ELSEVIER

Il Farmaco 53 (1998) 504–512

IL FARMACO

2-Substituted-3-oxoisothiazolo[5,4-b]pyridines as potential central nervous system and antimycobacterial agents

Wiesław Malinka [a,*], Stanisław Ryng [b], Maria Sieklucka-Dziuba [c], Grażyna Rajtar [c], Andrzej Głowniak [c], Zdzisław Kleinrok [c]

[a] *Department of Chemistry of Drugs, Wrocław University of Medicine, ul. Tamka 1, 50-137 Wrocław, Poland*
[b] *Department of Organic Chemistry, Wrocław University of Medicine, ul. Grodzka 9, 50-137 Wrocław, Poland*
[c] *Department of Pharmacology, Medical Academy, ul. Jaczewskiego 8, 20-090 Lublin, Poland*

Received 12 November 1997; accepted 7 July 1998

Abstract

The 2-[3-(substituted-amino)-2-hydroxypropyl]-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridines **3** were synthesized and pharmacologically evaluated in animal models. The preliminary pharmacological screening study showed that the investigated compounds were toxic and had no significant activity in central nervous system (CNS) tests. Additionally, compounds **3**, and several other 2-substituted-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridines described here (**2**), together with those (**4**) reported in a previous paper, were evaluated in vitro against *Mycobacterium tuberculosis* H37Rv. For comparison, products of the rearrangement of some isothiazolopyridine 1,1-dioxides (**4a,b**) with the corresponding pyrido[3,2-e]-1,2-thiazines (**5a,b**) and different N_2-substituted derivatives of the latter (**5c–i**) were also prepared and investigated in antimycobacterial tests. The most potent antituberculars of the 23 compounds assayed are 2-[3-(4-benzylpiperidin-1-yl)-2-hydroxypropyl]-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine **3d** and ethyl (4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridin-2-yl)acetate **4c** (MIC < 12.5 μg/ml, 100 and 98% inhibition, respectively).

Keywords: Isothiazolo[5,4-b]pyradines; Antimycobacterial activity

1. Introduction

Our particular interest was previously focused on the synthesis and preliminary pharmacological screening (in vivo) of derivatives of 2-[4-phenyl(heteroaryl)piperazin-1-ylalkyl]isothiazolo[5,4-b]pyridine and its 1,1-dioxide (Fig. 1, **I**), structurally related to ipsapirone (Fig. 1), a serotonine 5-HT_{1A} receptor agonist with anxiolytic and antidepressive properties [1].

Compounds **I** (Fig. 1) were evaluated in vivo on mice and rats to determine their action on the central nervous system (CNS) and arterial blood pressure. These efforts have led to the identification of an interesting anorectic agent (Fig. 1, **IA:** X = S; $n = 0$; T,Z = CH) [2] and showed that the incorporation of the hydroxyl (Fig. 1, **IB**) [3] or ethereal (Fig. 1, **IC**) [4] function at the central alkyl chain had generally negative influence on CNS effectiveness of the isothiazolopyridines investigated. On the other hand, many of the 3-oxoisothiazolo[5,4-b]pyridines, bearing a diverse substituent (benzyl, (un)substituted phenyl, alkyl, hydroxyalkyl, alkenyl, cycloalkyl, carbamoylalkyl(cycloalkyl, phenyl)) at position 2 of the isothiazole ring, received considerable attention for their antibacterial properties [5–8].

The object of the present study was twofold. First, as a further extension of our previous research on isothiazolo-[5,4-b]pyridines of type **IB** (Fig. 1) we prepared a series of compounds **3** (Scheme 2), containing only one base nitrogen atom in the side chain structure. The nitrogen atom of the base centres was located on the terminal carbon atom of the 2-hydroxypropyl chain and was substituted with different isoalkyl, cycloalkyl, and aromatic groups. Compounds **3** were prepared with the aim of comparing their CNS and arterial blood pressure actions with those of the previously studied phenyl(heteroaryl)piperazine analogues of type **IB** (Fig. 1) [3]. Secondly, the pronounced antibacterial activity of many isothiazolopyridines prompted us to investigate 3-oxoisothiazolo[5,4-b]pyridines (also synthesized by our group) against *Mycobacterium tuberculosis*. Therefore, beside **3**

* Corresponding author.

