Department of Chemistry of Drugs[1], Wrocław Medical University, and Department of Pharmacology[2], Medical Academy, Lublin, Poland

Synthesis and preliminary screening of derivatives of 2-(4-arylpiperazin-1-ylalkyl)-3-oxoisothiazolo[5,4-*b*]pyridines as CNS and antimycobacterial agents

W. Malinka[1], M. Sieklucka-Dziuba[2], G. Rajtar[2], W. Zgodziński[2] and Z. Kleinrok[2]

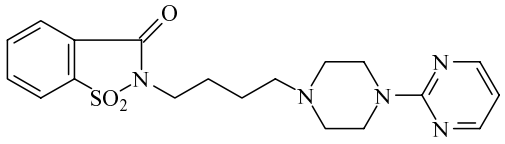
We have synthesized several new isothiazolopyridines possessing a side chain at the isothiazole ring typical, among others, for trazodone or NAN-195. Representatives of the novel isothiazolopyridines were examined for acute toxicity and in several commonly used CNS tests in mice and for arterial blood pressure in rats. Three of the five compounds tested showed significant analgesic activity. The most active compound (**3b**) exhibited analgesic action in the "writhing" test in a dose 1/1280 of LD_{50} (LD_{50} = 1135.5 mg/kg) after administration i.p. to mice. Additionally, the compounds described here and related isothiazolopyridines obtained previously were evaluated against *Mycobacterium tuberculosis* $H_{37}Rv$ at 12.5 μg/ml in *in vitro* assays. Seven of the nineteen compounds tested showed 100% inhibition of that mycobacterium.

1. Introduction

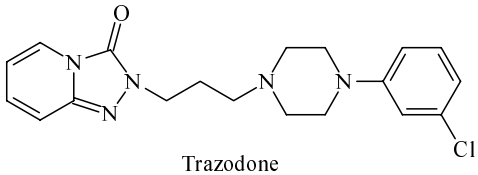
We have published the synthesis of a series of *N*-[4-phenyl(heteroaryl)piperazin-1-ylalkyl]-3-oxoisothiazolopyridines of the general formula **I** and we examined the role of the central alkanyl chain length, the introduction at the central alkanyl chain of an ether oxygen atom or a hydroxyl group, and variation of the 4-substitution (phenyl, heteroaryl) of the piperazine ring [1–4].

The most pharmacologically interesting compound within the series of isothiazolopyridines **I** was 2*H*-4,6-dimethyl-2-[(4-phenylpiperazin-1-yl)methyl]-3-oxo-2,3-dihydroisothiazolo[5,4-*b*]pyridine (**A**). This compound showed anorectic activity in a dose of 2 mg/kg i.p. and of 8.5 mg/kg after oral administration to rats [4]. It is also noteworthy that the synthesized compound **B**, a 4,6-dimethyl-7-azaanalogue of ipsapirone, a non-benzodiazepine anxiolytic agent [5], lacks biological activity [3]. To continue our systematic study within the group of isothiazolopyridines bearing the side chain of the arylpiperazinylalkyl type, we obtained a series of compounds **3**, **4** (Table 1) related to **I** for pharmacological screening in animal models.

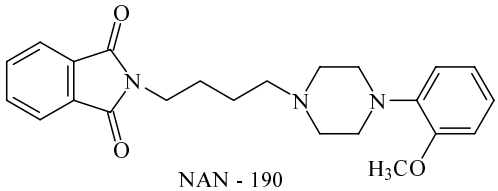
Compounds **3b**, **4b** and **3d**, **4e** were characterized by the presence of the side chain which represents the partial structure of trazodone, a non-tricyclic antidepressive agent [5], and NAN-190, a well recognized antagonist of postsynaptic receptors 5-HT_{1A} [6]. Additionally, an analogue of



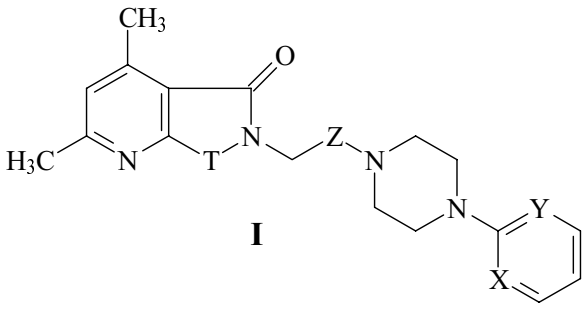
Ipsapirone



Trazodone



NAN - 190



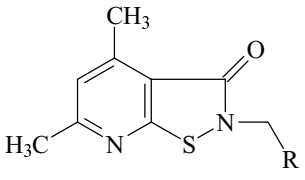
I

$Z = (CH_2)_{0–3}$, $CH_2OCH_2CH_2$, $CH(OH)CH_2$;

X, Y = CH or N; T = S, SO_2

A : T = S; X,Y = CH; $Z = (CH_2)_0$

B : T = SO_2, X,Y = N; $Z = (CH_2)_3$



R

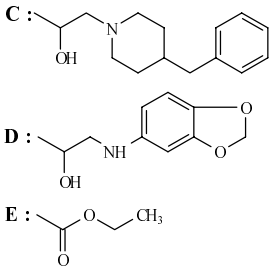
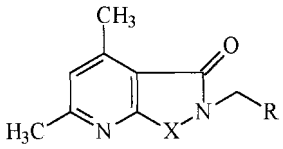
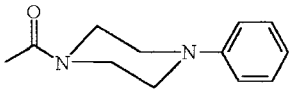
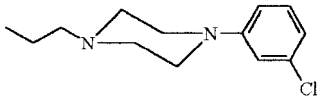
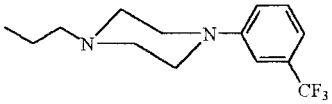
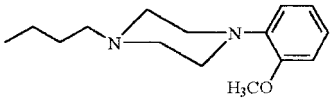
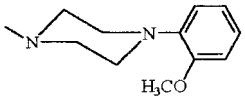
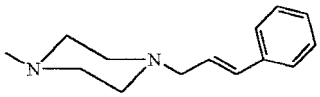
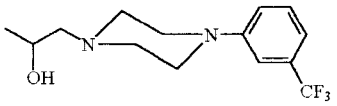
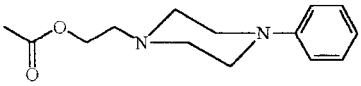
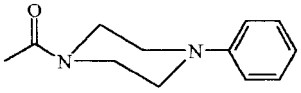
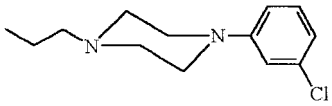
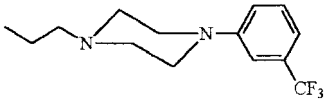
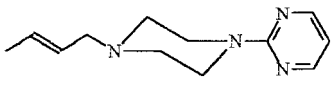
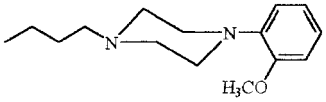


