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Preparation of novel derivatives of pyridothiazine-1,1-dioxide and their CNS and antioxidant properties

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Abstract

Starting from isothiazolopyridine-1,1-dioxide (**1**), corresponding derivatives of 3-aryl-4-hydroxypyrido[3,2-*e*]-1,2-thiazine-1,1-dioxide (**6**) possessing the 3-[4-(substituted-phenyl)piperazinyl]propyl or 3-(4-substituted-piperidinyl)propyl side chain by the nitrogen atom of the thiazine ring were prepared. Under pharmacological central nervous system (CNS) screening in animal models (mice), all of the six pyridothiazines **6** tested exhibited analgesic action as the predominant profile of their activity ('writhing' test 12.5–50 mg/kg). Moreover, the radical scavenging activity against peroxyl radicals of the representative pyridothiazines **6** was evaluated in vitro in water environment and some of them proved to be moderate antioxidants.

Keywords: Pyrido[3,2-*e*]-1,2-thiazine-1,1-dioxides; Analgesic activity; Antioxidant properties

1. Introduction

In our earlier papers, we described synthesis, anti-inflammatory and CNS (central nervous system) activities of some derivatives of the new biheterocylic system pyrido[3,2-*e*]-1,2-thiazine of the general structure **I** (Fig. 1) [1–3]. Initially, we found that 3-carbamoyl-2-methyl derivatives **IA**, designed as 8-aza-analogues of piroxicam (Fig. 1), an antiphlogistic agent, showed a similar profile of pharmacological activity as piroxicam [2]. Furthermore, we found that replacement of the 3-carbamoyl in **IA** with the acetyl(benzoyl) group and of the 2-methyl substituent with 2-(4-phenylpiperazinylpropyl) chain lead to pyridothiazines (**IB**) which exhibited high psychopharmacological activity [3]. The compounds strongly decreased spontaneous locomotor activity (1/40–1/80 LD_{50}) (sedative action), and also showed antiamphetamine (1/80 LD_{50}) and hypothermic (1/80 LD_{50}) effects (neuroleptic action).

Introduction of a substituent to the aromatic ring of 4-phenylpiperazinyl alkyl side chain of derivatives of heterocyclic system lead in some cases to an increase in the CNS effects and behavioural responses which resulted from action on different receptor systems (mainly serotoninergic and dopaminergic [4–6]). Therefore, to enhance the central activity in 3-benzoyl derivative **IB** ($R = C_6H_5$), a series of its analogues **6a–l** (Scheme 1) variously substituted ($\mathbf{X} \neq \mathbf{H}$) at the terminal aromatic ring of the side chain were prepared (Scheme 1). Moreover, additional substituents **Y** were introduced at the *para* position of the 3-benzoyl group of certain compounds **6** to modify lipophilic properties of molecules.

This paper describes the synthesis and some of the preliminary CNS pharmacology of such derivatives of pyrido[3,2-*e*]-1,2-thiazine (**6**, Scheme 1).

Furthermore, pyridothiazines **6** were evaluated to test their peroxyl radical scavenging activity.

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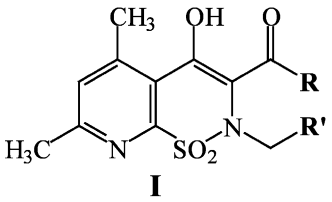
Free radical mediated process have been implicated in the pathogenesis of several diseases i.e. cancer, Alzheimer's dementia, rheumatoid arthritis [7]. Tenoxicam (Fig. 1), which is widely employed in treatment of rheumatic diseases because of its cyclo/lipooxygenase inhibitory properties [8], is also a good hydroxyl, superoxide and peroxyl radicals scavenger as it was evidenced in cell-free experiments [9]. The probable mechanism of inactivation of radical species is multiple and leads, among other species, to 4-oxyradical which is stabilized by delocalization on the β-dicarbonyl grouping (Fig. 2) [9].

Therefore, it was not illogical to test scavenging activity of pyridothiazines **6** which possess similarly to tenoxicam the β-dicarbonyl grouping partially incorporated in the 1,2-thiazine ring. For such a test, we chose compounds **6a**, **6c**, **6g**, **6g**·HCl, **6l** and their piperidine **6m**, **n** and heterocyclic (pyrimidine) **6o** analogues (Scheme 1). The synthesis of the last compounds was described recently [3].

2. Synthesis of compounds

The desired compounds of general formula **6** were synthesized as shown in Scheme 1.

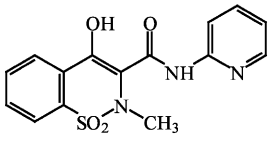
The synthesis of the key intermediates **3a**–**e** was achieved by condensation of isothiazolopyridine-1,1-dioxide (**1**) [10] with ω-bromoacetophenone or *p*-substituted ω-bromoacetophenones (commercially available) with formation of **2a**–**e** and the subsequent rearrangement of these compounds to the corresponding pyridothiazines **3a**–**e**. The mechanism of rearrangement of isothiazolopyridine **2a** to pyridothiazine **3a** under basic condition (anhydrous ethanol, $NaOC_2H_5$) has been studied recently in our laboratory [1]. The expected pyridothiazines **3b**–**e** were obtained similarly.



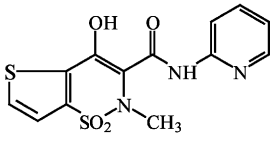
I

IA **R** = NH(C_6H_5, hetroarom.), **R'** = H

IB **R** = CH_3, C_6H_5, **R'** =



Piroxicam



Tenoxicam

Fig. 1.