0014-827X/98/$ - see front matter
PII S0014-827X(98)00056-1

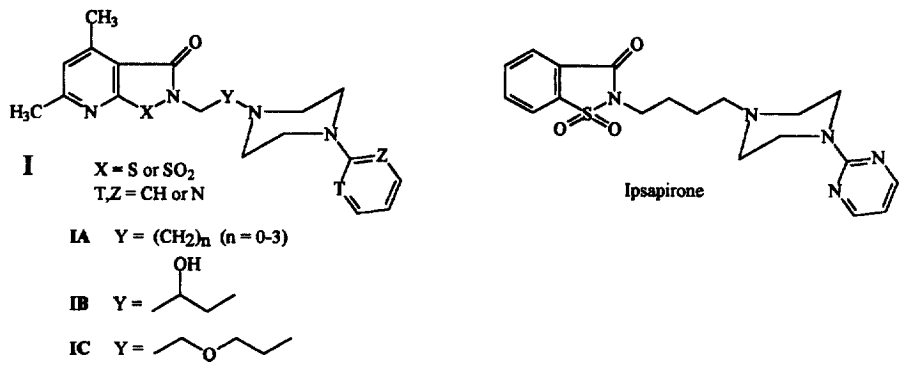
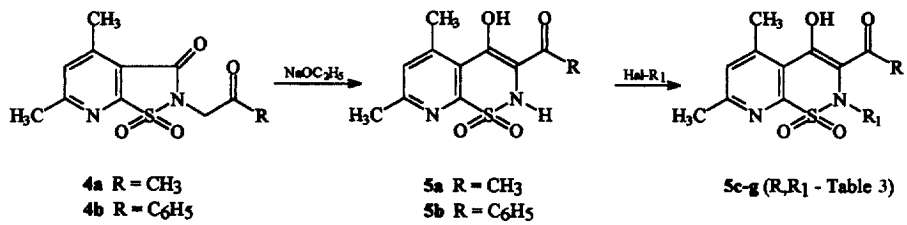


Fig. 1.



Scheme 1. Synthesis of pyrido-1,2-thiazines 5.

(Scheme 2), we prepared a set of 2-substituted-3-oxoisothiazolopyridines **2** (Scheme 2) and **4** (Table 3), characterized by the presence of widely diverse functional groups (amino, carboxyl, carbamoyl, ester, sulfoester, hydroxyl, aminoalcohol, unsaturated bond) at the 2-substituent structure. For comparison, the products of rearrangement of some isothiazolopyridine 1,1-dioxides (**4a,b**) to pyrido[3,2-e]-1,2-thiazines (**5a,b**, Scheme 1) and N_2 derivatives (**5c–g**, Table 3) of the latter were prepared as well.

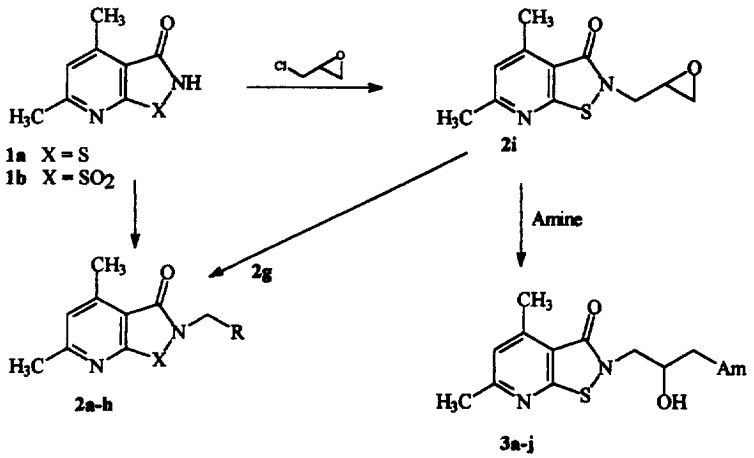
Syntheses of the new isothiazolopyridines **2** and **3** (Scheme 2) are described in this paper, whereas the procedures of preparation of isothiazolopyridines **4** [2,9,10] and pyrido-1,2-thiazines **5** [11,13] (Scheme 1) were described previously by us.

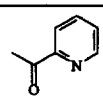
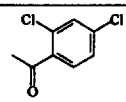
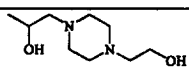
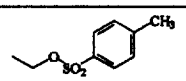
2. Chemistry

In order to synthesize the compounds **2a,c–e**, isothiazolopyridine **1a** [9] or its 1,1-dioxide **1b** [10] were alkylated with the respective bromo derivatives (Scheme 2, Section 3) using methods similar to those described for preparation **4** [9,10] (Table 3). Desired compounds **2a,c–e** were obtained in satisfactory yield (40–60%, Table 1). Amide **2b** was prepared by treating the ester **4c** (Table 3) with ammonia, sulfoester **2g** in tosylation of the known 2-hydroxyethylisothiazolopyridine (**2h**, Scheme 2) [12], whereas **2f** was obtained analogously to the synthesis of compounds **3** (Scheme 2).

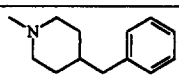
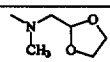
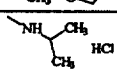
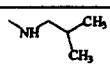
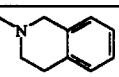
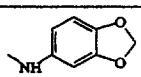
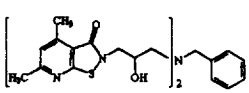
Isothiazolopyridines **3** and **2f** were synthesized using a similar procedure to the one we have previously used for the preparation of compounds of series **IB** (Fig. 1). According to this method (Scheme 2), the key intermediate, epoxypropylisothiazolopyridine **2i** [3], was prepared from isothiazolopyridine **1a** [9] and 1-chloro-2,3-epoxypropane. The compounds **3** planned, characterized by the presence of the secondary or tertiary amine grouping on the terminal carbon atom of the 2-hydroxypropyl side chain, were obtained in aminolysis of the resulting epoxide **2i** by the corresponding, commercially available, primary or secondary amines (Section 3). Reaction **2i** with primary amines in a protic (ethanol) or in an aprotic (xylene) solvent results, however, in a complex mixture (thin-layer chromatography). The predominant product of the mixture was separated by chromatography on a silica-gel column, giving the expected aminoalcohols **3f,g,i** or bis-aminoalcohol of type **3j** in low yield ($\sim$20%, Table 1).

