropharmacological tests to characterise their activity spectrum and to reveal the influence of the structure on activity which may indicate further steps in psychotropic drug design.

2. Chemistry

There are two satisfactory methods for the synthesis of 3-cyano-6-phenyl-4-(3′-pyridyl)pyridine-2(1H)-thione **1** *(figure 1)*: A) the Michael reaction of 1-phenyl-3-(3′-pyridyl)-2-propen-1-one and cyanothioacetamide with the subsequent heterocyclisation, dehydration and dehydrogenation to yield **1** (80%), and B) the Knoevenagel reaction of 1-phenyl-3-(3′-pyridyl)-propane-1,3-dione and cyanothioacetamide with the subsequent heterocyclisation to yield **1** (72%). The shortcoming of the second method is a rather complicated synthesis of the 1,3-diketone [33].

* Correspondence and reprints



a R = H, **b** R = Me, **c** R = COOEt, **d** R = CN, **e** R = $CONH_2$, **f** R = COPh; X = Cl, Br, I

Figure 1. Synthesis of 3-cyano-6-phenyl-4-(3′-pyridyl)pyridine-2(1H)-thione **1**.

The treatment of pyridine-2(1H)-thione **1** with piperidine yielded piperidinium thiolate **2** which was an intermediate for further reactions. The oxidation of piperidinium pyridine-2-thiolate **2** with iodine gave 2,2′-bispyridyldisulfide **3** (70%) and small amount of 2-piperidylthiopyridine **4** (8%) as by-product. A slightly higher yield of **3** (75%) was obtained by the oxidation of thione **1** with iodine in ethanol solution in the presence of sodium hydroxide.

2-Alkylthio-3-cyanopyridines **5** were obtained in high yields by alkylation of piperidinium thiolate **2** with the corresponding alkyl halides. The subsequent Thorpe cyclisation of **5c–f** in the presence of sodium methylate gave 3-aminothieno[2,3-b]pyridines **6c–f** in high yields. Compound **6e** was also obtained by heating thione **1** with iodoacetamide in the presence of an excess of sodium methylate without isolation of **5e**, but in this case the yield was appreciably lower. The structures of synthesised compounds were confirmed by elemental analysis and IR and ^{1}H NMR spectra.

3. Pharmacological results and discussion

As it can be seen from *table I*, all tested compounds showed low toxicity in mice (ED_{50} > 1 000 mg/kg i.p.). A general pharmacological examination (i.p., in mice) of the influence of all compounds on coordination and body temperature revealed two groups of compounds. Group 1 [2-alkylthiopyridines **5** (with the exception of **5a**) and thienopyridines **6c**, **6e** and **6f**] showed very weak (average ED_{50} doses > 100–500 mg/kg) depressing activity on muscle tone and coordination (rota rod and traction tests) and weak hypothermic effects *(table I)*. At 50 mg/kg these compounds possessed moderate anticonvulsant activity, since pentylenetetrazol (PTZ) dose had to be increased to cause the PTZ-induced clonic and tonic seizures *(table II)*. The anticonvulsant activity of these compounds (with the exception of **6f**) was slightly less than Diazepam (5 mg/kg). Group 2 (pyridine-2(1H)-thione **1**, the corresponding 2,2 -bispyridyldisulfide **3** and 2-methylthiopyridine **5a**) lacked anticonvulsant activity in comparison with Group 1, with exception of **3**, which showed slight anticlonic action at 5 mg/kg *(table II)*. Moreover, they gave a sharp rise in hypothermic and myorelaxing activities (in comparison to compounds of group 1) at doses of about 3–10 mg/kg *(table I)*. The myorelaxing and hypothermic activities indicate that the compounds **1**, **3** and **5a** may possess tranquillising features, since their activities were comparable with Diazepam (a well-known tranquilliser) effects *(table I)*. The data obtained in the studies allowed us to select the compounds **1**, **3** and **5a** for more detailed examination to assess their interaction with cholin-, dopamin- and serotoninergic processes. The compounds were administered orally at 5 and 50 mg/kg (in separate experiments the dose range was broadened). *Table II* shows that the compounds **1** and **3** at the highest tested dose (50 mg/kg) exerted a slight anticholinergic effect as they prolonged time prior to arecoline-induced tremors. However, nicotine tremorogenic action was not antagonised by the tested compounds *(table II)*. When amphetamine, a dopamine agonist, was used as a test-drug in stereotypy tests, compounds **3** and **5a** slightly reduced the duration of stereotypic behaviour caused by amphetamine only at 5 mg/kg *(figure 2)*; this effect was comparable to that of Piracetam (100 mg/kg). No effect was observed at lower and higher doses. These data indicate weak and dose-independent antidopamine action for compounds **3** and **5a**. An antagonistic influence on serotoninergic processes (reduction of the number of head shakes induced by 5-hydroxytryptophane (5-HTP), a serotonin precursor), was found only for compound **3** at the highest dose tested (50 mg/kg); this effect was comparable to that caused by Piracetam at 50 mg/kg *(figure 3)*. Surprisingly, in the memory test (passive avoidance response behaviour, electroconvulsive shock-induced amnesia), only compound **3** showed high antiamnesic activity in mice *(figure 4)*. The effects were observed at 0.05 mg/kg and maintained at the plateau level at 0.5–25 mg/kg doses, and they were significantly higher than the effects caused by Piracetam (a well-known nootropic drug) at 50 mg/kg. Other tested compounds **1** and **5a** were inactive. In the open field test *(table III)* the compound **3** in doses which exerted the high antiamnesic activity, did not change the rats locomotion (with the exception of dose 5 mg/kg which caused a slight reduction in vertical activity). Compounds **1** and **5a** also did not affect locomotor responses at doses of 5 and 25 mg/kg (with the exception of compound **1**, which slightly increased vertical activity at 25 mg/kg). It is worth noting that the antiamnesic activity of compound **3** occurred at much smaller doses (already at 0.05 mg/kg p.o.) than those which affected muscle tone and body temperature (on average, 5–10 mg/kg i.p.). So, our preliminary pharmacological data have revealed the selective antiamnesic activity of compound **3**; however, the question of which mechanisms could be taken as crucial for this activity remains open. A slight anticonvulsant activity (PTZ-test) which can be related to GABAergic processes, and negligible antidopamine (at 5 mg/kg, but not 50 mg/kg), antiserotoninergic and anticholinergic (both 50 mg/kg) activities, as well as Diazepine-like myorelaxing and hypothermic effects, may indicate that these properties can appear at