The new final compounds **6a**–**n** (Scheme 1) were prepared by alkylation of pyridothiazines **3** with corresponding 4-(substituted-phenyl)-1-(3-chloropropyl)piperazines (**4**) or -piperidines (**5**). The reaction was carried out in close analogy to the methods described in our previous paper [3], and produced the expected compounds **6** in moderate yield (40–55%). Piperazine intermediates **4b**, **c** were obtained from corresponding commercially accessible *N*-arylpiperazines and 1-bromo-3-chloropropane according to the method described in Ref. [11] and our preceding paper [12]. Compound **4a** and piperidine intermediates **5a**, **b** were prepared analogously (exp. part).

The physical data associated with the final compounds **6a**–**n** and intermediates (**2b**–**e**, **3b**–**e**, **4a**, **5a**, **b**) are summarized in Table 1 and exp. part, respectively. It is noteworthy that pyridothiazines **3** and **6**, as fully enolized β-diketones, did not show the typical carbonyl absorption, instead a broad, intense band at range 1600–1560 cm^{-1} was observed. The enolization of **3** was confirmed by the ^1H NMR spectra, which showed the presence of a peak ~16 ppm (OH enole) in addition to that of sufonamide NH (9.2 ppm **3a** [1]). The position of the enol proton in ^1H NMR spectra of series **6**, like in **IB** (Fig. 1) [3], was not established; however these compounds, similar to **3**, give intense colouration with iron(III) chloride and in the IR (KBr) spectra a broad band between 2750 and 2200 cm^{-1} indicating the presence of an enolic OH group. Because the spectral data within the series of the new compounds **2b**–**e**, **3b**–**e** and **6a**–**n** did not show remarkable differences, they are presented for selected derivatives in the Section 5.

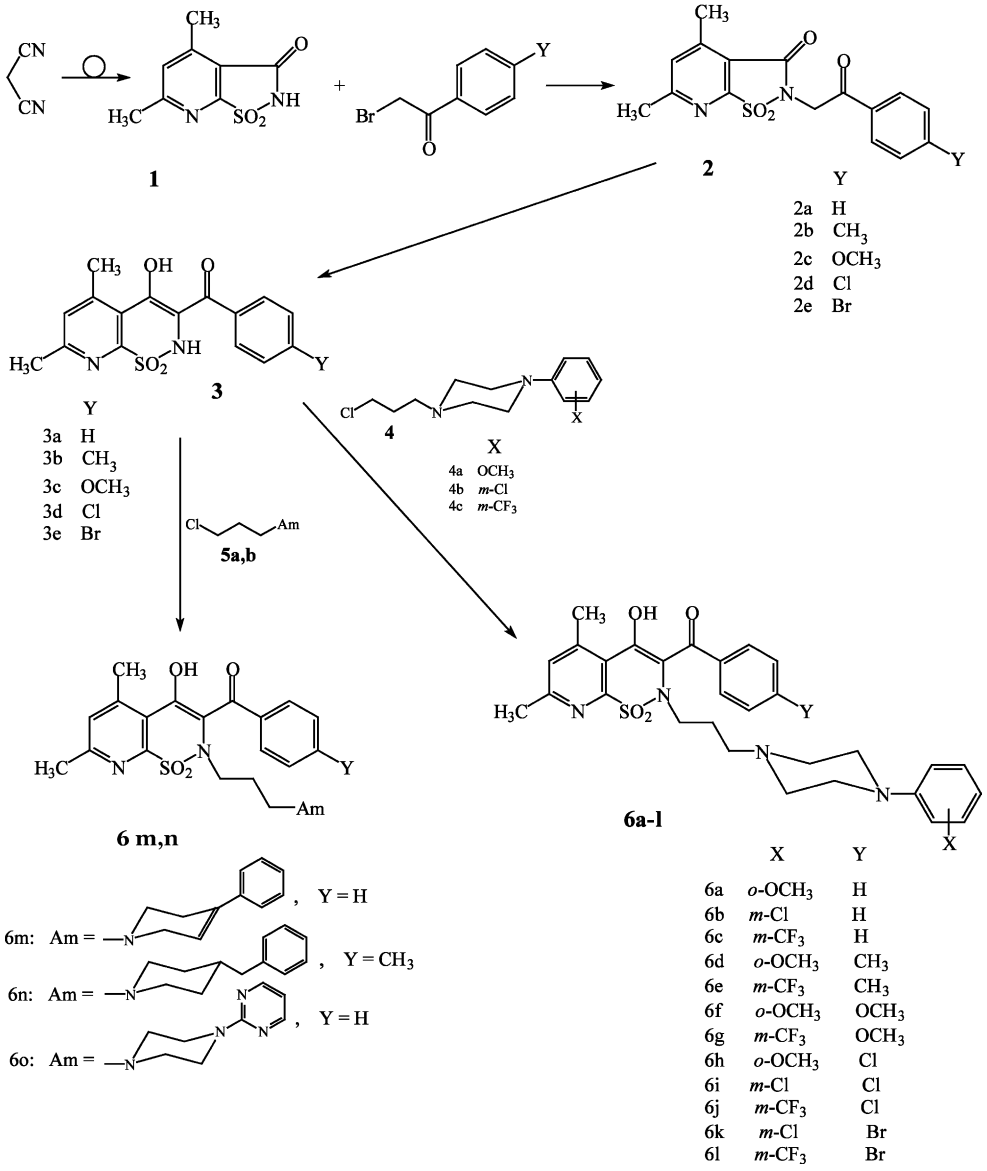
Finally, for each compound **6** tested at the pharmacological screening, the log of the octanol–water partition coefficient was calculated (log P_{calc}). The calculations of the log P_{calc} values were made for the free bases, using the CHEMPLUS program from Hypercube, Inc., IBM PC version. The calculated lipophilicities of these compounds are shown in Table 1.