Table 1: Data of compounds 3,4



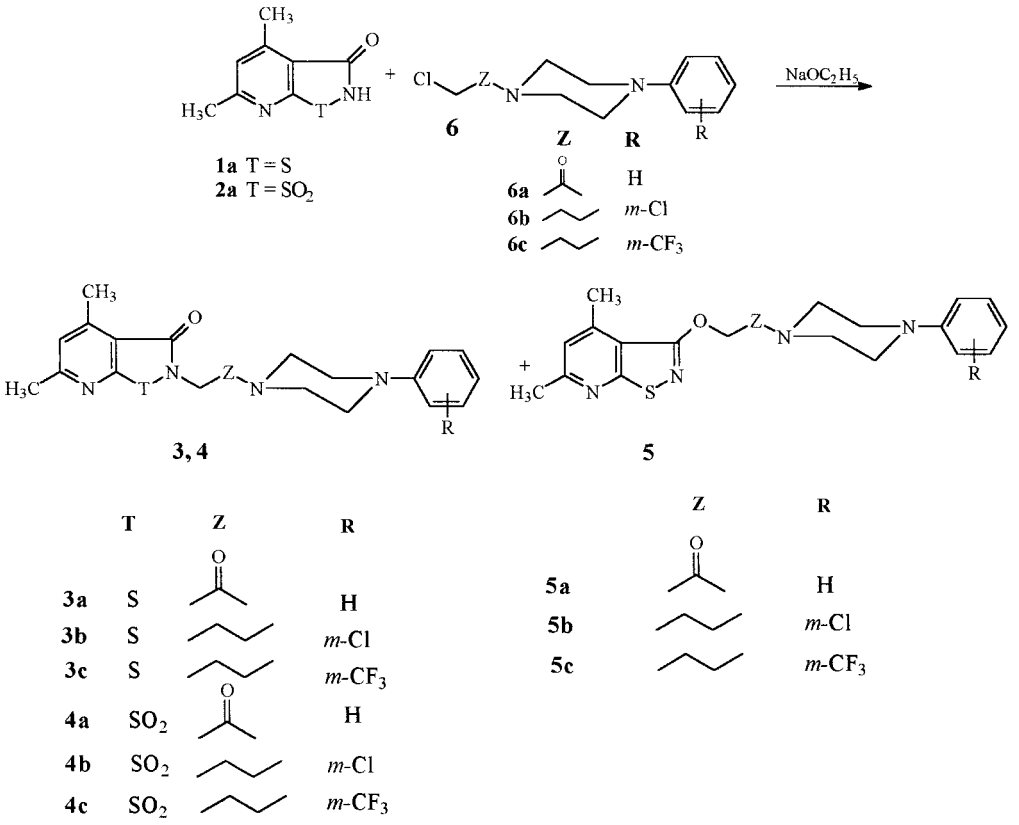
Compd.	X	R	Formula Molecular mass	m.p. (°C) (solvent)	Yield (%)
3a	S		$C_{20}H_{22}N_4O_2S$ 328.5	174–176 (methanol)	47
3b	S		$C_{21}H_{25}ClN_4OS$ 417.0	117–119 (*n*-heptane)	53
3c	S		$C_{22}H_{25}F_3N_4OS$ 417.0	59–61 (*n*-hexane)	49
3d	S		$C_{23}H_{30}N_4O_2S$ 426.6	122–124 (*n*-heptane)	42
3e	S		$C_{20}H_{24}N_4O_2S$ 384.5	126–128 (*n*-heptane)	65
3f	S		$C_{22}H_{26}N_4OS$ 394.5	81–83 (*n*-hexane)	59
3g	S		$C_{22}H_{25}F_3N_4O_2S$ 466.5	99–101 (cyclohexane)	40
3h	S		$C_{22}H_{26}N_4O_3S$ 426.5	103–105 (cyclohexane)	18
4a	SO_2		$C_{20}H_{22}N_4O_4S$ 414.5	222–234 (ethyl acetate)	65
4b	SO_2		$C_{21}H_{25}ClN_4O_3S$ 449.0	114–116 (ethanol)	51
4c	SO_2		$C_{22}H_{25}F_3N_4O_3S$ 483.0	98–100 (ethanol)	42
4d	SO_2		$C_{20}H_{24}N_6O_3S$ 428.5	116–118 (ethanol)	70
4e	SO_2		$C_{23}H_{30}N_4O_4S$ 458.6	117–119 (ethanol)	55

4,6-dimethyl-7-azaipsapirone **B** with a *trans*-2-butenyl central chain (**4d**) and a cinnamyl analogue (**3f**) of our anorectic agent **A** were prepared. Finally, to reduce the basicity of the compounds, a carbonyl group was introduced to the carbon atom of the central alkanyl chain adjacent to N-1 of the piperazine ring (**3a**, **4a**).

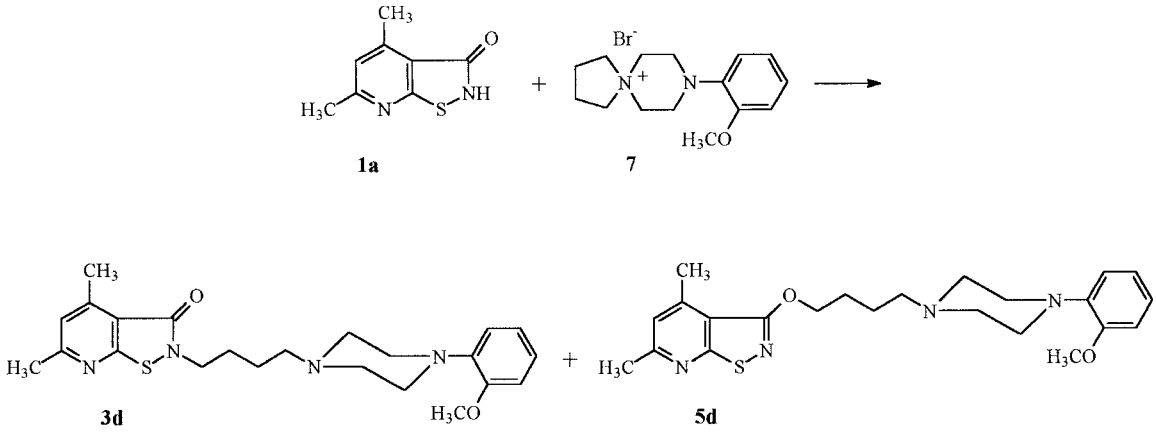
Representatives of compounds **3**, **4** were assayed for acute toxicity as well as in several commonly used CNS (central nervous system) behavioral tests in mice and for arterial blood pressure in rats.

Recently, we also reported that some derivatives of isothiazolo[5,4-*b*]pyridine, variously substituted at the nitrogen atom of the isothiazole ring, demonstrate antimycobacterial activity [7]. The most potent antimycobacterial agents (100%–93% inhibition of *Mycobacterium tuberculosis* $H_{37}Rv$ at 12.5 μg/ml) were compounds **C**, **D** and **E**.

Scheme 1



Scheme 2



In this context the antimycobacterial activity of compounds **3a**–**h**, **4d**, **e**, **5b**, **d** and related aryl-piperazinylalkyl isothiazolopyridines **8**, for which syntheses have been already reported, was assayed (Table 3).