Table I. Neurotropic activity and toxicity of 3-cyano-6-phenyl-4-(3′pyridyl)pyridine-2(1H)-thione derivatives administered i.p. in ICR mice (n = 6 per group).

Compounds	Activity (ED50, mg/kg)			Toxicity (LD50, mg/kg)
	Rota rod	Traction	Hypothermia	
1	5.6* (3.4-8.1)	6.5* (4.4-8.9)	3.7 (1.1-7.7)	> 5000
3	9.2* (3.3-17.5)	11.6* (4.0-22.0)	4.8* (2.5-7.7)	> 5000
5a	3.0 (1.6-4.9)	3.0 (1.6-4.8)	4.5* (2.6-6.4)	> 10000
5b	650* (438-886)	224* (144-285)	547* (200-1027)	> 2000
5c	> 500*	325* (214-455)	410* (268-552)	> 1000
5d	274* (99-524)	258* (168-357)	224* (144-298)	> 1000
5e	410* (268-552)	258* (168-357)	300* (158-489)	> 5000
5f	564* (342-814)	282* (183-372)	325* (172-502)	> 5000
6c	410* (268-552)	325* (219-455)	282* (159-419)	> 8000
6e	346* (120-662)	258* (168-357)	282* (159-419)	> 5000
6f	325* (219-455)	129* (61-202)	178* (136-230)	> 4000
Diazepam	2.6 (1.5-4.4)	3.3 (1.4-7.6)	1.6 (0.6-4.0)	-

* $P < 0.05$ vs. Diazepam.

higher doses than those required for antiamnesic action and cannot be considered as essential for antiamnesic action.

Taking into account the effectiveness and low toxicity of the compound **3** (> 5 000 mg/kg, i.p.), it may be considered as a leading compound with an eventual nootropic repertoire in the pyridinethioether series tested, and can be selected for the further studies to clarify its mechanisms of action. One may suggest that the structural peculiarities (two bipyridyl moieties connected via a

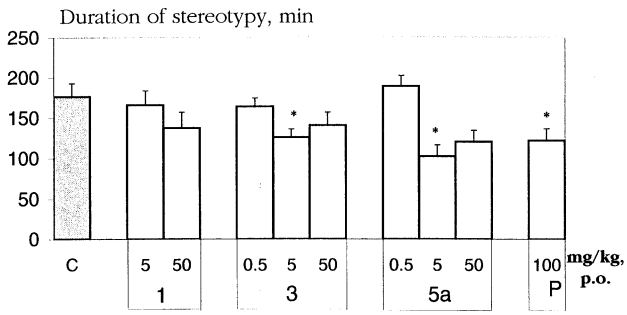


Figure 2. Influence of the compounds **1, 3, 5a** and Piracetam (P) on amphetamine-induced stereotypy in Wistar rats (n = 6). * $P < 0.05$ vs. Amphetamine control (C).