3. Pharmacological screening and discussion

Compounds **6b**, **f**–**h**, **j**, **k** representing a new series of pyrido[3,2-*e*]-1,2-thiazines, were evaluated in animal models for acute toxicity (ip) and for their influence on the spontaneous locomotor activity, thiopental anaesthesia, carrageenan induced edema and analgesic activity in the 'writing syndrome' and 'hot-plate' tests.

3.1. Acute toxicity

All the investigated compounds **6b**, **6f**–**h**, **j**, **k** were not toxic. The LD_{50} values of the compounds assayed after their intraperitoneal administration were above 2000



Scheme 1.

and 200 mg/kg (1/10 of LD_{50}) was taken to be the initial dose in the further experiments.

3.2. Locomotor activity

The pyridothiazines **6**, given intraperitoneally in doses of 100 and 50 mg/kg (1/20 and 1/40 of LD_{50}), decreased locomotor activity during 30 min observation period. The most potent effect was produced by compounds **6b**, **6j** and **6g**, which significantly inhibited the spontaneous locomotor activity of mice at the doses up to 50 mg/kg (1/40 of LD_{50}). Compounds **6h**, **6k** and **6f** produced a significant decrease in locomotor activity at the doses up to 100 mg/kg (1/20 of LD_{50}). The ED_{50} values and therapeutic index for the compounds investigated are presented in Table 2.

3.3. Thiopental anaesthesia

The effect of investigated compounds on the thiopental anaesthesia is presented in Table 3. Compounds **6b**, **6f**, **6h**, **6j** and **6k**, given in doses of 100 and 50 mg/kg (1/20 and 1/40 of LD_{50}), significantly prolonged barbiturate sleep in mice by 292–193 and 172–129%, respectively. Compound **6g** also prolonged the time of anaesthesia (by ca. 244%), but this effect was significant only after dose of 100 mg/kg (1/20 of LD_{50}).

3.4. 'Writhing syndrome' test in mice

The investigated compounds were tested for analgesic activity by intraperitioneal administration in mice in terms of the inhibition of the 'writhing syndrome'

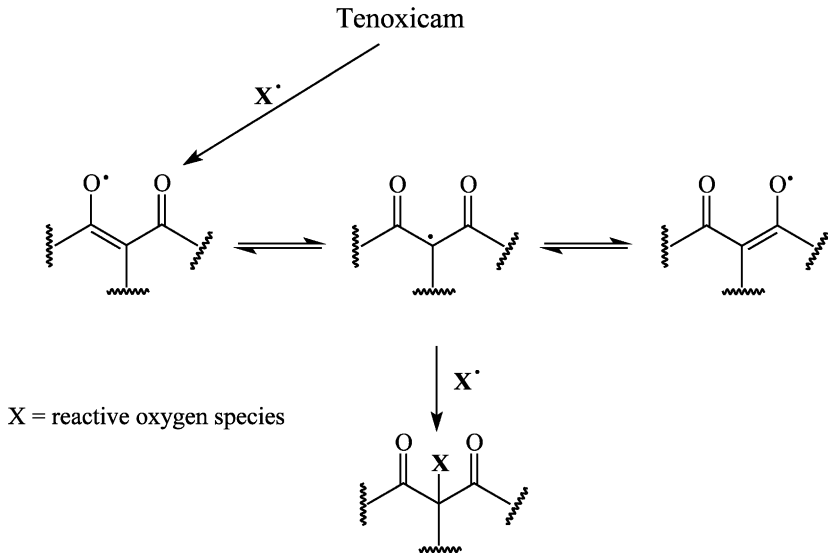


Fig. 2.

induced by phenylbenzoquinone. All compounds tested showed strong analgesic activity in this test. The most potent effects were produced by compounds **6b** and **6j** which were significant active up to a dose of 12.5 mg/kg (1/160 of LD_{50}). Compounds **6h**, **6f** and **6g** were significantly active in the 'writhing syndrome' test in mice up to a dose of 25 mg/kg (1/80 of LD_{50}), while compound **6k** had analgesic activity in doses up to 50 mg/kg (1/40 of LD_{50}). The ED_{50} values of the compounds tested and standards (acetylsalicylic acid, morphine) are summarized in Table 4.

3.5. 'Hot plate' test in mice

All investigated compounds showed significant activity in the 'hot plate' test only at the dose of 200 mg/kg (1/10 of LD_{50}; data not shown).

3.6. Influence on the blood pressure

All investigated compounds, injected intraperitoneally in a single dose corresponding to ED_{50} in the 'writhing syndrome' test did not affected the pulse rate and arterial blood pressure in the normotensive rats (data not shown).

3.7. Carrageenan-induced edema

The tested compounds, given at a dose of 100 mg/kg 1 h before carrageenan injection, did not inhibit the postcarrageenan edema of rat paw, which suggest, that these compounds have no influence on the inflammatory process.