The analytical data of all new compounds **2** and **3** were in agreement with the assigned structures (Tables 1 and 2). The ^{1}H NMR spectra of **2f,g** and **3a–j** revealed the presence of singlets at $\sim$2.6 ppm (s, 3H, CH_3) and $\sim$2.7 ppm (s, 3H, CH_3) for the methyl groups of pyridine and a singlet at $\sim$6.9 ppm (s, 1H), due to the β-pyridine proton of the isothiazolopyridine system. In the case of the 1,1-dioxides **2c–e**, these protons resonate at $\sim$2.71 ppm (s, 3H, CH_3), $\sim$2.76 ppm (s, 3H, CH_3) and $\sim$7.37 ppm (s, 1H), respectively. ^{1}H NMR data of the side chain (R) of the new compounds **2c–g**, **3a–j** are summarized in Table 2. Finally, for each compound **2–5** (Table 3) tested against *M. tuberculosis* the log of the octanol–water partition coefficient was calculated (log-P_{calc}). The calculations of the log P_{calc} values were made for the free bases, using the ChemPlus program from Hypercube (IBM PC version).



Comp.	X	R
2a	S	
2b	S	
2c	SO_2	
2d	SO_2	
2e	SO_2	
2f	S	
2g	S	
2h*	S	

*Lit.[12]

Comp.	Am
3a	
3b	
3c	
3d	
3e	
3f	
3g	
3h	
3i	
3j	

Scheme 2. Syntheses of isothiazolopyridines **2** and **3**.

3. Experimental

3.1. Chemistry

Melting points are uncorrected. Proton ^1H NMR spectra (Table 2) were obtained with a Tesla spectrometer (80 MHz). IR spectra (Table 2) were recorded on a Specord-75 IR spectrometer. All the results of the C, H, and N determinations were within $\pm 0.4\%$ of the values calculated for the corresponding formulae (Table 1). Chromatographic separations were performed on a silica-gel column [Kieselgel 60 (70–230 mesh), Merck]. Analytical thin-layer chromatography (TLC, R_f) was carried on Merck silica gel, $60F_{254}$ (alufolien), and visualized by UV.

3.1.1. (4,6-Dimethyl-3-oxo-2,3-dihydroisothiazolo-[5,4-b]pyridin-2-yl)acetic acid **2a**

A solution of 1.4 g (0.01 mol) of bromoacetic acid neutralized with 0.5N KOH to pH 7–8 was added to a solution of 1.8 g (0.01 mol) of isothiazolopyridine **1a** [9] in 20 ml 0.5N KOH water solution. The solution was stirred and heated at 40°C for 4 h, filtered with charcoal and the filtrate was acidified with hydrochloric acid and left overnight. The separated solid was filtered off, heated to boiling with 50 ml $CHCl_3$ and filtered while hot to give 0.85 g of undissolved in $CHCl_3$ substance (m.p. 208–211°C (decomp.)). The chloroform filtrate was evaporated and the residue was crystallized from benzene to give 0.3 g of the starting isothiazolo-

Table 1
Physical data of compounds 2 and 3

Comp.	Yield (%)	M.p. (°C) Solvent	Formula Molecular wt.
2a	40	210–212 (decomp.)	$C_{10}H_{10}N_2O_3S$ 238.26
2b	70	225–227 ethanol	$C_{10}H_{11}N_3O_2S$ 237.27
2c	62	214–216 ethanol	$C_{15}H_{13}N_3O_4S$ 331.34
2d	53	155–157 ethanol	$C_{16}H_{12}Cl_2N_2O_4S$ 399.24
2e	55	126–128 cyclohexane	$C_{11}H_{10}N_2O_3S$ 250.27
2f	35	137–139 acetone	$C_{17}H_{26}N_4O_3S$ 366.47
2g	60	122–124 ethanol	$C_{17}H_{18}N_2O_5S_2$ 395.46
3a	65	135–138 n-heptane	$C_{15}H_{21}N_3O_2S$ 307.41
3b	60	81–83 n-heptane	$C_{16}H_{23}N_3O_2S$ 321.43
3c	70	77–79 n-heptane	$C_{17}H_{25}N_3O_2S$ 335.46
3d	60	102–104 n-hexane	$C_{23}H_{29}N_3O_2S$ 411.56
3e	65	81–83 n-hexane	$C_{17}H_{26}N_3O_4S$ 368.47
3f	20	252–255 ethanol	$C_{13}H_{20}ClN_3O_2S$ 317.83
3g	22	70–72 n-hexane	$C_{15}H_{23}N_3O_2S$ 309.42
3h	45	116–119 cyclohexane	$C_{20}H_{23}N_3O_2S$ 369.48
3i	20	140–142 ethyl acetate	$C_{18}H_{19}N_3O_4S$ 373.42
3j	19	>95 tetrahydrofuran + ethyl ether	$C_{29}H_{33}N_5O_4S_2$ 579.71

pyridine **1a**. The crude product (m.p. 208–211°C (decomp.)) was dissolved in dilute ammonium hydroxide solution, heated to boil, filtered and the hot filtrate acidified with hydrochloric acid. After cooling the separated product **2a** (Tables 1 and 2) was filtered off.