2. Investigations, results and discussion

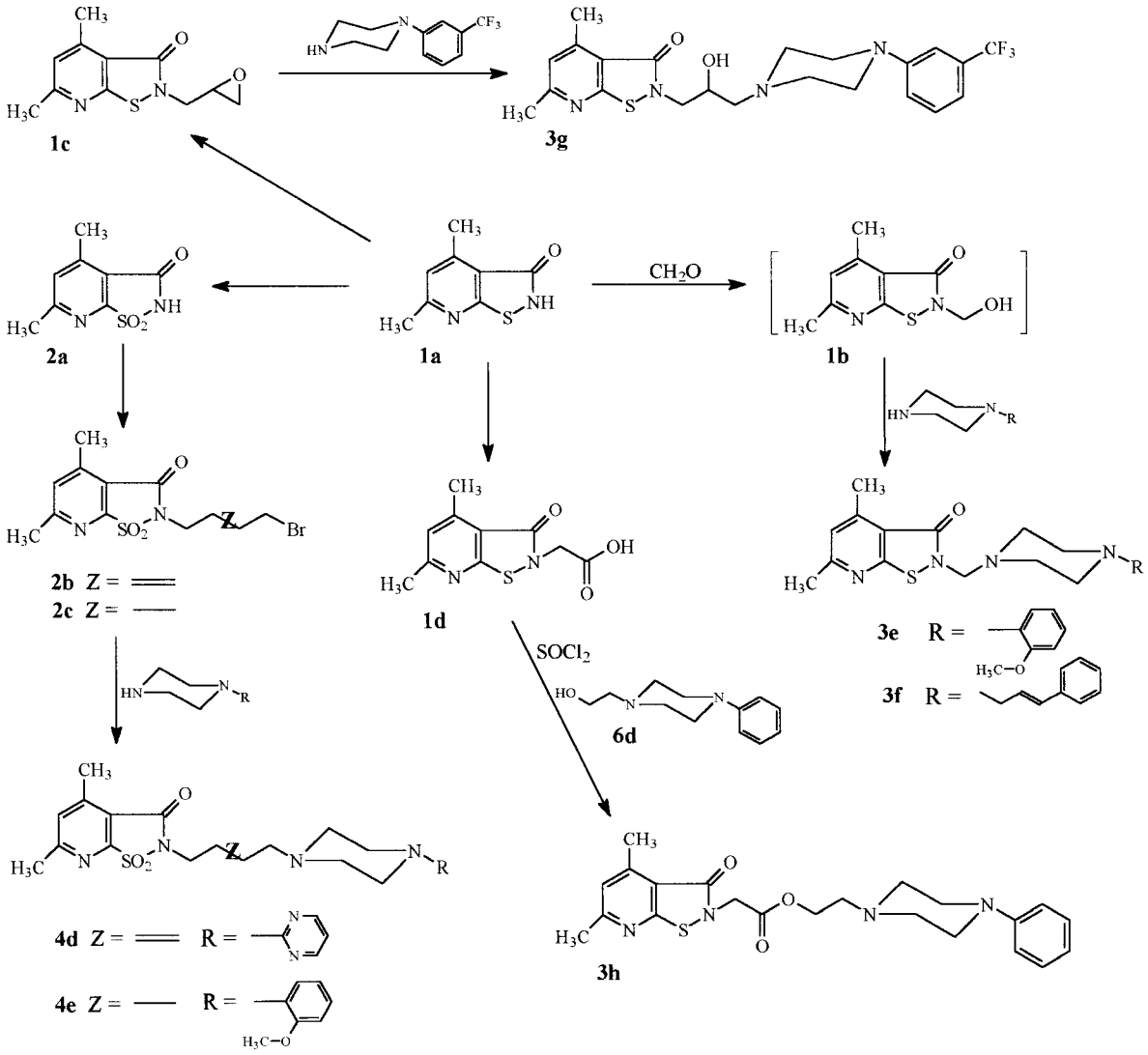
2.1. Synthesis of the compounds

The procedures leading to the final compounds **3**, **4** (Table 1) are shown in Schemes 1–3. These compounds were synthesized in a simple one-step alkylation of isothiazolopyridine **1a** [8] or its 1,1-dioxide **2a** [9] with corresponding 1,4-disubstitued piperazines (Method A) or alternatively by reaction of isothiazolopyridine derivatives (**1b**–**d**, **2b**, **c**; Scheme 3) with *N*-monosubstituted piperazines (Method B).

2.1.1. Method A

Simple alkylation of isothiazolopyridine **1a** [8] or its 1,1-dioxide **2a** [9] with corresponding 1,4-disubstituted-piperazines **6a**–**c** and **7** gave the target compounds **3a**–**d** and **4a**–**c**, respectively, in 42–65% yield (Schemes 1 and 2; Table 1). The piperazine intermediates **6a**–**c** and **7** were obtained from commercially available *N*-arylpiperazines. However, alkylation of **1a** led to a mixture of 2-N-(**3**) and 3-O-alkylated (**5**, Table 2) isomers which were separated by CC. In the reaction of isothiazolopyridine-1,1-dioxide

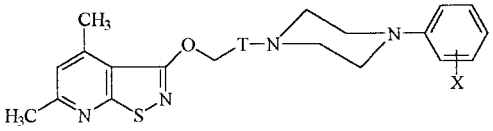
Scheme 3

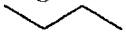
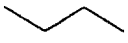
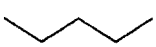


2a with the piperazine derivatives **6**, formation of *O*-alkylated by-products were not observed.

Isomers **3a**–**d** and **5a**–**d** were differentiated on the basis of IR and ^{1}H NMR spectra. IR absorption for the 3-C=O group of isothiazolopyridines **3** was observed for all compounds around 1670 cm^{-1} (for 1,1-dioxides **4** around 1720 cm^{-1}). In the IR spectra of the O-isomers **5** the absence of the bands characteristic of the 3-C=O (1670 cm^{-1}) were observed. Furthermore, in the ^{1}H NMR spectra the *O*-methylene protons of the central alkanyl chains were shifted downfield (4.53–4.59 ppm) when compared to those of the *N*-isomers (4.03–3.91 ppm); for

Table 2: Data of compounds 5



Compd.	T	X	Formula Molecular mass	m.p. (°C) (solvent)	Yield (%)
5a		H	$C_{20}H_{22}N_4O_2S$ 382.5	174–178 (ethanol)	8
5b		*m*-Cl	$C_{21}H_{25}ClN_4OS$ 417.0	81–83 (*n*-heptane)	9.5
5c		*m*-CF_3	$C_{22}H_{25}F_3N_4OS$ 450.5	74–76 (*n*-hexane)	10
5d		*o*-CH_3O	$C_{23}H_{30}N_4O_2S$ 426.6	72–74 (*n*-hexane)	11

isomers **5a** and **3a** the shift values are 5.11 and 4.62 ppm, respectively.

2.1.2. Method B

The structures of compounds **3**, **4** prepared in this manner are shown in Scheme 3. The starting isothiazolopyridine intermediates **1b–d**, **2c** (Scheme 3) were prepared according to published procedures [2, 3, 4, 7], whereas intermediate **2b** was obtained by reaction of **2a** with *trans*-1,4-dibromo-2-butene. The final products **3e–g**, **4d**, **e** were synthesized from **1b**, **c**, **2b**, **c** and corresponding commercially available *N*-substituted piperazines in analogy to published procedures [2–4] in yields ranging from 40 to 70% (Table 1). Scheme 3 illustrates also the method leading to the target ester **3h**. Yield of compound **3h** was low (18%) and no attempts were made to improve it.

Tables 1 and 2 summarize the data associated with compounds **3–5**. The analytical data (elementary analyses, IR and ^{1}H NMR spectra) of the new products **2d**, **3–5** are in agreement with the assigned structures. The spectral data within the series **3**, **4** and **5** did not show remarkable differences and are presented for selected compounds in the Experimental section.

2.2. Pharmacological screening

2.2.1. Antimycobacterial activity

The primary screening of **antimycobacterial activity** of the compounds synthesized here (**3a–h**, **4d**, **e** **5b**, **d**) and others described previously (**8a–g**) in the form of bases was conducted *in vitro* at a concentration of 12.5 μg/ml against *Mycobacterium tuberculosis* $H_{37}Rv$ in BACTEC 12B medium using the BACTEC 460 radiometric system. The antimycobacterial activities of the compounds tested are summarized in Table 3.