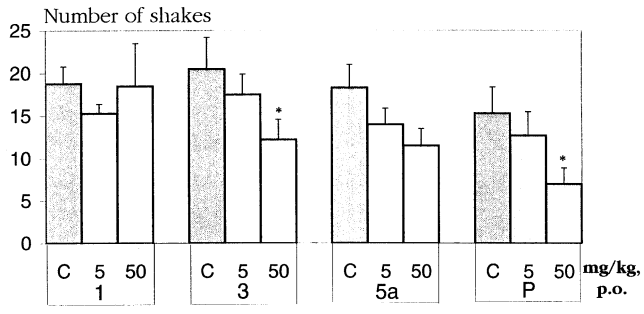


Figure 3. Influence of the compounds **1**, **3**, **5a** and Piracetam (P) on the 5-HTP-induced head shakes in BALB/c mice (n = 6). * $P < 0.05$ vs. 5-HTP control (C).

Table II. Influence of the compounds[a] and Diazepam (i.p.) on pentylenetetrazole (PTZ)-induced convulsions and nicotine (N)-, and arecoline (A)-induced tremor in BALB/c mice (n = 6 per group).

Compounds	Dose (mg/kg)	PTZ (mean ± SEM, mg/kg) seizures Clonic	Tonic	A (mean ± SEM), min Tremor	N (mean ± SEM, mg/kg) Tremor
Control (saline)	0	31.4 ± 0.6**	86.5 ± 6.2**	11.7 ± 1.1	1.3 ± 0.1
1	5	33.8 ± 3.0**	108.9 ± 8.6**	17.5 ± 1.4	1.4 ± 0.1
	50	31.4 ± 0.6**	77.3 ± 10.2**	19.2 ± 1.2***	1.8 ± 0.3
3	5	40.0 ± 3.8*	107.3 ± 7.1**	11.3 ± 0.6**	1.5 ± 0.1
	50	37.8 ± 0.4**	102.1 ± 11.0**	19.5 ± 2.0***	1.4 ± 0.2
5a	5	34.3 ± 4.1**	98.5 ± 12.4**	7.6 ± 3.1**	1.3 ± 0.2
	50	32.3 ± 2.3**	113.9 ± 16.1**	10.7 ± 2.1	1.4 ± 0.1
5b	50	39.3 ± 2.6*	133.3 ± 15.5**	-	-
5c	50	45.2 ± 1.9*	155.0 ± 24.2***	-	-
5d	50	43.6 ± 3.2*	166.7 ± 17.6***	-	-
5e	50	39.3 ± 3.3*	106.8 ± 7.7***	-	-
5f	50	50.0 ± 3.5*	106.8 ± 6.1***	-	-
6c	50	43.3 ± 1.3*	102.6 ± 6.2**	-	-
6e	50	42.0 ± 2.2*	177.8 ± 9.8***	-	-
6f	50	46.2 ± 1.6*	207.6 ± 19.8*	-	-
Diazepam	5	43.8 ± 2.6*	213 ± 10.4*	14.5 ± 1.3	1.2 ± 0.3

* $P < 0.05$ vs. control. ** $P < 0.05$ vs. Diazepam.
[a]In PTZ-test, the compounds are administered i.p.; in (N)- and (A)-induced tremor they are administered p.o.

disulfide bridge) may play an important role in a series of substituted pyridine-2(1H)-thione or its pyridyl-2-thiol type derivatives for enhancing their neurotropic activities, and particularly for inducing the antiamnesic properties.

4. Experimental protocols

4.1. Chemistry

Melting points were determined on a Boetius apparatus and were uncorrected. Elemental analyses (C, H, N, S) were within ± 0.4% of the theoretical values. IR spectra were recorded on a Perkin-Elmer 580 B spectrometer (in Nujol) and peak positions ν_{max} are expressed as cm^{-1}. ^1H NMR spectra were recorded on a Bruker WH-90 spectrometer and chemical shifts are reported as δ values (ppm) relative to tetramethylsilane.