3.1.2. (4,6-Dimethyl-3-oxo-2,3-dihydroisothiazolo-[5,4-b]pyridin-2-yl)acetamide 2b

2.6 g (0.01 mol) of ester **4c** (Table 3) [9] was suspended in 20 ml of ammonium hydroxide (25% NH_3 in water) and the mixture was stirred at room temperature for 10 h. After completion of the reaction, the mixture was diluted with 20 ml of water, the product was filtered off and purified by crystallization (**2b**, Tables 1 and 2).

3.1.3. General procedure for the preparation of compounds 2c–e

0.4 g (0.01 mol) of NaH (60% suspension in mineral oil) was added to a stirred solution of 2.1 g (0.01 mol) of isothiazolopyridine **1b** (Scheme 2) [10] in 7 ml of anhydrous *N,N*-dimethylformamide (DMF). The mixture was stirred at room temperature for 2 h, and then 0.011 mol of the appropriate bromide was added (2-(bromoacetyl)pyridine to obtain **2c**, ω-bromo-2,4-dichloroacetophenone for **2d**, propargyl bromide for **2e**). The reaction mixture was stirred for 7 h at 70°C and after cooling to room temperature was poured into water (50 ml). The precipitate formed was filtered off and purified by crystallization (**2c–e**, Tables 1 and 2).

3.1.4. 2-(4,6-Dimethyl-3-oxo-2,3-dihydroisothiazolo-[5,4-b]pyridin-2-yl)ethyl p-toluenesulfonate 2g

2.1 g (11 mmol) of *p*-toluenesulfonyl chloride was added to a solution of 1.1 g (5 mmol) of 2-hydoxyethylisothiazolopyridine **2h** (Scheme 2) [12] in pyridine (20 ml). The solution was stirred at room temperature for 24 h and then poured into water (500 ml). The precipitate formed was filtered off yielding crude tosylate **2g**. The crude product was purified by crystallization (**2g**, Tables 1 and 2).

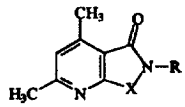
3.1.5. General procedure for the preparation of compounds 3a–e

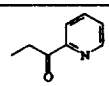
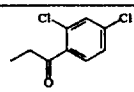
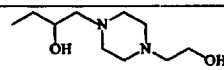
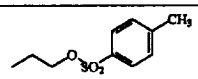
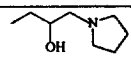
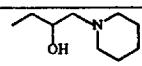
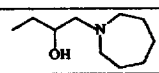
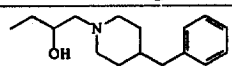
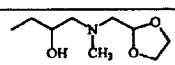
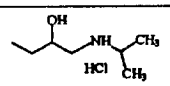
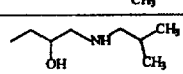
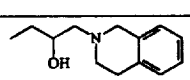
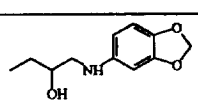
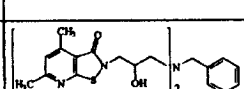
0.01 mol of the appropriate amine (pyrrolidine to obtain **3a**, cyclohexylamine for **3b**, cycloheptylamine for **3c**, 4-benzylpiperidine for **3d**, 2-methylaminomethyl-1,3-dioxolane for **3e**) was added to a stirred solution of 2.35 g (0.01 mol) of 2-epoxypropylisothiazolopyridine **2i** (Scheme 2) [3] in ethanol (30 ml). The solution was boiled under reflux for 2 h, filtered with charcoal and evaporated. The residue was purified by crystallization (**3a–e**, Tables 1 and 2).

3.1.6. General procedure for the preparation of compounds 2f and 3f–j

0.01 mol of the appropriate amine (1-(β-hydroxyethyl)piperazine to obtain **2f**, isopropylamine for **3f**, isobutylamine for **3g**, 1,2,3,4-tetrahydroisoquinoline for **3h**, 3,4-(methylenedioxy)aniline for **3i**, benzylamine for **3j**) was added to a stirred solution of 2.35 g (0.01 mol) of 2-epoxypropylisothiazolopyridine **2i** (Scheme 2) [3] in ethanol (30 ml; in xylene for compound **3i**). The solution was boiled under reflux for 2 h (5 h for **3i**), filtered with charcoal and evaporated. The residue was purified by chromatography on a silica-gel column with appropriate eluant to get the predominant product of the mixture (TLC) (for compound **2f** (R_f=0.33), methanol/acetone (1:1); for **3f** (R_f=0.3), methanol; for **3g** (R_f=0.5), methanol; for **3h** (R_f=0.33), ethyl acetate; for **3i** (R_f=0.7), ethyl acetate; for **3j** (R_f=0.4), methanol). The products **2f**, **3g–j** were crystallized from the appropriate solvents (Tables 1 and 2), whereas free base **3f** (oil, ^{1}H NMR, Table 2) was converted into hydrochloride by using dry HCl in an anhydrous Et_2O/EtOH (1:1) mixture. The precipitate formed was crystallized to give an analytically pure monohydrochloride salt **3f** (Tables 1 and 2).