As seen in Table 3, the antimycobacterial potential of the isothiazolopyridines **3**, **4**, **5**, **8** is largely determined by the presence of a substituent (*o*-CH_3, *m*-Cl, *m*-CF_3) at the terminal phenyl ring of the side chain. Lack of such a substituent or replacement of the terminal aromatic ring by a heteroaromatic one (pyridine, pyrimidine) drastically reduces activity (compare **3e** and **8f**, **3g** and **8a**, **c**, **d**). It is noteworthy that the length of the bridging alkyl unit separating the piperazine ring from the isothiazole has no substantial influence on the antimycobacterial potency. For example, *o*-methoxyphenyl isothiazolopyridine **3e** with the 1-carbon chain and its butylene analogue **3d** possess equipotent antimycobacterial activity (100% inhibition, 12.5 μg/ml). On the other hand, the high activity of both **3e** and **3d** may suggest that in its bioactive form compound **3d** prefers a folded conformation rather than an extended one. Furthermore, the antimycobacterial activity of the isothiazolopyridines tested is strongly dependent on the position of the side chain. In contrast to the active N-derivatives **3b**, **d** (100% inhibition), the activity of their *O*-analogues **5b** and **5d** or *O*-isomers **8b**, **e**, **g** was generally poor (12-0% inhibition).

Modification of the central alkanyl chain by introduction of the OH substituent (**3g**, **8a**, **c**, **d**), ester bound (**3h**), double bound (**4d**) or carbonyl group (**3a**), has a diverse effect on antimycobacterial activity (100-5% inhibition). However, in this series of compounds (**3a**, **g**, **h**, **4d**, **8a**, **c**, **d**), the type of the terminal aromatic (heteroaromatic) substitution of the side chain may also contribute to the observed variations in antimycobacterial activity.

Finally, it should be noted that in accordance to our previous observations [7], oxidation of the sulfur atom of the isothiazole ring of compounds **3** is also not beneficial and leads to practically inactive preparations (compare **3d** and **4e**).

Representatives of the isothiazolopyridines effecting >90% inhibition of *Mycobacterium tuberculosis* $H_{37}Rv$ in

Table 3: Activity *in vitro* of isothiazolopyridines 3, 4, 5 and 8 against *Mycobacterium tuberculosis* $H_{37}Rv$

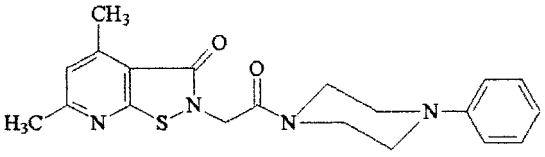
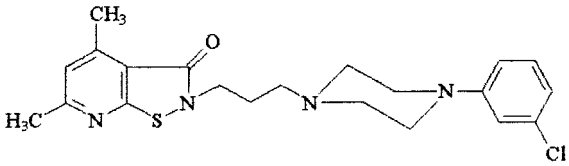
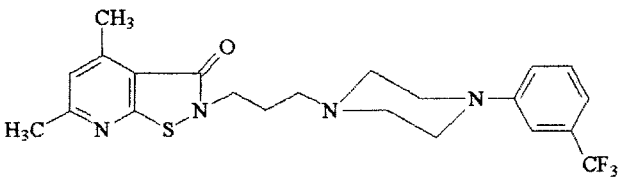
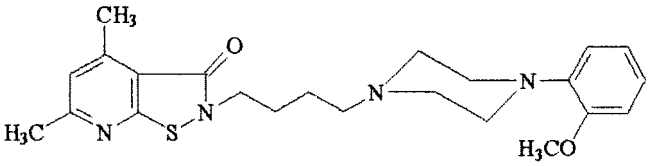
Compd.	Structure	% Inhibition (12.5 μ/ml)	Ref.
3a		100	
3b		100	
3c		100	
3d		100	

Table 3 (cont.)

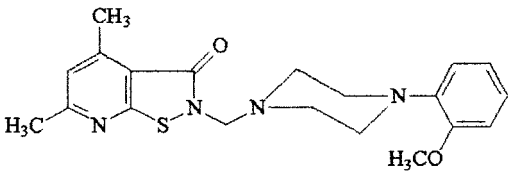
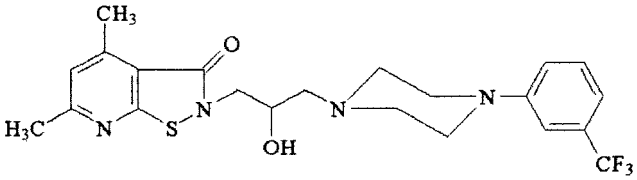
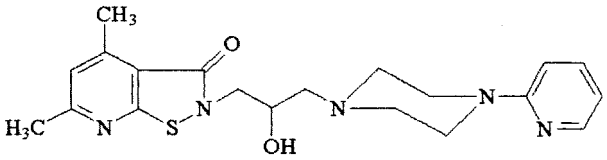
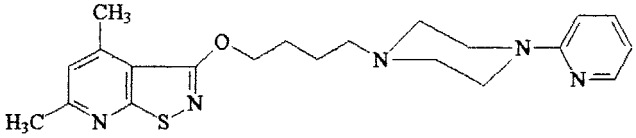
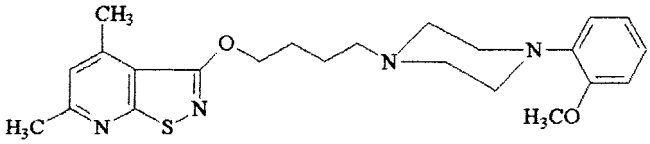
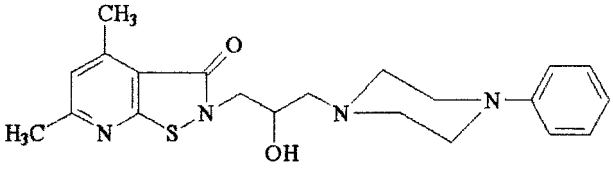
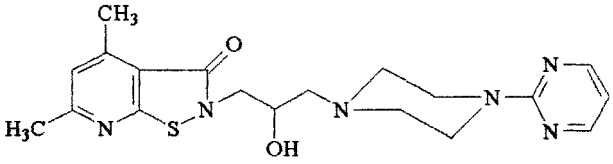
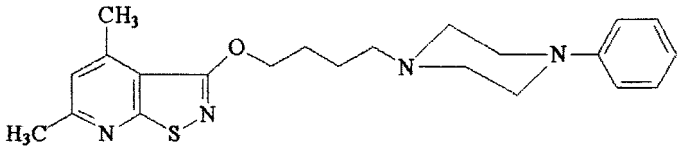
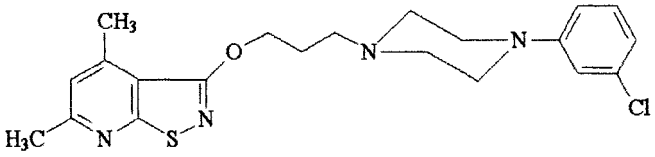
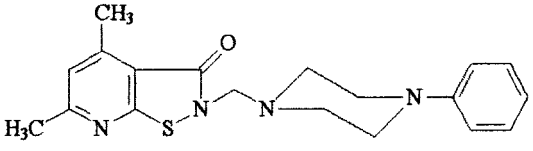
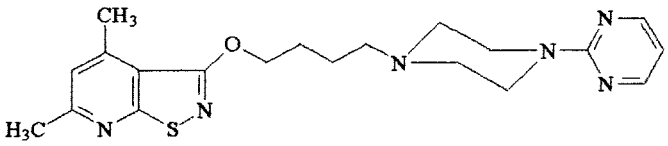
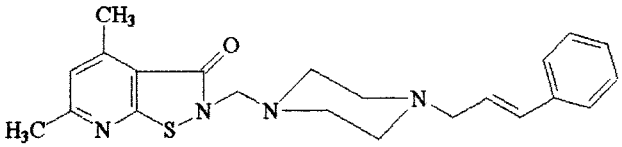
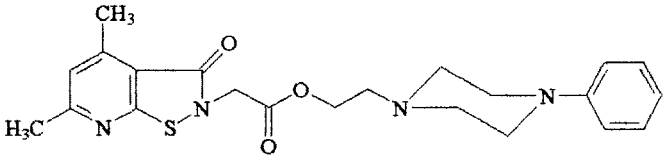
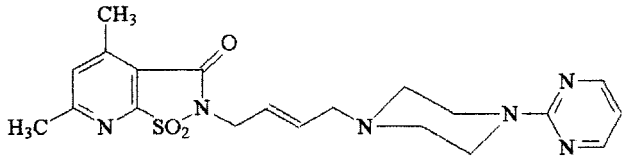
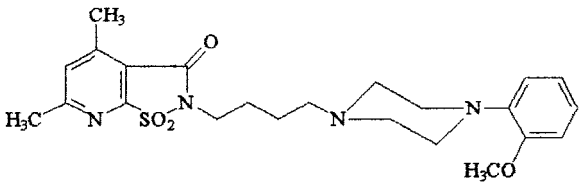
Compd.	Structure	% Inhibition (12.5 μ/ml)	Ref.
3e		100	
3g		100	
8a		12	[2]
8b		12	[3]
5d		10	
8c		5	[2]
8d		4	[2]
8e		2	[1]
5b		0	
8f		0	[4]

