Protective Effect of Thiazolo[5,4-b]Indole in Toxic Pulmonary Edema

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Antiedematous activity of new thiazolo[5,4-b]indole derivatives containing a fragment of isothiourea and characterized by higher antihypoxic activity compared to known antihypoxants was studied on a model of toxic edema of the lungs in mice. Compounds exhibiting high activity on two models of hypoxia (hypobaric and hemic) better protected from lung edema than compounds active only in hypobaric hypoxia.

Key Words: *thiazolo[5,4-b]indole; isothiourea; toxic pulmonary edema; hypoxia*

Lung edema is a prevalent condition in urgent medicine. It is caused by exposure to prooxidant toxicants used in industry and military objects (H_2O_2, acids, nitrogen oxides, hydrazine, missile fuel components, *etc.*). No etiotropic treatment of this condition was developed by the present time. The absence of specific therapy for lung edema necessitates the search for prevention and treatment of toxic lung edema, which is a practically important and pressing problem.

Augmenting hypoxia plays an important role in the pathogenesis of edemogenic processes. Hypoxia decelerates the synthesis surface active substances in the lungs (*e.g.* surfactant) [7]. Surfactant deficiency promotes the formation of numerous pathological changes in the lungs, including atelectases, liquid exudation into alveolar lumen, impedes bloodflow in alveolar capillaries and gas diffusion. In addition, local oxygen deficiency increases permeability of capillary endothelium.

Thiazolo[5,4-b]indole system compounds (1-4) were obtained within the framework of study of pharmacological activity of condensed indole systems with triazine, pyrimidine, and imidasole [8-11]; molecules of these new compounds contain active isothiourea group (Fig. 1). Pilot experiments demonstrated their high antihypoxic activity on models of hypobaric, hypercapnic, and hemic hypoxia [3]. Here we studied antiedematous activities of compounds 1-4 (Table 1) and its possible relationship with antihypoxic effect.

MATERIALS AND METHODS

Compounds 1-4 were synthesized at Department of Pharmacology, S. M. Kirov Military Medical Academy.

Experiments were carried out on outbred male mice (18-24 g). Toxicity of the test compounds (LD_{50}) was evaluated routinely after intraperitoneal injection [6]. Toxic pulmonary edema was induced by phosgene inhalation (4.2 mg×min/liter) for 4 min [5]. The level of injury was evaluated by actual death of control animals 24 h after inhalation. The results were evaluated 3 and 24 h after intoxication. Pulmonary coefficient (PC) was calculated for evaluating the severity of lung edema and animal survival 24 h after poisoning:

$$PC = \frac{\text{lung weight (g)}}{\text{animal weight (g)}} \times 1000.$$

The compounds were injected intraperitoneally 30-60 min before poisoning (preventive effect) in

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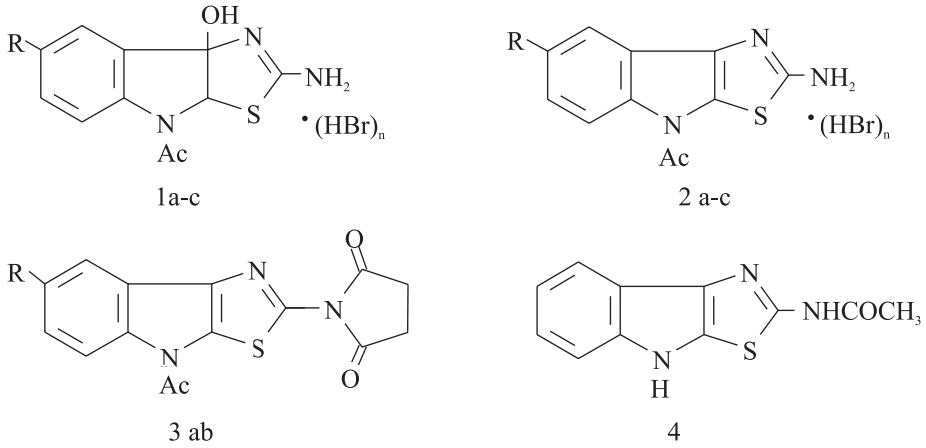

Fig. 1. Structural formulae of studied compounds. 1a-c: 2-amino-4-acetyl-8[b]-hydroxy-3[a],8[b]-dihydrothiazolo[5,4-b]indole derivatives (a: R=H, *n*=1; b: R=Br, *n*=1; c: R=Br, *n*=0). 2a-c: 2-amino-4-acetylthiazolo[5,4-b]indole derivatives (a: R=H, *n*=0; b: R=Br, *n*=1; c: R=Br, *n*=0). 3ab: 2-N-succinimido-4-acetylthiazolo[5,4-b]indole derivatives (*a*: R=H; b: R=Br). 4: 2-acetylaminothiazolo[5,4-b]indole.