Table 2
Spectroscopic data of compounds **2** and **3**



Comp.	X	R	IR (cm^{-1})	R ^{1}H-NMR ($CDCl_3$); δ, ppm
2a	S		3200-2400 (OH, acid), 1710 (C=O,acid), 1670 (C=O)	
2b	S		3330, 3180 (NH), 1720,1680 (C=O)	
2c	SO_2		1710 (C=O)	5.45 (s,2H,CH_2), 7.45-8.15 (m,3H,2×H_β + H_γ), 8.65-8.75 (m,1H,H_α)
2d	SO_2		1710 (C=O)	5.07 (s,2H,CH_2), 7.3-7.75 (m,3H, 3ArH)
2e	SO_2		3230 (=CH),1730 (C=O)	2.38 (tr,1H,CH, *J=2.5 Hz*), 4.53 (d,2H,CH_2, *J=2.5 Hz*)
2f	S		3350 (OH), 1650 (C=O)	2.4-2.65 [m,12H,2×N(CH_2)$_2$], 3.54-3.67 (m,2H,CH_2O), 3.87-4.2 (m,3H,CH_2CH)*
2g	S		1670 (C=O).	2.32 (s,3H,CH_3), 405 (tr,2H,CH_2, *J=5.2 Hz*), 4.36 (tr,2H,CH_2, *J=5.2 Hz*), 7.16 (d,2ArH, *J=8.3 Hz*), 7.73 (d,2ArH, *J=8.3 Hz*)
3a	S		3350-3150 (OH),1670 (C=O)	1.67-2.1 (m,4H,CH), 2.35-2.67 [m,6H,CH,N(CH_2)$_2$], 3.8 (br,1H,OH, exchangeable with D_2O), 3.87-4.17 (m,3H,CH_2CH)
3b	S		3350-3150 (OH),1670 (C=O)	1.42-1.66 (m,6H,CH), 2.13-2.66 [m,6H,N(CH_2)$_2$], 3.68 (br,1H,OH exchangeable with D_2O), 3.78 -4.2 (m,3H,CH_2CH).
3c	S		3350-3150 (OH),1670 (C=O)	1.1-1.74 (m,8H,CH), 2.2-2.68 [m,6H, N(CH_2)$_2$], 3.6-4.05 (m,4H,CH_2CH + OH exchangeable with D_2O).
3d	S		3350 (OH), 1670(C=O)	1.0-3.0 (m,13H,CH), 3.6 (br,1H,OH exchangeable with D_2O), 3.85-4.14 (m,3H,CH_2CH), 7.05-7.27 (m,5H,ArH).
3e	S		3400-3200 (OH), 1670 (C=O)	2.42 (s,3H,CH_3), 2.5-2.7 (m,2H, N-CH_2), 3.67 (br,1H,OH exchangeable with D_2O), 3.75-4.1 (m, 9H,CH_2CH + 4H dioxolane + CH_2 attached to C_2 of dioxolane), 4.94 (tr,1H, H_2 of dioxolane, *J=4 Hz*)
3f	S		3300 (OH), 3100-2600 (hydrochloride), 1670 (C=O)	1.05 (d,6H,2×CH_3, *J=6.3 Hz*), 2.05-3.1 (m, 3H, >CHNCH_2), 3.4 (br,2H,OH+ NH exchangeable with D_2O), 3.7-4.2 (m,3H,CH_2CH)**
3g	S		3300-2550 (NH, OH), 1670 (C=O)	0.89 (d,6H,2×CH_3, *J=6.4 Hz*), 1.7-1.85 (m,1H,-CH<), 2.12-2.92 [(m,4H, N(CH_2)$_2$] , 3.62-4.2 (m,4H,CH_2CH +OH exchangeable with D_2O)*
3h	S		3400-3200 (OH),1670 (C=O)	2.5-2.97 (m,6H, (CH_2NCH_2CH_2Ar), 3.51 (br,1H,OH, exchangeable with D_2O), 3.69-4.23 (m,5H,CH_2CH + NCH_2Ar), 6.92-7.16 (m,4H, 4ArH)
3i	S		3250-3000 (NH, OH), 1670 (C=O)	3.05-3.25 (m,2H, NCH_2), 3.5-4.25 (m,4H,CH_2CH + OH exchangeable with D_2O), 5.1(br,1H,NH exchangeable with D_2O), 5.83 (s,2H, OCH_2O), 6.07 (dd, 1H,ArH, *J= 1.9 Hz and 8.3 Hz*), 6.27 (d,1H,ArH, *J= 1.9 Hz*), 6.62 (d,1H,ArH, *J=8.3 Hz*)
3j			3380-3200 (OH), 1670 (C=O)	2.4-2.8 (m,16H,4×CH3 + 2×NCH_2), 3.0-4.1 (m, 10H, 2×CH_2CH + NCH_2Ar + 2×OH exchangeable with D_2O), 6.88 (s,2H,2×H_β), 7.24 (s,5H,5ArH)

* Position of the OH(NH) proton signal was not established.
** ^{1}H NMR for base.

3.2. Pharmacology

The primary screening of antimycobacterial activity [1] of the 23 compounds synthesized here (**2b,c,e–g, 3a,b,d,g–i**) and described previously (**4b–f, 5a–g**) in the form of bases was conducted in vitro at the level of 12.5 μg/ml against *Mycobacterium tuberculosis* H37Rv in BACTEC 12B medium using the BACTEC 460 radiometric system. The antibacterial activities as well as the lipophilic properties ($\log P_{calc}$) of the compounds tested are summarized in Table 3.