Table 3 (cont.)

Compd.	Structure	% Inhibition (12.5 μ/ml)	Ref.
8g		0	[3]
3f		0	
3h		0	
4d		0	
4e		0	

the primary screening [**D**, **3b**, **3c**, **3g**] were also tested against *Mycobacterium avium* complex, a naturally drug-resistant opportunistic pathogen, in broth microdilution Alamar Blue assay (MABA). However, these compounds did not exhibit activity in this experiment (0–1% inhibition, 12.5 μg/ml).

2.2.2. CNS activity

The five newly synthesized compounds **3a**, **b**, **d**, **f** and **4d** were subjected to preliminary pharmacological analysis to test their activity on CNS and arterial blood pressure in animal models.

The following pharmacological tests were performed: acute toxicity in mice, motor coordination in the rota-rod test in mice, spontaneous locomotor activity in mice, amphetamine-induced locomotor hyperactivity in mice, pain reactivity in the "writhing" test in mice, pain reactivity in the "hot-plate" test in mice, anxiolytic properties in "four plates" test in mice, pentetrazol-induced seizures in mice, maximal electric shock in mice, head twitches induced by 5-hydroxytryptophane (5-HTP) in mice and arterial blood pressure in rats.

2.2.2.1. Acute toxicity

The LD_{50} value of the investigated compounds after their i.p. administration to mice are presented in Table 4. The less toxic compounds were **3b**, **3a** with LD_{50} values of 1135 mg/kg and 774 mg/kg, respectively. Others compounds, e.g. **3d**, **3f**, and **4d** were quite toxic, with LD_{50} values between 159.0 and 291 mg/kg.

Table 4: Acute toxicity of investigated compounds (n = 8)

Compd.	LD_{50} (mg/kg i.p.)	Confidence limit
3a	773.8	[481.0–1245.0]
3b	1135.5	[854.6–1508.8]
3d	159.0	[91.2–277.5]
3f	291.3	[190.0–253.1]
4d	215.6	[98.0–374.6]

The LD_{50} values and confidence limit were calculated by the method of Litchfield and Wilcoxon [13]

2.2.2.2. Motor coordination

All the investigated compounds at the doses equivalent to 1/10 LD_{50} had no neurotoxic properties as they did not affect the motor coordination in the rota-rod test.

2.2.2.3. Locomotor activity

Only compound **3f** did not affect spontaneous locomotor activity. All others compounds suppressed the spontaneous locomotor activity during an observation period of 1 h. **3a** and **3d** acted at the dose of 1/10, **4d** up to the dose of 1/20, **3b** up to the dose of 1/40 of LD_{50} (Table 5).

2.2.2.4. Amphetamine-induced locomotor hyperactivity

Compounds **3b**, **3d**, and **3f** administered at the dose equivalent to 1/10 of LD_{50} did not affect the excitatory action of amphetamine in mice, **3a** and **4d** suppressed amphetamine-induced hyperactivity at the dose of 1/10 of LD_{50}.

Table 5: Influence of investigated compounds on the spontaneous locomotor activity in mice (n = 8)

Compd.	Dose (part of LD_{50})	Number of impulses ± SEM after time (min) 30	60
Control	–	408.8 ± 52.4	617.0 ± 74.2
3a	1/10	244.1 ± 45.3*	367.8 ± 24.0*
	1/20	389.6 ± 44.8	503.1 ± 71.5
3b	1/10	94.8 ± 34.3***	211.4 ± 56.1**
	1/20	145.6 ± 60.9**	293.2 ± 40.4**
	1/40	192.8 ± 50.5*	410.1 ± 42.9*
	1/80	326.4 ± 50.9	520.0 ± 34.1
3d	1/10	229.8 ± 67.2*	254.1 ± 65.9**
	1/20	217.5 ± 47.8	485.2 ± 67.7
3f	1/10	361.4 ± 68.6	510.8 ± 111.3
4d	1/10	145.6 ± 44.5***	292.7 ± 76.0*
	1/20	276.2 ± 19.6*	368.0 ± 26.1*
	1/40	329.5 ± 21.4	447.3 ± 46.2

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Student-*t*-test

Table 6: Influence of the investigated compounds on the pain reactivity in "writhing" test in mice (n = 8)

Compd.	Dose (part of LD_{50})	Mean number of writhings ± SEM
Control	–	9.24 ± 0.67
3a	1/10	1.10 ± 0.8***
	1/20	2.30 ± 1.1***
	1/40	3.10 ± 1.0***
	1/80	7.50 ± 2.1
3b	1/10	0.87 ± 0.5***
	1/20	1.44 ± 0.98***
	1/40	1.75 ± 0.72***
	1/80	2.50 ± 0.73***
	1/160	2.87 ± 1.14***
	1/320	3.75 ± 0.59***
	1/640	4.25 ± 1.12***
	1/1280	5.87 ± 1.3*
	1/2560	9.25 ± 1.3
3d	1/10	4.12 ± 0.6***
	1/20	7.60 ± 1.5
3f	1/10	0***
	1/20	0***
	1/40	0.25 ± 0.16***
	1/80	1.62 ± 0.49***
	1/160	3.75 ± 0.31***
	1/320	7.76 ± 0.92
4d	1/10	8.80 ± 1.1

*** $p < 0.001$; Student-*t*-test

Table 7: Influence of investigated compounds on the pain reactivity in "hot plate" test in mice (n = 8)

Compd.	Dose (part of LD_{50})	Time of reaction on pain stimulus in seconds SE
Control	–	4.44 ± 0.43
3a	1/10	8.26 ± 0.86**
	1/20	6.45 ± 0.82*
	1/40	6.15 ± 0.75
3b	1/10	9.75 ± 0.29***
	1/20	8.26 ± 0.45***
	1/40	6.58 ± 0.51**
	1/80	4.75 ± 0.29
3d	1/10	5.01 ± 0.52
3f	1/10	7.30 ± 1.42*
	1/20	6.85 ± 1.09*
	1/40	4.85 ± 0.49
4d	1/10	4.45 ± 0.43

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; Student-*t*-test

2.2.2.5. Pain reactivity

Most of the investigated compounds possess very strong analgesic activity. Assayed in the "writhing" test compound **3d** acted at the dose of 1/10, **3a** – 1/40, **3f** – 1/160 and the most active **3b** up to the dose of 1/1280 of LD_{50}. In the "hot plate" test compounds **3a** and **3f** acted at the dose up to 1/20, **3b** up to 1/40 of LD_{50}. Here, compound **3d** was not active whereas **4d** did not show analgesic activity in both tests (Tables 6 and 7).