Table 1
Physical data of the compounds **6**

Comp.	M.p. (°C) (crystal from)	Formula (M.w.)	Log P_{calc}
6a	123–125 (EtOH)	$C_{30}H_{34}N_4O_5S$ (562.68)	2.92
6b	175–177 (EtOH)	$C_{29}H_{31}ClN_4O_4S$ (567.10)	3.69
6c	179–181 (EtOH)	$C_{30}H_{31}F_3N_4O_4S$ (600.65)	4.06
6d	212–214 (EtOH)	$C_{31}H_{36}N_4O_5S$ (576.71)	3.39
6e	182–184 (EtOH)	$C_{31}H_{33}F_3N_4O_4S$ (614.68)	4.52
6f	182–184 (EtOH)	$C_{31}H_{38}N_4O_6S$ (592.71)	2.67
6g	130–132 (EtOH)	$C_{31}H_{33}F_3N_4O_5S$ (630.68)	3.80
6h	219–221 (EtOH–acetone 1:1)	$C_{30}H_{33}ClN_4O_5S$ (583.14)	3.44
6i	214-216 (EtOH–acetone 1:1)	$C_{29}H_{30}Cl_2N_4O_4S$ (601.54)	4.21
6j	190–192 (EtOH–acetone 1:1)	$C_{30}H_{30}ClF_3N_4O_4S$ (635.10)	4.57
6k	205–207 (acetone)	$C_{29}H_{30}BrClN_4O_4S$ (632.01)	4.48
6l	195–197 (EtOH)	$C_{30}H_{30}BrF_3N_4O_4S$ (678.55)	5.12
6m	175–177 (EtOH)	$C_{30}H_{31}N_3O_4S$ (529.65)	4.92
6n	199–201 (EtOH)	$C_{32}H_{37}N_3O_4S$ (559.72)	4.35
6o	Lit. [3]	2.00	

3.8. Conclusion

The results of the present CNS study of pyridothiazines **6b**, **f**–**h**, **j**, **k** clearly demonstrated that intraperitoneal administration of the compounds significantly suppressed the spontaneous locomotor activity and increased barbituric-induced sleep in the mice. These effects suggest that new pyridothiazines assayed possess sedative activity on the CNS of mice. Additionally, compounds exhibit significant analgesic action determined in the phenylbenzoquinone-induced 'writhing'

Table 2
Influence of the investigated compounds **6** on the spontaneous locomotor activity in mice

Comp.	Dose (mg/kg)	No. of impulses ± SEM after 30 min	ED_{50} (mg/kg)	Therap. index
Control		222.4 ± 48.3		
6b	100	34.0 ± 19.1 **	36.6	> 54.6
	50	84.2 ± 14.2 ***	(17.1–78.3)	
	25	145.0 ± 41.0		
6h	100	39.4 ± 16.2 **	37.1	> 53.9
	50	86.2 ± 21.0	(18.5–74.2)	
6j	100	26.4 ± 8.2 **	31.4	
	50	79.4 ± 17.4 *	(13.7–71.6)	> 63.7
	25	127.0 ± 19.6		
6k	100	74.3 ± 20.3 *	46.1	> 43.4
	50	92.4 ± 25.1	(17.1–124.5)	
6f	100	46.2 ± 19.0 **	34.4	> 58.1
	50	114.0 ± 17.6	(12.3–96.3)	
6g	100	35.6 ± 17.3 **	24.7	
	50	54.2 ± 20.0 *	(10.6–57.8)	> 81.0
	25	117.0 ± 16.4		

Each group consisted of 6–8 animals.
* $P < 0.05$.
** $P < 0.01$
*** $P < 0.001$.

Table 3
Influence of the investigated compounds **6** on the thiopental anaesthesia

Comp.	Dose (mg/kg)	Time of duration of anaesthesia ± SEM (min.)	% of control
Control		36.0 ± 10.1	
6b	100	110.0 ± 14.4 ****	205.5
	50	84.2 ± 14.2 ***	133.9
	25	42.1 ± 12.4	16.9
6h	100	141.0 ± 10.2 ****	291.7
	50	98.0 ± 12.1 ***	172.2
	25	42.0 ± 11.1	16.7
6j	100	138.0 ± 21.2 ***	283.3
	50	82.4 ± 18.2 **	128.9
	25	51.0 ± 12.4	41.7
6k	100	105.4 ± 29.1 **	192.8
	50	88.9 ± 18.2 *	146.9
	25	39.2 ± 17.1	8.9
6f	100	109.4 ± 18.8 ***	203.9
	50	84.2 ± 21.8 *	133.9
	25	44.8 ± 19.1	24.4
6g	100	124.0 ± 14.1 ***	244.4
	50	54.2 ± 12.5	50.5

Each group consisted of 6–8 animals.
* $P < 0.05$.
** $P < 0.02$.
*** $P < 0.01$.
**** $P < 0.001$.

test and weak analgesic effect in the 'hot-plate' test in mice, i.e. two behaviourally different models. The analgesic potency of the compounds in 'writhing' test was 1.5–4 times higher than that of acetylsalicylic acid and 4–10 times weaker than that of morphine (Table 4). The 'writhing' test in mice has been used by many investigators for measuring peripheral analgesic activity, whereas the 'hot-plate' test for evaluating central

analgesia [13]. Then we initially presumed that compounds **6** possess strong peripheral analgesic action [50–12.5 mg/kg (1/40–1/160 of LD_{50})] with partially weak central analgesic effect, observed only at the highest dose equivalent to 1/10 of LD_{50} (200 mg/kg). Therefore, the compounds were also screened for their anti-inflammatory activity using carrageenan-induced hind paw edema model in mice. However, all pyridothiazines **6** were inactive at this model of inflammation. Due to interesting analgesic properties of compounds **6**, further studies will be performed in order to define the mechanism of their analgesic action.

Lipophilicity of compounds **6b**, **f**–**h**, **j**, **k**, expressed as log P_{calc}, was differentiated and ranged from 2.7 to 4.6 (Table 1). However, it is difficult to correlate these parameters with potency of analgesic action of compounds observed in the 'writhing' test (Table 4). For example, the value of log P_{calc} (4.57) for the most active compound **6j** (12.5 mg/kg; **X** = *m*-CF_3, **Y** = *p*-Cl) was close to the value of log P_{calc} ~4.48 of the less active compound **6k** (50 mg/kg; **X** = *m*-Cl, **Y** = *p*-Br) in this test, although electron-attracting **X** and **Y** substituents in both compounds occupy the same positions at the aromatic rings.