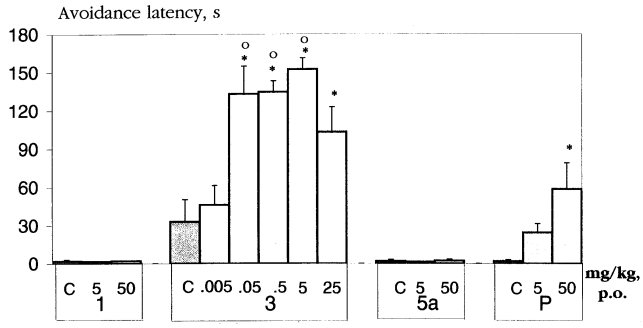


Figure 4. Antiamnesic activity of the compounds **1, 3, 5a** and Piracetam (P) in ECS-amnesia tests in BALB/c mice (n = 6). * $P < 0.05$ vs. control (C), saline; o $P < 0.05$ vs. Piracetam

4.1.1. 3-Cyano-6-phenyl-4-(3′-pyridyl)pyridine-2(1H)-thione **1**

Method A: A mixture of 1-phenyl-3-(3′-pyridyl)-2-propen-1-one [33] (4.19 g; 0.02 mol), cyanothioacetamide (2.0 g; 0.02 mol) and piperidine (0.85 g; 0.01 mol) in 25 mL of ethanol was refluxed for 30 min. Then 5 mL of acetic acid were added and the reaction mixture was stirred for 2 h at room temperature and cooled to 0 °C. The precipitated dark yellow crystals were filtered and washed with 20 mL of cold ethanol and 20 mL of water to yield **1** (4.63 g, 80%); m.p. 232–234 °C (from nitromethane); IR ν cm^{-1}: 3 160 (NH), 2 220 (CN); ^1H NMR (DMSO-d_6) δ: 7.26 (1H, s, 5-H); 7.4–8.8 (9H, m, C_6H_5 and C_5H_4N); 14.31 (1H, br.s, NH). Anal. $C_{17}H_{11}N_3S$ (C, H, N, S).

Method B: A mixture of 1-phenyl-3-(3′-pyridyl) propane-1,3-dione [34] (2.3 g; 0.01 mol), cyanothioacetamide (1.0 g, 0.01 mol) and triethylamine (1.0 g; 0.01 mol) in 20 mL ethanol was refluxed for 1 h. After

Table III. Influence of the compounds **1, 3, 5a** (p.o.) and Piracetam (p.o.), on locomotion behaviour in male Wistar rats in ″open field″ test (n = 6 per group**).**

		Number of locomotor responses (mean ± SEM)		
Compounds	Dose mg/kg	Horizontal activity	Vertical activity	Exploratory activity
Control (saline)	0	29.3 ± 3.7	6.8 ± 1.0	1.7 ± 0.6
1	5	32.5 ± 6.0	5.7 ± 0.5**	1.3 ± 1.8
	25	36.0 ± 4.8	13.5 ± 2.7*	1.2 ± 0.8
3	0.005	36.2 ± 3.2	5.6 ± 1.1	2.8 ± 0.8
	0.05	29.0 ± 4.2	3.2 ± 1.4	2.6 ± 0.5
	0.5	34.2 ± 4.0	3.5 ± 2.0	3.5 ± 1.0
	5	35.0 ± 8.1	3.6 ± 0.4***	3.6 ± 0.7
5a	5	34.3 ± 7.2	6.0 ± 2.3	1.1 ± 1.3
	25	33.3 ± 4.5	4.5 ± 1.8**	1.2 ± 1.4
Piracetam	5	27.4 ± 5.4	9.5 ± 1.8	2.3 ± 1.8
	25	26.3 ± 6.2	10.5 ± 1.9	1.7 ± 2.1

* $P < 0.05$ vs. control. ** $P < 0.05$ vs. Piracetam (corresponding dose).

that, 2 mL of acetic acid were added and then the reaction mixture was treated as in method A to yield **1** (2.08 g, 72%).

4.1.2. Piperidinium 3-cyano-6-phenyl-4-(3′-pyridyl) pyridine-2-thiolate **2**

Similar to **1** (method A), after refluxing the reaction mixture was stirred at room temperature. For 10 d the precipitated crystals were filtered, combined and washed with cold ethanol and dry ether to yield **2** (68%); m.p. 240–241 °C (from ethanol); IR ν cm^{-1}: 2 530, 2 448 (+ NH_2); 2 216 (CN). ^{1}H NMR (DMSO-d_6) δ: 1.60 [6H, m, $(CH_2)_3$]; 3.02 [4H, m, $N(CH_2)_2$]; 7.08 (1H, s, 5-H); 7.4–8.8 (9H, m, C_6H_5 and C_5H_4N). Anal. $C_{22}H_{22}N_4S$ (C, H, N, S).