The results presented in Table 3 showed that several of the isothiazolopyridines investigated expressed in vitro antimycobacterial activity (the column labelled '% Inhibition' lists the activity of each compound), whereas the values of $\log P_{calc}$ for compounds **2–5** were within the ranges 3.88–0.74 for **2** and **4**, 4.4–2.06 for **3** and 2.03–0.21 for **5**. However, it is difficult to correlate the $\log P_{calc}$ values with the observed biological effects. For example, the most potent compound **3d** was the most lipophilic ($\log P_{calc} = 4.4$), while sulfoester **2g** with similar lipophilic properties ($\log P_{calc} = 3.88$) had no activity.

Structure–activity relationships with respect to the 2-side chain of isothiazolopyridines of the series **2–4** (Table 3) revealed the following. Firstly, among the compounds of series **3** it is evident that the presence of the phenyl ring at the structure of a base grouping of the side chain (**3d,h,i**) produces a significant increase of antitubercular activity, whereas the occurrence of cycloaliphatic residue (**3b**) on the amine group leads to an inactive compound (there are no data for **3a,g**, Table 3). Secondly, the lack of a base nitrogen atom at the structure of the 2-substituent of isothiazolopyridine (**2b,g, 4d**) and its 1,1-dioxide (**2c,e, 4b,e,f**) reduces, in general, antimycobacterial activity. An exception was ester **4c** (MIC < 12.5 μm/ml, 98% inhibition).

Finally, products rearrangement of some 1,1-dioxides of isothiazolopyridine **4** to the corresponding pyridothiazines-1,2 (**5a,b**, Scheme 1, Table 3) and N_2-functionalized derivatives of the latter (**5c–g**, Table 3) demonstrated a weak antitubercular activity and little difference in potency inhibiting of mycobacterium (MIC > 12.5, 37–28% inhibition). The only exception was compound **5f** (MIC > 12.5, 10% inhibition).

The four newly synthesized compounds **3c–f** were also subjected to the preliminary pharmacological analysis to test their central activity [2].

3.2.1. Material and methods

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 compound were administered intraperitoneally (i.p.) as a suspension in 3% Tween 80 in the constant volume of 10 ml/kg in mice and 5 ml/kg in rats. The compounds were administered in doses equivalent to 1/10 and 1/20 of LD_{50}. Control animals received the equivalent volume of solvent. Each experimental group consisted of eight animals.

The following pharmacological tests were performed:

1. Acute toxicity in mice.
2. Motor coordination in the rota-rod test in mice.
3. Spontaneous locomotor activity in mice.
4. Amphetamine-induced locomotor hyperactivity in mice.
5. Pain reactivity in the 'writhing syndrome' test in mice.
6. Pain reactivity in the 'hot-plate' test in mice.
7. Anxiolytic properties in the 'four plates' test in mice.
8. Pentetrazol-induced seizures in mice.
9. Maximal electric shock in mice.
10. Head twitches induced by 5-hydroxytryptophane in mice.
11. Arterial blood pressure in rats.

Acute toxicity was assessed by the methods of Litchfield and Wilcoxon [14] and presented as LD_{50} calculated from the mortality of mice after 24 hours.

Motor coordination was measured according to the method of Gross et al. [15]. 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.

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

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

Pain reactivity was measured by the 'writhing syndrome' test of Koster et al. [16]. 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 administration of the investigated compounds the number of writhing episodes was counted for 30 min after the injection of 0.6% acetic acid.

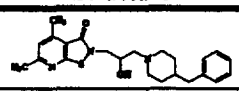
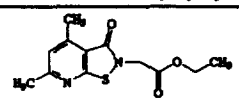
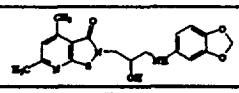
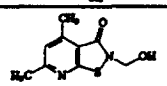
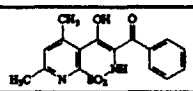
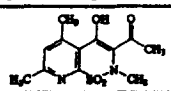
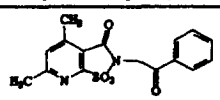
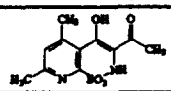
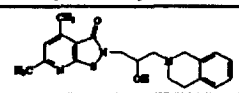
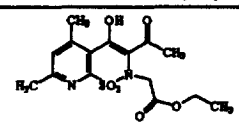
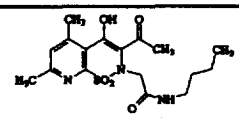
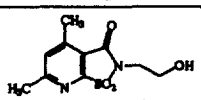
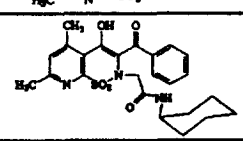
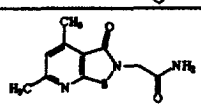
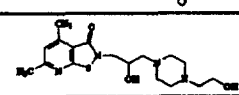
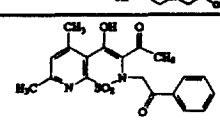
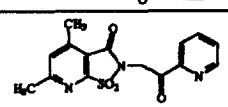
Pain reactivity was also measured in the 'hot-plate' test according to the method of Eddy and Leimbach [17]. Animals were placed individually on the metal plate heated to 56°C. The time (s) of appearance of the pain reaction (licking of the forepaws or jumping) was measured. Experiments were performed 30 min after administration of the investigated compounds.