2.2.2.6. Anxiolytic action

None of investigated compounds, administered at doses which not affected spontaneous locomotor activity, increased the number of punished crossings in the "four plates" test in mice.

2.2.2.7. Pentetrazol induced seizures

The investigated compounds administered at the doses equivalent to 1/10 of LD_{50} had no anticonvulsive properties in pentetrazol-induced seizures test in mice.

2.2.2.8. Maximal electric shock

Investigated compounds administered at the dose of 1/10 of LD_{50} showed lack of protection against tonic seizures in maximal electric shock in mice.

2.2.2.9. Head twitches

Only **3f** reduced the number of head twitches episodes induced by 5-HTP in mice at a dose of 1/10 of LD_{50}. All others compounds did not change the number of head twitches.

2.2.2.10. Arterial blood pressure

Compound **3d** decreased the arterial blood pressure at the dose of 1/10 of LD_{50}. All others compounds did not affect arterial blood pressure and pulse rate in rats.

2.2.2.11. Conclusion

The present study of 2-(4-substituted-piperazin-1-yl-alky)isothiazolo[5,4-*b*]pyridines **3a**, **b**, **d**, **f** and **4d** shows that the majority of the these compounds assayed in CNS tests, with the exception of compounds **3d** and **4d** with a 4-carbon chain, exhibit a similar pharmacological profile – predominant analgesic activity evidenced in "writhing" and "hot plate" tests in animal models (mice). An especially interesting analgesic effect is shown by compound **3b** (1/640 of LD_{50}; LD_{50} = 1135.5 mg/kg), bearing the *m*-chlorophenylpiperazinylpropyl chain characteristic for trazodone. It is noteworthy that the isothiazolopyridines **3a** and **3b** active in analgesic test also reveal high antimycobacterial activity (100% inhibition of *Mycobacterium tuberculosis* $H_{37}Rv$ at 12.5 μg/ml).

3. Experimental

3.1. Chemistry

Melting points are uncorrected. ^{1}H-NMR spectra were obtained with a Tesla spectrometer 80 MHz in $CDCl_3$; the chemical shifts are reported in δ (ppm). IR spectra were recorded on Specord-75 IR spectrometer. Elemental C, H, N analyses were run on a Carlo Erba NA-1500 analyzer, the results were within ±0.4% of the values calculated for the corresponding formulas. Chromatographic separations were performed on a silica gel [Kieselgel 60 (70–230 mesh), Merck] column (CC). Syntheses described in sections 3.1.2.–3.1.10. refer to method A, those in sections 3.1.11.–3.1.14. to method B (see 2.1.).

3.1.1. 2H-2-(4-Bromo-trans-2-buten-1-yl)-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine-1,1-dioxide (**2b**)

A mixture of 1.1 g (5 mmol) of isothiazolopyridine-1,1-dioxide **2a** [9], 0.7 g of K_2CO_3 and 3.2 g (15 mmol) of 1,4-dibromo-*trans*-2-butene in acetonitrile (50 ml) was refluxed with stirring for 1.5 h. Then the mixture was filtered and evaporated. The resulting crude product **2b** was purified by CC [benzene, $R_f = 0.15$, 0.7 g (40% yield)]. An analytical sample of **2b** (m.p. 88–90 °C) was obtained after crystallization of the product from a small amount of ethanol.
IR: 1730 (C=O). ^{1}H NMR: 2.70 s (3H, CH_3), 2.75 s (3H, CH_3), 3.93 d (2H, CH_2; J = 6.2 Hz), 4.37 d (2H, CH_2; J = 4.88 Hz), 5.72–6.34 m (2H, CH=CH), 7.34 s (1H, 5-H).
$C_{12}H_{13}BrN_2O_3S$ (345.2).

3.1.2. 2H-2-[2-Oxo-2-(4-phenylpiperazin-1-yl)ethyl]-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**3a**) *and 4,6-dimethyl-3-[2-oxo-2-(4-phenylpiperazin-1-yl)ethoxy]isothiazolo[5,4-b]pyridine* (**5a**)

To the solution of sodium ethoxide, prepared from 0.23 g of Na and 50 ml of anh. ethanol, 1.8 g (0.01 mol) of isothiazolopyridine **1a** [8] and 2.9 g (0.012 mol) of 1-chloroacetyl-4-phenylpiperazine (**6a**) [10] were added. The reaction mixture was refluxed with stirring for 5 h. Then the mixture was cooled, and the precipitated product was filtered off, washed with water and crystallized from methanol (60 ml) to give 1.4 g of compound **3a**. The filtrate was evaporated, and the resulting residue was chromatographed (CC). From the fraction eluted with chloroform 0.3 g of **5a** (Table 2) were obtained. The fraction eluted with ethyl acetate gave additionally 0.4 g of **3a** (Table 1).
3a: IR: 1670 (3-C=O and amide C=O). ^{1}H NMR: 2.52 s (3H, CH_3), 2.66 s (3H, CH_3), 3.02–3.18 m [4H, ArN$(CH_2)_2$], 3.51–3.80 m [4H, CON$(CH_2)_2$], 4.62 s (2H, CH_2), 6.65–7.29 m (6H, 5 ArH + 5-H).
5a: IR: 1670 (C=O, amide). ^{1}H NMR: 2.54 s (3H, CH_3), 2.67 s ((3H, CH_3), 3.04–3.18 m [4H, ArN$(CH_2)_2$], 3.45–3.81 m [4H, CON$(CH_2)_2$], 5.11 s (2H, CH_2), 6.66–7.29 m (6H, 5 ArH + 5-H).

3.1.3. 2H-2-{3-[4-(m-Chlorophenyl)piperazin-1-yl]propyl}-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**3b**) *and 4,6-dimethyl-3-{3-[4-(m-chlorophenyl)piperazin-1-yl]propoxy}isothiazolo[5,4-b]pyridine* (**5b**)

To the solution of sodium ethoxide, prepared from 0.23 g of Na and 50 ml of anh. ethanol, 1.8 g (0.01 mol) of isothiazolopyridine **1a** [8] and 3.3 g (0.012 mol) 1-(*m*-chlorophenyl)-4-(3-chloropropyl)piperazine (**6b**) [11] were added. The reaction mixture was refluxed for 15 h, filtered and evaporated. The residue was chromatographed (CC) with ethyl acetate. The fractions containing the compound of $R_f = 0.58$ were combined and evaporated to provide **5b** (Table 2), whereas fractions of $R_f = 0.36$ afforded **3b** (Table 1).
3b: IR: 1670 (3-C=O). ^{1}H NMR: 1.8–2.12 m (2H, C-CH_2-C), 2.39 to 2.62 m [9H, CH_3 + N$(CH_2)_3$], 2.73 s (3H, CH_3), 3.1–3.24 m [4H, Ar$(CH_2)_2$], 3.94 t (2H, $N_{isothiazole}CH_2$; J = 6.4 Hz), 6.7–7.31 m (5H, 4 ArH + 5-H).
5b: ^{1}H NMR: 1.97–2.25 m (2H, C-CH_2-C), 2.5 to 2.75 m [12H, 2 × CH_3 + N$(CH_2)_3$], 3.16–3.27 m [4H, Ar$(CH_2)_2$], 4.57 t (2H, OCH_2; J = 6.4 Hz), 6.74–7.27 m (5H, 4 ArH + 5-H).