4.1.3. 2,2′-Bis[3-cyano-6-phenyl-4-(3′-pyridyl)] disulfide **3**

A solution of 0.5 N ethanolic iodine (4 mL; 0.01 mol) was added under vigorous stirring to a suspension of thione **1** (2.9 g; 0.01 mol) and sodium hydroxide (0.48 g; 0.012 mol) in 70 mL of ethanol and stirred for 1 h. The reaction mixture was cooled to 0 °C, the precipitate was filtered and washed with 20 mL of ethanol and 50 mL of water. After drying in the oven, the crude product was recrystallised from chloroform to yield **3** (2.16 g, 75%); m.p. 239-241 °C; IR ν cm^{-1}: 2 220 (CN); ^{1}H NMR (DMSO-d_6) δ: 8.24 (1H, s, 5-H); 7.2–9.0 (9H, m, C_6H_5 and C_5H_4N). Anal. $C_{34}H_{20}N_6S_2$ (C, H, N, S).

4.1.4. 3-Cyano-6-phenyl-2-piperidylthio-4-(3′-pyridyl) pyridine **4**

A solution of 0.5 N ethanolic iodine (4 mL; 0.01 mol) was added under vigorous stirring to a solution of salt **2** (3.75 g, 0.01 mol) in 50 mL of ethanol and stirred for 1 h. The reaction mixture was cooled to 0 °C, the precipitate was filtered and washed with 10 mL of ethanol and 50 mL of water. The crude product (**3** and **4**) was recrystallised from the chloroform-ethanol (2:1) mixture. After separation of **3** (1.73 g; 60%), the filtrate was evaporated to approx. 1/5 of its initial volume. The precipitate was filtered and washed with 10 mL of hot ethanol. After separation of the additional **3** (0.29 g, 10%), the second filtrate was concentrated to approx. 1/3 of its initial volume and compound **4** (0.31 g; 8%) was filtered. M.p. 195–197 °C (from ethanol); IR ν cm^{-1}: 2 216 (CN); ^{1}H NMR ($CDCl_3$) δ: 1.62 [6H, m, $(CH_2)_3$]; 3.64 [4H, m, $N(CH_2)_2$]; 7.50 (1H, s, 5-H); 7.8–8.9 (9H, m, C_6H_5 and C_5H_4N). Anal. $C_{22}H_{20}N_4S$ (C, H, N, S).

4.1.5. General method for the synthesis of 2-alkylthio-3-cyano-6-phenyl-4-(3′-pyridyl)pyridines **5**

A mixture of thiolate **2** (3.75 g; 0.01 mol) and the corresponding R-substituted alkyl halide (0.011–0.04 mol) in 30–40 mL of ethanol was refluxed for 5–10 min and filtered. The reaction mixture was cooled to 0 °C, the precipitated crystals were filtered, washed with ethanol (5–10 mL) and water (20–30 mL) and recrystallised from ethanol.

4.1.6. 3-Cyano-2-methylthio-6-phenyl-4-(3′-pyridyl) pyridine **5a**

From thiolate **2** and methyl iodide (1:4). M.p. 202–204 °C; yield: 94%; IR ν cm^{-1}: 2 220 (CN); ^{1}H NMR ($CDCl_3$) δ: 2.80 (3H, s, SCH_3); 7.57 (1H, s, 5-H); 7.5–8.9 (9H, m, C_6H_5 and C_5H_4N). Anal. $C_{18}H_{13}N_3S$ (C, H, N, S).

4.1.7. 3-Cyano-2-ethylthio-6-phenyl-4-(3′-pyridyl) pyridine **5b**

From thiolate **2** and ethyl bromide (1:4). M.p. 137–139 °C; yield: 82%; IR ν cm^{-1}: 2 216 (CN); ^1H NMR ($CDCl_3$) δ: 1.48 (3H, t, CH_2CH_3); 3.43 (2H, q, CH_2CH_3); 7.48 (1H, s, 5-H); 7.4–8.8 (9H, m, C_6H_5 and C_5H_4N). Anal. $C_{19}H_{15}N_3S$ (C, H, N, S).