Anxiolytic properties were assessed by the 'four plates' test in mice, according to Aron et al. [18], 60 min after administration of the investigated compounds at doses which had no effect on the spontaneous locomotor activity. Mice

[1] The antimycobacterial activity of compounds was assayed by TAACF (Tuberculosis Antimicrobial Acquisition and Coordinating Facility), National Institute of Allergy and Infectious Diseases, Southern Research Institute/GWL Hansen's Disease Center/Colorado State University.

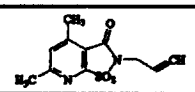
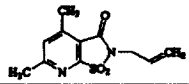
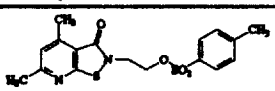
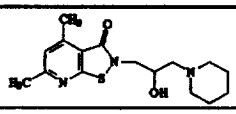
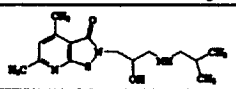
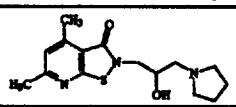
[2] Department of Pharmacology, Medical Academy, ul. Jaczewskiego 8, 20-090 Lublin, Poland.

Table 3
Antibacterial activity in vitro of isothiazolopyridines **2**, **3** and **4** and pyridothiazines-1,2 **5** against *Mycobacterium tuberculosis* H37Rv

Comp.	lit.	Structure	$logP_{calc.}$	MIC (μg/ml)*	% Inhibition
3d			4.40	< 12.5	100
4c	[9]		2.04	< 12.5	98
3i			2.88	> 12.5	93
4d	[2]		2.05	> 12.5	61
5b	[11]		1.27	> 12.5	37
5c	[11]		0.21	> 12.5	37
4b	[10]		2.25	> 12.5	36
5a	[11]		0.30	> 12.5	33
3h			3.49	> 12.5	33
5d	[11]		1.10	> 12.5	31
5e	[13]		1.65	> 12.5	29
4e	[3]		0.74	> 12.5	29
5g	[13]		2.03	> 12.5	28
2b			1.71	> 12.5	24
2f			1.09	> 12.5	15
5f	[11]		1.27	> 12.5	10
2c			1.33	> 12.5	1

(*continued*)

Table 3 (*continued*)

2e			1.45	> 12.5	0
4f	[10]		1.92	> 12.5	0
2g			3.88	> 12.5	0
3b			2.45	> 12.5	0
3g			2.59	> 12.5	**
3a			2.06	> 12.5	**

* MIC (minimum inhibitory concentration) of the standard antimycobacterial drug RMP (rifampin) = 0.25 μg/ml, 98% inhibition vs. *M. tuberculosis*.
** Compound not sufficiently mixed with assay medium, no yield.

were placed in the cages with four plate floors (11×7 cm) with a 4 mm gap between each. After 15 s of adaptation the number of crossings was counted during 1 min. Each crossing was punished with direct current (180 V, 0.5 A), but no more often than every 3 s.

Pentetrazol seizures in mice were induced by pentetrazol administration at a dose of 100 mg/kg s.c. 30 min after administration of the investigated compounds. Animals were observed for 30 min and the number of mice developing clonic and tonic seizures as well as mortality was recorded in that period.

Maximal electric shock was induced by means of alternating current (50 Hz, 25 mA, 0.2 s) with the use of ear clip electrodes according to the method of Swinyard et al. [19]. The criterion of the convulsive response was the tonic extension of the hind limbs. The test was performed 60 min after administration of the investigated compounds.

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

Arterial blood pressure was determined according to the method of Gerold and Tschirky [20] using the UGO-BASILE equipment (blood pressure recorder, Cat. No 8006). Systolic blood pressure on the tail artery was measured 30 min after administration of the investigated compounds.

3.2.2. Statistics

Results obtained were presented as means ± SEM and evaluated statistically using Student's *t*-test or the exact Fisher test.

4. Results

4.1. Acute toxicity

The LD_{50} values of the investigated compounds after their i.p. administration to mice are presented in Table 4. All tested compounds were toxic, with LD_{50} ranging from 72 (**3f**) to 149 mg/kg (**3e**).

4.2. Motor coordination

None of the investigated compounds at doses equivalent to 1/10 of LD_{50} had neurotoxic properties as they did not affect motor coordination in the rota-rod test.

4.3. Locomotor activity

Compounds **3d** and **3e** suppressed spontaneous locomotor activity during the 1 h observation period at the dose equivalent to 1/10 of LD_{50}.

4.4. Amphetamine-induced locomotor hyperactivity

Compound **3c** at a dose equivalent to 1/10 of LD_{50} decreased amphetamine-induced locomotor hyperactivity;

Table 4
Acute toxicity of compounds **3c–f**

Comp.	LD_{50} (mg/kg i.p.)	Confidence limits
3c	100.0	71.6–139.8
3d	119.6	92.3–155.0
3e	149.6	99.5–224.7
3f	72.0	51.5–100.0

other compounds (**3d**, **3e** and **3f**) administered at a dose equivalent to 1/10 of LD_{50} did not affect the excitatory action of amphetamine in mice.