4. Peroxyl radical scavenging activity of compounds 6

Compounds **6a**, **6c**, **6g**, **6g**·HCl, **6l**–**o** were evaluated for the free peroxyl radical scavenging activity using total peroxyl radical-trapping antioxidant potential (TRAP) measurements. The modified Valkonen and Kuusi method was used in this study [14]. All investigated compounds and reference substances [*N*-acetyl-L-cysteine (NAC), cyanidin (CC)] were evaluated in the final concentration 0.1 mM. However, it should be noted that for compounds **6a**, **6c**, **6m** there are not data on account of their not sufficient solubility in the test system.

Table 4
Influence of the investigated compounds **6** on the pain reactivity in the 'writhing syndrome' test in mice

Comp.	Dose (mg/kg)	Mean no. of writhings ± SEM	ED_{50} (mg/kg)
Control	0	19.8 ± 2.0	
6b	50	2.5 ± 1.2 ****	
	25	5.2 ± 2.7 ****	10.2
	12.5	6.6 ± 3.8 ***	(4.3–24.0)
	6.25	14.1 ± 3.1	
6h	50	4.7 ± 2.0 ****	
	25	5.9 ± 2.2 **	14.8
	12.5	11.5 ± 4.3	(5.5–40.0)
6j	50	5.8 ± 2.4 ***	
	25	5.2 ± 1.6 ***	9.4
	12.5	6.8 ± 2.8 **	(2.8–31.5)
6k	6.25	13.2 ± 2.4	
	50	4.6 ± 2.4 ***	25.05
	25	12.4 ± 4.3	(15.3–41.1)
6f	50	7.7 ± 2.4 ***	
	25	9.2 ± 2.2 *	26.4
	12.5	13.2 ± 2.4	(7.5–92.9)
6g	50	4.7 ± 2.3 ****	
	25	7.7 ± 3.2 ***	22.2
	12.5	14.7 ± 3.9	(11.4–43.3)
Control	0	19.2 ± 3.2	
Acetylsalicylic acid	100	3.2 ± 1.2 ****	
	50	8.5 ± 1.3 **	39.15
	30	11.2 ± 2.1	(29.1–48.4)
Morphine	10	1.2 ± 0.8 ****	
	3	7.5 ± 2.9 **	2.44
	1	16.2 ± 3.5	(1.18–5.02)

Each group consisted of 6–8 animals.
* $P < 0.05$.
** $P < 0.02$.
*** $P < 0.01$.
**** $P < 0.001$.

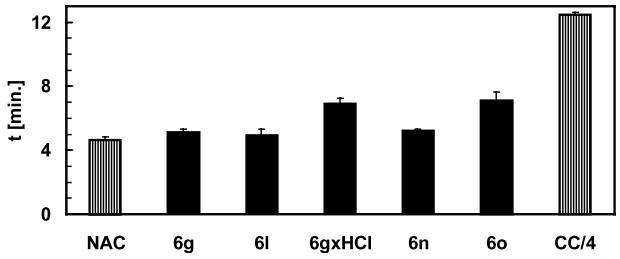


Fig. 3. Antioxidant capacity of NAC, compounds 6 and CC. Data presented as means ± SD.

The ability of pyridothiazines **6** to inhibit peroxyl radicals generated by thermal decomposition of AAPH is summarized in Fig. 3.

The data from Fig. 3 furnished evidence that every pyridothiazines **6** tested, without exception, possess antioxidant component at their structure. The antioxidant potential of these compounds is similar to that of NAC and four times lower then cyanidin used as standards. Based on the partial similarity of compounds **6** and tenoxicam we may assume that mechanism of their antioxidant action may by similar to that proposed for tenoxicam (Fig. 2).

In addition the relationship between the log of the octanol–water partition coefficients (log P_{calc}; Table 1) and potency of antioxidant properties of compounds **6** (Fig. 3) was investigated. The compounds (bases) exhibited a wide range value of log P_{calc} (1.72–4.85) and in general increase of lipophilicity lower their antioxidant properties [compare **6g** (log $P_{calc} = 3.80$) and its salt **6g**·HCl; **6g** and **6o** (log $P_{calc} = 2.00$)]. The same trend was observed in series of derivatives of pyrrolo[1,2-b]pyridazine [15]. Moreover, similar effects of antioxidant action and lipophilicity of compounds **6g**, **6l** (log $P_{calc} = 3.80$ and 4.85, respectively) and their 4-benzylpiperidine analogue **6n** (log $P_{calc} = 4.35$) demonstrated that terminal arylpiperazinyl grouping of the 2-side chain is not essential for antioxidant action in the series of pyridothiazines type **6**.

4.1. Conclusion

The results of the preliminary evaluation of pyridothiazines **6** suggest that these compounds are not toxic ($LD_{50} > 2000$ mg/kg), possess sedative influence on the CNS in mice and significant peripheral analgesic activity, notably **6b** and **6j** (12.5 mg/kg in 'writhing' test). However, the analgesic activity shown by compounds **6** in the 'writhing' test was not accompanied by their inhibition of carrageenan induced endema. Moreover, several of title pyridothiazines possess peroxyl radical scavenging activity of equal potency to that found in NAC. In the light of nonspecific CNS effects (analgesia, depression of locomotor activity, prolongation of barbiturate induced sleeping time) further investigations in series **6** should be directed towards syntheses of analogues with reduced central activity in relation to antioxidant properties. Syntheses of such compounds are under consideration.

5. Chemical experimental section

M.p.s 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(KBr) spectra were recorded on Specord-75 IR spectrometer. Elemental C, H, N analyses were run on a Carlo Erba NA-1500 analyser, 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).