4.1.8. 3-Cyano-2-ethoxycarbonylmethylthio-6-phenyl-4-(3′-pyridyl)pyridine **5c**

From thiolate **2** and ethyl bromoacetate (1:1.2). M.p. 164–165 °C; yield 55%; IR ν cm^{-1}: 1 736 (CO); 2 218(CN); ^1H NMR ($CDCl_3$) δ: 1.25 (3H, t, CH_2CH_3); 4.21 (2H, q, CH_2CH_3); 4.13 (2H, s, SCH_2); 7.57 (1H, s, 5-H); 7.4–8.9 (9H, m, C_6H_5 and C_5H_4N). Anal. $C_{21}H_{17}N_3O_2S$ (C, H, N, S).

4.1.9. 3-Cyano-2-cyanomethylthio-6-phenyl-4-(3′-pyridyl)pyridine **5d**

From thiolate **2** and chloroacetonitrile (1:1.2). M.p. 222–224 °C; yield 95%; IR ν cm^{-1}: 2 214 (3-CN); 2 248 (CH_2CN); ^1H NMR (DMSO-d_6) δ: 4.55 (2H, s, SCH_2); 8.14 (1H, s, 5-H); 7.5–9.0 (9H, m, C_6H_5 and C_5H_4N). Anal. $C_{19}H_{12}N_4S$ (C, H, N, S).

4.1.10. 2-Carbamoylmethylthio-3-cyano-6-phenyl-4-(3′-pyridyl)pyridine **5e**

From thiolate **2** and iodoacetamide (1:1.1). M.p. 245–247 °C; yield 96%; IR ν cm^{-1}: 1 654, 1 697 (CO); 2 216 (CN); 3 180 (NH_2); ^1H NMR (DMSO-d_6) δ: 4.08 (2H, s, SCH_2); 7.28 and 7.50 (2H, s and s, $CONH_2$); 8.00 (1H, s, 5-H); 7.5–9.0 (9H, m, C_6H_5 and C_5H_4N). Anal. $C_{19}H_{14}N_4OS$ (C, H, N, S).

4.1.11. 2-Benzoylmethylthio-3-cyano-6-phenyl-4-(3′-pyridyl)pyridine **5f**

From thiolate **2** and 2-bromoacetophenone (1:1.1). M.p. 201–204 °C; yield 96%; IR ν cm^{-1}: 1 692 (CO); 2 222 (CN); ^1H NMR ($CDCl_3$) δ: 4.81 (2H, s, SCH_2); 7.47 (1H, s, 5-H); 7.2–8.8 (14H, m, $2C_6H_5$ and C_5H_4N). Anal. $C_{15}H_{12}N_3OS$ (C, H, N, S).

4.1.12. General method for the synthesis of 3-amino-6-phenyl-4-(3′-pyridyl)thieno-[2,3-b]pyridines **6**

A 5 mL solution of 1 N sodium methylate in methanol was added to a mixture of 0.05 mol of the corresponding 2-alkylthio-3-cyanopyridine **5 c–f** in 30 mL of ethanol. The reaction mixture was refluxed for 5 min and cooled to 0 °C. The precipitated crystals were filtered and recrystallised from ethanol-chloroform (1:1) mixture.

4.1.13. 3-Amino-2-ethoxycarbonyl-6-phenyl-4-(3′-pyridyl)-thieno[2,3-b]-pyridine **6c**

M.p. 235–236 °C; yield 93%; IR ν cm^{-1}: 3 494, 3 344 (NH_2); 1 680 (CO); ^1H NMR ($CDCl_3$) δ: 1.38 (3H, t, CH_2CH_3); 4.36 (2H, q, CH_2CH_3); 5.52 (2H, s, 3-NH_2), 7.50 (1H, s, 5-H); 7.4–8.8 (9H, m, C_6H_5 and C_5H_4N). Anal. $C_{21}H_{17}N_3O_2S$ (C, H, N, S).

4.1.14. 3-Amino-2-cyano-6-phenyl-4-(3′-pyridyl)thieno [2,3-b]pyridine **6d**

M.p. 238–240 °C; yield 90%; IR ν cm^{-1}: 3 284 (NH_2); 2 192 (CN); ^1H NMR (DMSO-d_6) δ: 5.68 (2H, s, 3-NH_2); 7.96 (1H, s, 5-H); 7.5–8.8 (9H, m, C_6H_5 and C_5H_4N). Anal. $C_{19}H_{12}N_4S$ (C, H, N, S).