3.1.4. 2H-2-{3-[4-(m-Trifluoromethylphenyl)piperazin-1-yl]propyl}-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**3c**) *and 4,6-dimethyl-3-{3-[4-(m-trifluoromethylphenyl)piperazin-1-yl]propoxy}isothiazolo[5,4-b]-pyridine* (**5c**)

Compounds **3c** (Table 1) and **5c** (Table 2) were prepared by alkylation of 1.8 g (0.01 mol) isothiazolopyridine **1a** [8] with 3.7 g (0.012 mol) of 1-(*m*-trifluoromethylphenyl)-4-(3-chloropropyl)piperazine **6c** [11] as described for **3b** and **5b**. Isolation of the products **3c** and **5c** was performed by CC [ethyl acetate; **3c** ($R_f = 0.26$), **5c** ($R_f = 0.54$)].

3.1.5. 2H-2-{4-[4-(o-Methoxyphenyl)piperazin-1-yl]butyl}-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**3d**) *and 4,6-dimethyl-3-{4-[4-(o-methoxyphenyl)piperazin-1-yl]butoxy}isothiazolo[5,4-b]pyridine* (**5d**)

A mixture of 1.8 g, (0.01 mol) of isothiazolopyridine **1a** [8], 1.4 g of anh. potassium carbonate and 3.8 g (0.011 mol) of 8-(*o*-methoxyphenyl)-8-aza-5-azoniaspiro[4,5]decane bromide (**7**) [11] in xylene (80 ml) was refluxed with stirring for 20 h. After filtration and evaporation the resulting mixture of **3d** and **5d** was treated with 30 ml of ethyl acetate. Undissolved product was filtered off and crystallized from ethyl acetate to give 1.6 g of pure **3d** (Table 1). Both filtrates of ethyl acetate were evaporated, and the resulting residue was chromatographed (CC; ethyl acetate). The fractions containing the product of $R_f = 0.36$ afforded **5d** (Table 2), whereas fractions of $R_f = 0.1$ gave additionally 0.2 g of **3d**.

3.1.6. 2H-2-{[4-(o-Methoxyphenyl)piperazin-1-yl]methyl}-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**3e**)

Isothiazolopyridine **1a** [8] (1.8 g 0.01 mol) and 1 ml of 37% formaldehyde (w/v) in ethanol (18 ml) was refluxed for 15 min. After cooling, to the separated precipitate of hydroxymethylisothiazolopyridine **1b** [4], 1.9 g (0.01 mol) of 1-(*o*-methoxyphenyl)piperazine was added and the reaction mixture was stirred at RT for 12 h. Then the mixture was poured into ice-cold water. The insoluble waxy product was separated by decantation of the water phase, dissolved in chloroform and dried ($MgSO_4$). The solvent was distilled off and the resulting residue was purified by crystallization to give **3e** (Table 1).
3e: IR: 1670 (3-C=O). ^{1}H NMR: 2.6 s (3H, CH_3), 2.74 s (3H, CH_3), 2.9 to 3.5 m [8H, 2 × N$(CH_2)_2$], 3.83 s (3H, OCH_3), 4.73 s (2H, CH_2), 6.92 s (5H, 4ArH + 5-H).

3.1.7. 2H-2-{[4-(trans-Cinnamyl)piperazin-1-yl]methyl}-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**3f**)

Compound **3f** (Table 1) was obtained from 1.8 g (0.01 mol) isothiazolopyridine **1a**, formaldehyde and 2.0 g (0.01 mol) of *trans*-1-cinnamylpiperazine as described for **3e**.

3.1.8. 2H-2-[2-Oxo-2-(4-phenylpiperazin-1-yl)ethyl]-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine-1,1-dioxide (**4a**)

To the solution of sodium ethoxide, prepared from 0.115 g of Na and 25 ml of anh. ethanol, 1.1 g (5 mmol) of isothiazolopyridine-1,1-dioxide **2a** [9] and 1.2 g (5 mmol) of 1-chloroacetyl-4-phenylpiperazine (**6a**) [10] were added. The reaction mixture was refluxed with stirring for 5 h. Then the mixture was cooled and the precipitated product was filtered, washed with water and purified by crystallization (**4a**, Table 1).
IR: 1735 (3-C=O) and 1680 (C=O amide). ^{1}H NMR: 2.58 s (3H, CH_3), 2.63 s (3H, CH_3), 3.0–3.23 m [4H, ArN$(CH_2)_2$], 3.48–3.76 m [4H, CON$(CH_2)_2$], 4.45 s (2H, CH_2), 6.71–7.28 m (6H, 5 ArH + 5-H).

3.1.9. 2H-2-{3-[4-(m-Chlorophenyl)piperazin-1-yl]propyl}-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine-1,1-dioxide (**4b**)

To a stirred mixture of 2.1 g (0.01 mol) of isothiazolopyridine-1,1-dioxide **2a** [9] and 0.4 g (0.01 mol) of sodium hydride (~60% dispersion in mineral oil) in anh. DMF (20 ml) 3.3 g (0.012 mol) of 1-(*m*-chlorophenyl)-4-(3-chloropropyl)piperazine (**6b**) [11] was added. The reaction mixture was heated at 100 °C for 5 h and after cooling was poured into water. The separated waxy material was isolated by decantation of the water phase, dissolved in chloroform dried ($MgSO_4$), filtered and evaporated. The resulting residue was crystallized to give **4b** (Table 1).
IR: 1720 (3-C=O). ^{1}H NMR: 1.89–2.23 m (2H, C–CH_2–C), 2.23 to 2.74 m [12H, 2 × CH_3 + N$(CH_2)_3$], 3.18 t [4H, ArN$(CH_2)_2$; J = 4.8 Hz], 3.89 t (2H, $N_{isothiazole}CH_2$; J = 7.2 Hz), 6.69–7.16 m (4H, ArH), 7.32 s (1H, 5-H).

3.1.10. 2H-2-{3-[4-(m-Trifluoromethylphenyl)piperazin-1-yl]propyl}-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine-1,1-dioxide (**4c**)

Compound **4c** (Table 1) was obtained by alkylation of 2.1 g (0.01 mol) of isothiazolopyridine-1,1-dioxide **2a** [9] with 3.7 g (0.012 mol) of 1-(*m*-trifluoromethylphenyl)-4-(3-chloropropyl)piperazine (**6c**) [11] as described for **4b**. Crude **4c** was purified by CC (ethyl acetate, $R_f = 0.77$); further purification by crystallization (Table 1).

3.1.11. 2H-4,6-Dimethyl-3-oxo-2-[2-hydroxy-3-(4-phenylpiperazin-1-yl)-propyl]-2,3-dihydroisothiazolo[5,4-b]pyridine (**3g**)

A solution of 1.2 g (5 mmol) of 2-(2,3-epoxypropyl)isothiazolopyridine **1c** [2] and 1.15 g (5 mmol) of 1-(*m*-trifluoromethylphenyl)piperazine in 25 ml of ethanol was refluxed for 2 h. The solvent was distilled off and the residue was purified by CC [benzene-ethyl acetate (5 : 2)]. Fractions of $R_f = 0.3$ afforded **3g**; further purification by crystallization (Table 1).
IR: 3350 br(OH), 1670 (3-C=O). ^{1}H NMR: 2.5–2.97 m [12H, 2 × CH_3 + N$(CH_2)_3$], 3.25 t [4H, ArN$(CH_2)_2$; J = 4.88 Hz], 3.77–4.25 m [4H, CH_2CH(OH); 1H (OH), D_2O exchange], 6.95–7.45 m (5H, 4ArH + 5-H).