5.1. General procedure for the preparation of 2-(4-substituted-phenacyl)isothiazolopyridine-1,1-dioxides 2b–e

A solution of 1.05g (5 mmol) of isothiazolopyridine-1,1-dioxide (**1**) [10], 0.7 ml (5 mmol) of $N(C_2H_5)_3$ and 5 mmol of corresponding ω-bromoacetophenone (2-bromo-4′-methylacetophenone for obtaining (**2b**), 2-bromo-4′-methoxyacetophenone (**2c**), 2-bromo-4′-chloroacetophenone (**2d**), 2,4′-dibromoacetophenone (**2e**)) in 7 ml of DMF was stirred overnight at room temperature. The mixture was then diluted with ice-water and the separated precipitate was filtered off. The resulting crude products **2b–e** were purified by crystallization from appropriate solvents; yield: 50–70%.

2b: *Anal*. $C_{17}H_{16}N_2O_4S$ (m.w. 344.38); m.p. 177–179 °C (EtOH). ^{1}H NMR: 2.43 s (3H, CH_3–Ar), 2.71 s (3H, CH_3), 2.74 s (3H, CH_3), 5.11 s (2H, CH_2), 7.3 d (2H, 2ArH) and 7.8 d (2H, 2ArH). $J = 7.8$ Hz, 7.36 s (1H, 5-H_β-pyridine). IR: 1730 and 1700 (CO).

Similar ^{1}H NMR and IR data occur in all derivatives of general formula **2** [**2c**: $C_{17}H_{16}N_2O_5S$ (m.w. 360.38), m.p. 206–208 °C (MeOH); **2d**: $C_{16}H_{13}ClN_2O_4S$ (m.w. 364.80), m.p. 229–231 °C ($CHCl_3$); **2e**: $C_{16}H_{13}BrN_2O_4S$ (m.w. 409.25), m.p. 202–204 °C (EtOH)].

5.2. Rearrangement of isothiazolopyridines 2b–e to the corresponding derivatives of 3-(4′-substituted-aroyl)-4-hydroxypyridothiazines 3b–e

Three millimole of corresponding isothiazolopyridine-1,1-dioxide **2b–e** (**2b** for obtaining **3b**, **2c** for **3c**, **2d** for **3d**, **2d** for **3d**) in EtOH solution of EtONa (7.5 ml), prepared from 2.3 g of Na and 100 ml of anhydrous EtOH, was warmed up to 55–60 °C and maintained at these temperatures with well stirring for 15 min. The resulting mixture was then diluted with ice-cold water (40 ml), filtered with charcoal and the filtrate was

acidified with 5% aq. HCl. Precipitated product was filtered off and purified by crystallization from EtOH to give **3b–e** (yield: 45–60%).

3b: *Anal*. $C_{17}H_{16}N_2O_4S$ (m.w. 344.38)); m.p. 230–232°C. ^{1}H NMR: 2.42 s (3H, CH_3–Ar), 2.63 s (3H, CH_3), 2.77 s (3H, CH_3), 7.27 d (2ArH) and 7.97 d (2ArH). $J = 8.2$ Hz, 7.28 s (1H, 6-H_β-pyridine), 16.4 br (1H, 4-OH, enol, D_2O exchangeable), position of 2-NH signal was not established. IR: 3200 (NH), 1610–1540 [C(OH)=C–C(O)].

Similar ^{1}H NMR and IR data occur in all derivatives of general formula **3** [**3c**: $C_{17}H_{16}N_2O_5S$ (m.w. 360.38), m.p. 226–228°C; **3d**: $C_{16}H_{13}ClN_2O_4S$ (m.w. 364.80), m.p. 240–242°C; **3e**: $C_{16}H_{13}BrN_2O_4S$ (m.w. 409.25), m.p. 242–244°C].

5.3. 4-o-Methoxyphenyl-1-(3-chloropropl)piperazine (*4a*)

Compound **4a** was prepared in analogy to the procedure described in Ref. [12], starting from 1.9 g (12 mmol) of 1-bromo-3-chloropropane and 1.9 g (10 mmol) of *N*-(*o*-methoxyphenyl)piperazine. The crude product was purified with CC [$R_f = 0.6$ (EtOAc), m.p. 41–43°C (65% yield; 51–52 °C (petroleum ether)].

^{1}H NMR: 1.85–2.2 m (2H, $CH_2\mathbf{CH_2}CH_2$), 2.5–2.8 m [6H, $CH_2N(CH_2)_2$], 3.05–3.2 m [4H, $ArN(CH_2)_2$], 3.61 t (2H, CH_2Cl, $J = 6.4$ Hz), 3.84 s (3H, OCH_3), 6.9–7.05 m (4H, ArH).

5.4. 4-Phenyl-1-(3-chloropropyl)-1,2,3,6-tetrahydropyridine (*5a*) and 4-benzyl-1-(3-chloropropyl)piperidine (*5b*)

Compounds **5a** and **5b** were prepared from 1-bromo-3-chloropropane and 4-phenyl-1,2,3,6-tetrahydropyridine or 4-benzylpiperidine, respectively, in analogy to the procedure described in Ref. [12]. The crude products were purified with CC (EtOAc): **5a** ($R_f = 0.7$, m.p. 38–40°C, 45% yield); **5b** ($R_f = 0.57$, oil, 55% yield). **5b** was dissolved at C_3H_6O, acidified with HCl (saturated EtOH solution) and the resulting precipitate was filtered off to give **5b**·HCl [*Anal*. $C_{15}H_{22}ClN$·HCl (m.w. 288.26), m.p. 162–165°C].