4.1.15. 3-Amino-2-carbamoyl-6-phenyl-4-(3′-pyridyl) thieno[2,3-b]pyridine **6e**

Method A (general method): M.p. 245–246 °C; yield 91%; IR ν cm^{-1}: 3 444, 3 324, 3 152 (NH_2); 1 660 (CO); ^1H NMR (DMSO-d_6) δ: 5.90 (2H, s, 3-NH_2); 7.30 (2H, s, $CONH_2$); 7.85 (1H, s, 5-H); 7.5–8.8 (9H, m, C_6H_5 and C_5H_4N). Anal. $C_{19}H_{14}N_4OS$ (C, H, N, S).

Method B: A 20 mL solution of 1 N sodium methylate in methanol was added to a mixture of thione **1** (2.90 g; 0.01 mol) and iodoacetamide (2.04 g, 0.011 mol) in 20 mL of methanol, then the reaction mixture was refluxed for 30 min and cooled to 0 °C. The precipitated crystals were filtered and recrystallised from chloroform ethanol (1:1) to yield **6e** (2.40 g, 69%); m.p. 245–246 °C.

4.1.16. 3-Amino-2-benzoyl-6-phenyl-4-(3′-pyridyl)thieno [2,3-b]pyridine **6f**

General method: M.p. 190–191 °C; yield: 91%; IR ν cm^{-1}: 3 484, 3 300 (NH_2); 1 600 (CO); ^1H NMR ($CDCl_3$) δ: 6.65 (2H, s, 3-NH_2); 7.54 (1H, s, 5-H); 7.4–8.8 (14H, m, C_6H_5 and C_5H_4N). Anal. $C_{15}H_{12}N_3OS$ (C, H, N, S).

4.2. Biological evaluation

The experiments were performed in winter/spring season on BALB/c and ICR mice of both sexes weighing 18–24 g, and on Wistar rats weighing 200 ± 20 g. Animals used were from the breeding colony of the GRINDEX, Riga, Latvia. The animals were kept in plastic cages which were equipped with drinking bowls and bunkers-feeder. The animals had free access to water and food (Altromin Standard Diets, Germany). Ambient temperature in the animal colony and laboratory during experiments was maintained at 21 ± 2 °C, air humidity 50–70%, lighting from 7 a.m. to 7 p.m. The compounds **1**, **3**, **5a-5f**, **6c**, **6e**, **6f** were prepared as aqueous suspensions (by using 1–2 drops of 0.6% Tween-80) and injected intraperitoneally (i.p.) 1 h prior to testing in ICR

mice for general examination of the pharmacological profile of these compounds. In more detailed studies, the compounds **1**, **3**, and **5a** were administered orally (p.o.) as aqueous suspensions in BALB/c mice or Wistar rats 1 h prior to testing performance. Control animals received a corresponding volume of solvent. Piracetam (p.o.) and Diazepam (i.p.) were used as reference drugs, and they were administered according to the same schedule as the tested compounds. For general pharmacological examination of the compounds, the tests according to the Pharmacological Screening Program PANLABS, Inc, 1973, were used [35]. Brief descriptions of the procedures are given below. Some specific tests (e.g. antiamnesic) were performed according to methods described elsewhere and are explained in detail.

4.2.1. Coordination and muscle tone

Coordination and muscle tone were evaluated in ICR mice by using routine rota rod and traction tests. The compounds were administered i.p. 1 h prior to testing. Animals were placed on rota rod (Ugo Basile apparatus, Model Nr. 7600, Italy) and the number of animals which had fallen from the rotating cylinder (diameter 3 cm, rotating speed 8 rpm) during 2 min was registered. In the traction test, animals (compounds administered i.p.1 h prior to testing) were forced to hang by their forelegs on a metal wire (diameter 2 mm). The number of animals unable to hang for 30 s was registered.

4.2.2. Body temperature

Body temperature was measured by electrothermometer in ICR mice rectum before the experiment and 1 h after i.p. administration of each compound. The number of animals with a drop in temperature of 3 °C or more was considered as a hypothermic effect. Statistical evaluation of the results obtained in coordination and temperature tests was carried out by calculation of mean effective doses (ED_{50}) and confidence interval according to Litchfield and Wilcoxon.

4.2.3. Acute toxicity

Acute toxicity in ICR mice was determined at doses of 1 000–10 000 mg/kg i.p. The animals were observed for 3 d.

4.2.4. Anticonvulsant activity (pentylenetetrazol (PTZ)-titration test)

Anticonvulsant activity (pentylenetetrazol (PTZ)-titration test) was evaluated in BALB/c mice. The compounds **1**, **3**, and **5a** were administered in doses of 5 and 50 mg/kg, compounds **5b**–**5f** and **6c**, **6e**, **6f** in doses of 50 mg/kg. Diazepam (5 mg/kg, i.p.) was used as a reference drug. The PTZ dose (1% solution, i.v.) required for the inducing clonic and tonic seizures in the compound-treated and non-treated mice (PTZ control) was measured. The compounds were administered i.p. 1 h prior to the PTZ infusion.