4.5. Pain reactivity

Compounds **3c** at a dose of 1/10 of LD_{50} possessed analgesic activity assayed in the 'writhing syndrome' test. In the 'hot-plate' test, none of the investigated compounds expressed analgesic activity.

4.6. Anxiolytic action

Compound **3f** administered at a dose which did not affect spontaneous locomotor activity decreased the number of punished crossings in the 'four plates' test in mice; other compounds were without effect.

4.7. Pentetrazol-induced seizures

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

4.8. Maximal electric shock

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

4.9. Head twitches

None of the investigated compounds changed the number of head twitches induced by 5-HTP in mice.

4.10. Arterial blood pressure

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

5. Conclusions

The pharmacological study showed that all investigated compounds **3** are toxic and had no significant biological activity. Compound **3c** possessed a weak analgesic effect which was evident for the highest dose used and only in one of the analgesic tests performed. However, this effect may be a consequence of a general depressive action of compound **3c** on the CNS since **3c** in this dose decreased amphetamine-induced hyperactivity and decreased the arterial blood pressure.

References

[1] R. Glennon, R. Westkaemper, P. Bartyzel, Serotonin Receptor Subtypes: Basic and Clinical Aspects, Wiley-Liss, New York, 1991.
[2] W. Malinka, M. Rutkowska, Synthesis and the anorectic activity of 2*H*-4,6-dimethyl-2-[(4-phenylpiperazinyl)methyl]-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine, Farmaco 52 (1997) 595–601.
[3] W. Malinka, Synthesis and biological evaluation of some piperazine derivatives of isothiazolo[5,4-b]pyridin-3-one and its 1,1-dioxide, Acta Pol. Pharm. — Drug Res. 48 (1991) 19–21.
[4] W. Malinka, M. Sieklucka-Dziuba, G. Rajtar, D. Marowska, Z. Kleinrok, Synthesis and biological evaluation of 7-azaanalogues of ipsapirone, Farmaco 50 (1995) 769–778.
[5] J. Maignan, B. Shroot, Acne treatment with isothiazolopyridinones, Ger. Patent 3 342 538 (1984) [Chem. Abstr. 101 (1984) 116736c].
[6] J. Maignan, B. Shroot, *N*-Carbamoylisothiazolo[5,4-b]pyridin-3-one derivatives and anti-acne agent containing these compounds, Ger. Patent 3 313 778 (1983) [Chem. Abstr. 100 (1984) 121052k].
[7] B. Shroot, J. Maignan, Anti-acne composition containing isothiazolo[5,4-b]-pyridin-3-one derivative, Fr. Patent 2 555 450 (1985) [Chem. Abstr. 103 (1985) 215278c].
[8] J.L. Rainey, M.C. Seidel, Isothiazolopyridinones, US Patent 3 965 107 (1976) [Chem. Abstr. 85 (1976) 160072h].
[9] T. Zawisza, W. Malinka, Synthesis and properties of some derivatives of 2*H*-4,6-dimethylpyrido[3,2-e]isothiazolin-3-one, Farmaco 40 (1985) 124–132.
[10] T. Zawisza, W. Malinka, Synthesis and properties of some derivatives of 2*H*-4,6-dimethylpyrido[3,2-e]isothiazolin-3-one 1,1-dioxide, Farmaco 41 (1986) 676–683.
[11] T. Zawisza, W. Malinka, A novel system: 2*H*-pyrido[3,2-e]-1,2-thiazine 1,1-dioxide. Synthesis and properties of some derivatives, Farmaco 41 (1986) 819–826.
[12] W. Malinka, Synthesis and properties of 2*H*-2-(4-substituted-1-piperazinylalkyl)-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridines, Acta Pol. Pharm. — Drug Res. 47 (1990) 51–56.
[13] W. Malinka, A. Dereń, Synthesis of some amides of 4-hydroxy-5,7-dimethyl-2H-pyrido[3,2-e]-1,2-thiazine-2-acetic acid 1,1-dioxide, Pol. J. Chem. 66 (1992) 1953–1960.
[14] I.T. Litchfield, F. Wilcoxon, A simplified method of evaluating dose–effect experiments, J. Pharmacol. Exp. Ther. 96 (1949) 99–113.
[15] F. Gross, J. Tripod, R. Meier, Zur pharmakologischen Charakterisierung des Schlafmittels Doriden, Med. Wschr. 85 (1955) 305–309.
[16] R. Koster, M. Anderson, E.J. de Bear, Acetic acid for analgesic screening, Fed. Proc. Fed. Am. Soc. Exp. Biol. 18 (1959) 412.
[17] N.B. Eddy, D. Leimbach, Synthetic analgesics, II. Dithienylbutenyl and dithienylbutylamines, J. Pharmacol. Exp. Ther. 107 (1953) 385–389.
[18] C. Aron, D. Simon, C. Larousse, J.R. Boissier, Evaluation of a rapid technique for detecting minor tranquillisers, Neuropharmacology 10 (1971) 459–469.
[19] E.A. Swinyard, W.C. Brown, L.S. Goodman, Comparative assays of antiepileptic drugs in mice and rats, J. Pharmacol. Exp. Ther. 106 (1952) 319–330.
[20] M. Gerold, H. Tschirky, Measurement of blood pressure in unanaesthetized rats, Artzneim.-Forsch. 18 (1968) 1285–1287.