5a: *Anal*. $C_{14}H_{18}ClN$ (m.w. 235.75). ^{1}H NMR: 1.85–2.25 m (2H, $CH_2\mathbf{CH_2}CH_2$), 2.55–2.77 m (6H, NCH_2+2′,3′-CH–piperidine), 3.2 d (2H, 6′CH–piperidine, J = 2.4 Hz), 3.65 t (2H, Cl–CH_2, $J = 6.4$ Hz), 6.06 t (1H, 5′CH–piperidine, $J = 2.4$ Hz), 7.25–7.5 m (5H, ArH).

5b: ^{1}H NMR: 1.2–2.1 m (9H, $CH_2\mathbf{CH_2}CH_2$+3′,4,′5′-CH–piperidine+NCH_2), 2.3–2.95 m [6H, 2′, 6′CH–piperidine+$ArCH_2$], 3.55 t (2H, Cl–CH_2, $J = 6.8$ Hz), 7.0–7.4 m (5H, ArH).

5.5. General procedure for the preparation of pyridothiazines *6a–n*

To the stirred mixture of 5 mmol of pyridothiazine **3** in 20 ml of anhydrous EtOH, was added 5 ml of EtOH solution of EtONa, prepared from 2.3 g of Na and 100 ml of anhydrous EtOH. After 1 h, 5 mmol of corresponding 4-(substituted-phenyl)-1-(3-chloropropyl)piperazine **4** or 4-substituted-1-(3-chloropropyl)piperidine **5** [**3a** [1] and 4-*o*-methoxyphenyl-1-(3-chloropropyl)piperazine (**4a**) was added for obtaining **6a**; **3a** and 4-*m*-chlorophenyl-1-(3-chloropropyl)piperazine (**4b**) [11,12] for **6b**, **3a** and 4-*m*-trifluoromethylphenyl-1-(3-chloropropyl)piperazine (**4c**) [12] for **6c**; **3b** and **4a** for **6d**; **3b** and **4c** for **6e**; **3c** and **4a** for **6f**; **3c** and **4c** for **6g**; **3d** and **4a** for **6h**; **3d** and **4b** for **6i**; **3d** and **4c** for **6j**; **3e** and **4b** for **6k**; **3e** and **4c** for **6l**; **3a** and 4-phenyl-1-(3-chloropropyl)-1,2,3,6-terahydropyridine (**5a**) for **6m**, **3b** and 4-benzyl-1-(3-chloropropyl)piperidine (**5b**) for **6n**] and the reaction mixture was refluxed with stirring for 15 h. Ethanol was distilled off, the residue was treated with 50 ml of $CHCl_3$ and insoluble materials were filtered off. The filtrate was then evaporated and the residue was purified by crystallization from appropriate solvents (Table 1) to give **6** (yield: 45–55%).

6d: ^{1}H NMR: 1.1–1.5 m (2H, $CH_2\mathbf{CH_2}CH_2$), 1.95–2.1 m (2H, $CH_2CH_2\mathbf{CH_2}$N–piperazine), 2.25–2.4 m [4H, $N(CH_2)_2$], 2.44 s (3H, CH_3Ar), 2.69 s (3H, CH_3), 2.79 s (3H, CH_3), 2.9–3.4 m [6H, $(CH_2)_2$N–Ar+2-NCH_2], 3.83 s (3H, OCH_3), 6.8–7.0 m (4H, ArH), 7.2–7.4 m (3H, 2ArH+H_β-pyridine), 7.95–8.1 m (2H, ArH), position of 4–OH (enol) signal was not established. IR: 2750–2200 (OH, enol), 1600–1560 [C(OH)=C–C(O)].

6k: ^{1}H NMR ($CDCl_3$): 1.05–1.7 m (2H, $CH_2\mathbf{CH_2}CH_2$), 1.9–2.4 m [6H, $CH_2N(CH_2)_2$], 2.7 s (3H, CH_3), 2.78 s (3H, CH_3), 2.95–3.3 m [6H, 2-NCH_2+(CH_2)N–Ar], 6.7–7.35 m (5H, 4ArH+H_β-pyridine), 7.64 d (2H, ArH, $J = 8.6$ Hz), 8.0 d (2H, ArH, $J = 8.6$ Hz), position of 4-OH (enol) signal was not established, IR: 2750–2200 (OH, enol), 1600–1560 [C(OH)=C–C(O)].

Similar ^{1}H NMR and IR data occur in derivatives **6a–c**, **e–j**, **l**.

6m: ^{1}H NMR: 1.25–1.6 m (2H, $CH_2\mathbf{CH_2}CH_2$), 2.1–2.6 m (6H, N–CH_2+2′,3′CH–piperidine), 2.68 s (3H, CH_3), 2.78 s (3H, CH_3), 2.9–3.05 m (2H, 6′CH–piperidine), 3.1–3.4 m (2H, 2–NCH_2), 5.9–6.05 m (1H, 5′CH–piperidine), 7.25–7.65 m (9H, 8ArH+H_β-pyridine), 8.0–8.35 (m, 2H, ArH), position of 4-OH (enol) signal was not established, IR: 2750–2200 (OH, enol), 1600–1560 [C(OH)=C–C(O)].

6n: ^{1}H NMR: 0.95–2.0 m (7H, $CH_2\mathbf{CH_2}CH_2$+3′,4′,5′-CH–piperidine), 2.35–2.8 m (17H, 3×CH_3+$CH_2N(CH_2)_2$+CH_2Ar), 3.0–3.3 m (2H, 2-NCH_2), 7.0–7.4 m (8H, 7ArH+H_β-pyridine), 7.7–8.2 (m, 2H, ArH), position of 4-OH (enol) signal was not esta-

blished, IR: 2750–2200 (OH, enol), 1600–1560 [C(OH)=C–C(O)].