4.2.5. Nicotine tremor test

The i.v. titration was carried out with nicotine base (0.01% solution) and the doses required for inducing tremor were registered in treated and non-treated (nicotine control) mice. The compounds were administered p.o. 1 h prior to nicotine infusion.

4.2.6. Arecoline-tremor test

Arecoline-tremor tests were performed by subcutaneous (s.c.) administration of arecoline, 25 mg/kg. The compounds were administered p.o. 1 h prior to arecoline injection. The time (min) before the arecoline-induced tremors began in the compound-treated and non-treated (arecoline control) mice was registered. The data obtained in the tests (4–6) were expressed as the mean values ± SEM and were statistically analysed by Student's *t*-test at $P < 0.05$.

4.2.7. Amphetamine stereotypy

Amphetamine stereotypy in Wistar rats was induced by administration of D-amphetamine in a dose of 10 mg/kg, s.c., 30 min following compound p.o. administration. Compounds **1**, **3** and **5a** were administered at 0.5, 5 and 50 mg/kg p.o. (with the exception of **1** which was administered at 5 and 50 mg/kg). The duration (min) of stereotypic behaviour was registered 1 h after the compound administration and compared to the data obtained in amphetamine control rats (without compound-treatment). Piracetam (100 mg/kg) served as reference drug. The mean values in doses ± SEM were calculated and statistical significance was evaluated vs. amphetamine control at $P < 0.05$.

4.2.8. 5-HTP-induced head shake test

5-HTP-induced head shake tests were performed in BALB/c mice by i.p. administration of 5-HTP at a dose of 300 mg/kg. Compounds **1**, **3** and **5a** were administered at 5 and 50 mg/kg, p.o. 1 h prior to 5-HTP administration. The number of head shake incidences was registered for 1 min at 10, 20, 30 and 40 min after 5-HTP injection. Total number of head shakes (4 min) was calculated. A statistical analysis of the treated and non-treated (5-HTP control) animal groups was the same as in amphetamine test.

4.2.9. Antiamnesic activity

Antiamnesic activity was studied in BALB/c mice in passive avoidance response (PAR) tests according to De Wied [36]. Amnesia was induced by electroconvulsive shock (ECS). The following method was used. PAR training was started by placing the mice individually into the lighted side of the two-compartment (light-dark) PAR apparatus (Model Nr. 7530, Ugo Basile, Italy). Following 10 min accommodation in the light compartment, the door was opened to provide access to the dark compartment. Once a mouse moved into the dark compartment (the first entry latency was measured), the door was closed completely. Mice not entering the dark compartment within 90 s were removed from experiment. After 5 s an inescapable foot-shock (1.0 mA, 5s) was applied immediately through the steel grid floor of the dark compartment. Then the mouse was removed from dark compartment and electroshocked (50 mA, 0.2 s) through corneal electrodes and returned to its home cage. The latency to enter the dark compartment after 24 hrs followed the foot-shock trial (the retention test or the second entry). The latter procedure was performed in the same manner as previously (according to the experimental schedule), with the exception that the foot-shock was not applied. An access to the dark compartment was provided for 3 min. The difference between latencies of the second and the first entries in the ECS-shocked mice served as a parameter showing the antiamnesic activity. Piracetam at 5 and 50 mg/kg was used as a reference. The mean values ± SEM were statistically evaluated by Student's *t*-test using $P < 0.05$ as the difference criterion (vs. saline and Piracetam controls).

4.2.10. Open field test

Open field tests in BALB/c mice were estimated 1 h after the compounds **1**, **3** and **5a** p.o. were administered (saline for the control group) as described earlier [37]. The animals were placed into the brightly illuminated (60 W bulb, 1 m above the centre of the area) black-painted regular octagonal-shaped (diagonal length of 36 cm) wooden box. The black floor was divided into eight equal triangle-shaped sections. The animals were allowed 1 min of habituation to the experimental conditions in order to minimise interference with additional stress factors. Subsequently, horizontal activity (passage of horizontal lines with all four paws), vertical activity (the number of rearings) and exploratory activity (hole inspection in the vertical walls) were registered as a number of corresponding locomotor activities. The results were expressed as means ± SEM, and a probability level of $P < 0.05$ was accepted as statistically significant (vs. saline control values, Student's *t*-test).

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