3.1.12. 2-(4-Phenylpiperazin-1-yl)ethyl (2H-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridin-2-yl)acetate (**3h**)

A mixture of 1.2 g (5 mmol) of isothiazolopyridinylacetic acid **1d** [7] in 50 ml of anh. chloroform containing 1 ml of $SOCl_2$ was refluxed for 1 h. Then the solvent was distilled off and 1.05 g (5 mmol) of 1-(2-hydroxyethyl)-4-phenylpiperazine (**6d**; see below) in chloroform (50 ml) was added to the residue. The mixture was refluxed with stirring for 2 h, then the solvent was distilled off and the resulting solid was treated with 2% sodium hydrogen carbonate aqueous solution. Insoluble material was filtered, dissolved in chloroform, dried ($MgSO_4$) and evaporated. The resulting residue was chromatographed (CC; ethyl acetate). Fractions of $R_f = 0.33$ afforded **3h** (Table 1). An analytical sample of **3h** was obtained after crystallization (Table 1).
IR: 1730 (C=O, ester), 1670 (3-C=O). ^{1}H NMR: 2.25–2.72 m [12H, 2 × CH_3 + N$(CH_2)_3$], 3.09 t [4H, ArN$(CH_2)_2$; J = 4.4 Hz], 4.35 t (2H, OCH_2; J = 5.4 Hz], 4.59 s (2H, NCH_2CO), 6.83–7.35 m (6H, 5 ArH + 5-H).

3.1.13. 2H-2-{4-[4-(2-Pyrimidinyl)piperazin-1-yl]-trans-2-butenyl}-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo-[5,4-b]pyridine-1,1-dioxide (**4d**)

A mixture of 1.75 g (5 mmol) of *trans*-4-bromobutenylisothiazolopyridine **2b**, 0.7 g of anh. potassium carbonate and 0.8 g (5 mmol) of *N*-(2-pyrimidinyl)piperazine in acetonitrile (40 ml) was refluxed for 5 h. After filtration the solvent was evaporated and the resulting residue was crystallized to give **4d** (Table 1).

3.1.14. 2H-2-{4-[4-(o-Methoxyphenyl)piperazin-1-yl]butyl}-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine-1,1-dioxide (**4e**)

Compound **4e** (Table 1) was prepared from 1.75 g (5 mmol) of 4-bromobutylisothiazolopyridine-1,1-dioxide (**2c**) [3] and 0.95 g (5 mmol) of 1-(*o*-methoxyphenyl)piperazine as described for **4d**. Crude **4e** was purified by CC (ethyl acetate, $R_f = 0.5$).
IR: 1720 (3-C=O). ^{1}H NMR: 1.5 m (4H, CH_2CH_2), 2.41–2.85 m [12H, $2 \times CH_3 + N(CH_2)_3$], 3.1 t [4H, $ArN(CH_2)_2$; J = 4.88], 3.72–3.90 m (5H, $OCH_3 + N_{isothiazole}CH_2$), 6.87–7.0 m (4H, ArH), 7.32 s (1H, 5-H).

3.1.15. 1-(2-Hydroxyethyl)-4-phenylpiperazine (**6d**)

A mixture of 1.6 g (0.01 mol) of N-phenylpiperazine, 2.1 g of anh. potassium carbonate and 1.2 g (0.02 mol) of 2-chloroethanol in ethanol (30 ml) was refluxed with stirring for 17 h. After filtration, the solvent was distilled off. The oily residue was dissolved in ethyl ether (30 ml) and filtered with charcoal. The filtrate was evaporated to give 1.3 g of crude **6d** (m.p. 76–79 °C). An analytical sample was obtained by crystallization from *n*-hexane (m.p. 80–82 °C; lit. m.p. 82.5–83 °C [12]).

3.2. Pharmacology

3.2.1. Animals

The experiments were carried out on male and female Albino-Swiss mice (body weight of 20–25 g) and male Wistar rats (200–250 g). Investigated compounds were administered intraperitoneally (i.p.) as a suspension in 3% Tween® 80 in a volume of 10 ml/kg in mice and 5 ml/kg in rats. The compounds were administered in doses equivalent to 1/10, 1/20, 1/40, 1/80, 1/160, 1/320, 1/640, 1/1280 or 1/2560 of LD_{50}. Control animals received the equivalent volume of solvent. Each experimental group consisted of 8 animals.

3.2.2. Acute toxicity test

Acute toxicity was assessed by the methods of Litchfield and Wilcoxon [13] and presented as LD_{50} and confidence limit calculated from the mortality of mice after 24 h.

3.2.3. Motor coordination test

Motor coordination was measured according to Gross et al. [14]. The mice were placed for 2 min on the rod rotating with the speed of 4 rpm. The effects were evaluated 15, 30, 45, 60, 75, 90 and 105 min after the administration of the investigated compounds.

3.2.4. Locomotor activity test

Spontaneous locomotor activity in mice was measured in a circular photoresistor actometers (32 cm in diameter). 30 min after the injection of the investigated compounds, the animals were placed in the actometers for 1 h. Each crossing of the light beam was recorded automatically. The amount of impulses was noted after 30 and 60 min.

3.2.5. Amphetamine hyperactivity test

Amphetamine hyperactivity in mice was induced by D,L-amphetamine 2.5 mg/kg s.c. Investigated compounds were injected 30 min before amphetamine. The locomotor hyperactivity was measured 30 and 60 min later in the photoresistor actometers.

3.2.6. Pain reactivity test

Pain reactivity was measured by the "writhing" test of Koster et al. [15]. The test was performed in mice by the i.p. injection of a 0.6% solution of acetic acid in a volume of 10 ml/kg 60 min after the administration of investigated compounds. The number of writhing episodes was counted for 30 min after the injection of 0.6% acetic acid.
Pain reactivity was also measured in the "hot plate" test according to Eddy and Leimbach [16]. Animals were placed individually on the metal plate heated to 56° C. The time (s) of appearance of the pain reaction (licking of the forepaws or jumping) was measured. Experiments were performed 60 min after the administration of investigated compounds.

3.2.7. Anxiolytic properties

Anxiolytic properties were assessed by the "four plate" test in mice, according to Aron et al. [17], 60 min after administration of the investigated compounds at doses which had no effect on the spontaneous locomotor activity. Mice were placed in the cages with four plates floor (11 × 7 cm) with 4 mm gape between each. After 15 s of adaptation the number of crossing was counted during 1 min. Each crossing was punished with direct current (180 V, 0.5 A) but not more often than every 3 s.

3.2.8. Pentetrazol seizures

Seizures in mice were induced by pentetrazol administration (100 mg/kg s.c.) 60 min after the investigated compounds. Animals were observed during 30 min and the number of mice developing clonic and tonic seizures as well as mortality was recorded in that period.

3.2.9. Maximal electric shock

Maximal electric shock was induced by means of alternating current (50 Hz, 25 mA, 0.2 sec) with the use of ear clip electrodes according to Swinyard et al. [18]. The criterion of the convulsive response was the tonic extension of the hind limbs. The test was performed 60 min after the administration of the investigated compounds.

3.2.10. Head twitches

Head twitches behaviour was induced by the administration of 5-hydroxytryptophan (5-HTP) at a dose of 180 mg/kg i.p. 30 min after the investigated compounds. Animals were observed 60 min after 5-HTP administration.

3.2.11. Arterial blood pressure

Arterial blood pressure was determined according to Gerold and Tschirky [19] using the UGO-BASILE equipment (Blood Pressure Recorder, cat. No 8006). Systolic blood pressure on the tail artery was measured 30 min after the administration of investigated compounds.

3.2.12. Statistics

Results obtained were presented as means and evaluated statistically using Student's t-test or exact Fischer's test. $P < 0.05$ was the criterion of the significance.

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Dr. habil. W. Malinka
Department of Chemistry of Drugs
Wrocław Medical University
ul. Tamka 1
50-137 Wrocław
Poland