For compound **6g**, hydrochloride was prepared by dissolving base **6** in C_3H_6O, acidification with HCl MeOH solution and filtration of the resulting precipitate.

6. Pharmacological experimental section

6.1. Substances used

Thiopental Natrium (HEFA-FRENON Arzneimittel, Germany), phenylbenzoquinone (INC Pharmaceuticals, Inc. NY), Carrageenin (Viscarin, Marine Colloids Inc.)

6.2. Animals

The experiments were carried out on male Wistar rats (body weight 120–250 g) and male Albino-Swiss mice (body weight 18–26 g). Animals were housed in constant temperature facilities exposed to 12:12 h light–dark cycle and maintained on a standard pellet diet and tap water was given ad libitum. Control and experimental groups consisted of 6–8 animals each. The investigated compounds were administered intraperitoneally as the suspension in 0.5% methylcellulose in constant volume of 10 ml/kg (mice) and 1 ml/kg (rats). The statistical significance was calculated using a one-way ANOVA or Student's *t*-test.

6.3. Acute toxicity

The investigated compounds were injected intraperitoneally in increasing doses up to 2000 mg/kg. Each dose was given to six animals. The number of dead mice was assessed 24 h after the injection. LD_{50} were calculated according to the method of Litchfield and Wilcoxon [16].

6.4. Locomotor activity

The investigated compounds were injected intraperitoneally in doses of 100–25 mg/kg, equivalent to 1/80–1/20 of LD_{50}. Thirty minutes later, the mice were placed in a cage with a photocell which registered the numbers of movements of the animals, during the first 30 min.

6.5. Thiopental anaesthesia

Thiopental in narcotic dose (55 mg/kg) was injected intraperitoneally 30 min after administration of the tested compounds. Duration of narcotic sleep was counted from disappearance to return of the righting reflex.

6.6. 'Writhing syndrome' test in mice according to Hendershot and Forsaith [17]

The investigated compounds were administered intraperitoneally in doses 50–6.25 mg/kg, corresponding to 1/40–1/320 of LD_{50}. Twenty-five minutes later, 0.2% phenylbenzoquinone was injected intrapertioneally in a constant volume of 0.25 ml. Five minutes after injection of the irritating agent, the number of 'writhing' episodes in the course of 10 min was counted. Analgesic activity was expressed by the following formula:

$$100 - \frac{\sum \text{ of writhing incidents in experimental group}}{\sum \text{ of writhing incidents in control group}} \times 100 = \%\ \text{inhibition}$$

The ED_{50} values and their confidence limits were calculated according to the method of Litchfield and Wilcoxon [16]. Acetylsalicylic acid and morphine were used as reference analgesics.

6.7. 'Hot plate' in mice according to Eddy and Leimbach [18]

Animals were placed individually on the metal plate heated to 55–56 °C. The time (s) of appearance of the pain reaction (licking or jumping) was recorded by a stop-watch. The experiments were performed 30 min after administration of the investigated compounds. Morphine was used as a reference analgesic.

6.8. Influence on the blood pressure

Arterial blood pressure in the common carotic artery of normotensive anaesthetized rats was measured using a Datamax apparatus (Columbus Instruments). The investigated compounds were injected intraperitoneally in dose corresponding to ED_{50}. The influence on the blood pressure was monitored for 1 h.

6.9. Carrageenan-induced edema

The method based on the assay of Winter et al. [19] was used in groups of five Wistar rats (120–150 g). Hind paw edema was induced by a subcutaneous injection of 0.1 ml of 1% carrageenan aqueous solution into the left hind paw. The drugs as suspension in 0.5% methylcellulose were administered 1 h before carrageenan. The control animals received an equal volume of vehicle (10 ml/kg). The edema value was determined immediately before and 1, 2, and 3 h after carrageenan injection. Percent edema inhibition was calculated according to the following formula:

$$\%\ \text{edema inhibition} = \frac{C - D}{C}$$

C, mean value of edema volume of control animals; D, mean value of edema volume of treated animals.

7. Peroxyl radical scavenging activity

7.1. Substances used

2′,7′-Dichlorodihydrofluorescein diacetate (DCFH·DA), 2,2′-diazobis(2-amidinopropane) dihydrochloride (AAPH) and phosphate buffered saline (PBS) tablets were obtained from Sigma (St. Louis, MO, USA) and cyanidin chloride (CC) from Alexis Biochemicals (San Diego, CA, USA). Other reagents being of analytical reagent grade were used without further purification. The Milli-Q (Millipore, Bedford, USA) water system was used to prepare all solutions.

7.2. Apparatus

TRAP measurements were performed using DigiScan photometer (AsysHitech, Eugendorf, Austria).

7.3. Measurements

Peroxyl radicals were obtained from thermal decomposition of AAPH (final concentration, 56 mM). In the first step carbon radicals are formed in pairs which react rapidly with oxygen molecules to give peroxyl radicals. Their concentration was monitored photometrically, at 504 nm, measuring the conversion of DCFH·DA to dichlorofluorescein (DCF). Reaction was performed in 50 mM PBS solution at temperature 24 °C. DCFH·DA was dissolved firstly in DMSO followed by the same amount of water to obtain its final concentration, 14 μM. Analysed sample (final concentration, 0.1 mM) shifts measured S-shape kinetic curve. Results are the delay time (measured at half time of the reaction) of competition kinetics during which antioxidant is consumed. This parameter measures the total antioxidant reactivity and is defined as the sum, over all the antioxidant present in the sample, of the product of reaction rate constant and concentration. The measurements of the antioxidant capacity were repeated three times for each sample and the results were averaged and expressed relative to the average result for the control samples containing no sample.

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