TABLE 1. Efficiency of Compounds 1-4 in a dose of 25 mg/kg in Prevention and Treatment of Toxic Edema of the Lungs Caused by Phosgene in Mice (*M*±*m*)

Group		PC, arb. units		Survival, %	K_p
		3 h	24 h		
Intact		7.5±0.5			
Control		10.2±0.2	19.7±0.8	20	
1b, 25 mg/kg	prevention	16.1±0.5*	16.3±2.2	25	1.04
	treatment	10.5±1.5	17.6±0.5*	25	1.04
1c, 25 mg/kg	prevention	12.9±3.7	18.8±0.5	50	1.25
	treatment	12.7±3.8	17.6±0.5*	0	0.83
2b, 25 mg/kg	prevention	9.2±0.3*	13.3±0.3*	75*	1.46
	treatment	10.2±0.7	16.5±1.5	40	1.17
Control		12.7±1.7	19.4±1.5	25	
2c, 25 mg/kg	prevention	8.4±0.6*	15.9±3.3	25	1.00
	treatment	14.5±0.7	18.0±1.3	50	1.20
3a, 25 mg/kg	prevention	8.8±0.4	20.6±3.0	75*	1.40
	treatment	9.7±1.0	19.6±1.6	50	1.20
3b, 25 mg/kg	prevention	9.2±0.4	16.7±1.5	25	1.00
	treatment	8.0±0.7*	16.7±1.5	67	1.34
Intact		8.2±0.5			
Control	10.2±0.2	21.0±0.8	20		
1a, 5 mg/kg	prevention	9.5±0.9	21.8±1.7	0	0.83
2a, 5 mg/kg	prevention	8.9±0.6	18.0±1.4	100**	1.67
4, 5 mg/kg	prevention	12.4±0.5	21.9±1.7	0	0.83
Control	11.0±0.8	20.4±1.1	60		
1a, 25 mg/kg	prevention	11.2±0.7	18.6±0.7	20	0.75
2a, 25 mg/kg	prevention	11.5±0.9	12.7±1.1*	100	1.25
4, 25 mg/kg	prevention	9.2±0.5	16.4±0.7*	40	0.88

Note. *$p \le 0.05$, **$p \le 0.025$ compared to the control.

a dose of 25 mg/kg in suspension with Twin-80 (0.2 ml) or 20-30 min after intoxication (therapeutic effect). Controls were injected with the solvent.

Protection coefficient (K_p) was calculated by the formula:

$$K_p = \frac{a/b+1}{c/d+1},$$

where *a* and *c* are the numbers of survivors in experiment and control, respectively, *b* and *d* numbers of animals per group.

The sampling for each dose of preparation was 10-12 animals. The results were processed using Student's *t* test (for life span and PC values) and exact Fisher's test (for survival values) [2]. Amtisole, reference antihypoxant [4], served as the reference drug in studies of antihypoxic activity.

RESULTS

For evaluation of the efficiency of the test compounds we determined the correlation between PC and animal survival 1 day after the start of experiment. Reduction of PC paralleled by a decrease in survival in the treated group compared to controls indicated inefficiency of the compound, because it aggravated the animal status.

The study of acute toxicity of the compounds showed that LD_{50} surpassed 500 mg/kg for the majority of the preparations (Table 2), this classifying these compounds as moderately or low toxic.

The study of antiedematous activity showed that compounds 1c, 3a, 2b in a dose of 25 mg/kg increased animal survival after poisoning 2.5, 3, and 3.75 times, respectively, compared to the control (Table 1). Compounds 1b and 2c reduced PC significantly by the 3rd hour, compound 4 by hour 24, and compound 2b by hours 3 and 24 of experiment. Compound 2a used in a dose of 5 mg/kg protected 100% animals from death; in a dose of 25 mg/kg it protected 100% animals and reduced PC significantly by the 24th hour of experiment. By the protection coefficient in preventive treatment the compounds can be ranked (in the order of decreasing activity) as follows: 2a>2b>3a>1c.

Analysis of the therapeutic efficiency showed positive effects of compounds 2b, 2c, and 3a, which 2-fold increased the survival of intoxicated animals. Compound 3b reduced significantly the PC of intoxicated animals by the 3rd hour of the experiment and increased the survival 2.67 times. Compound 1b reduced PC by hour 24. By the protection coefficient the most active compounds formed the following series: 3b>3a=2c>2b. Succinic acid imides 3 exhibited higher activity in therapy than in prevention, and the effect of bromine-containing 3b compound (K_p=1.34) was more pronounced than that of 3a compound (K_p=1.20). Compounds 2c and 2b (K_p 1.20 and 1.17, respectively) also contain a bromine atom in a benzene ring and represent the base and salt of the same structure.

Well-known antihypoxants amtisole, gutimine, and bemitil served as the reference drugs. None of these compounds possesses antiedematous activity (Table 2).

We compared K_p values of the studied compounds in relation to their antihypoxic and antiedematous effects (Table 3). Compounds 2a and 2b exhibited maximum preventive effect in lung edema; moreover, these compounds exhibited maximum antihypoxic activity in both types of hypoxia. The K_p of compounds 3a and 3b was the same (1.67) in hypobaric hypoxia; both compounds exhibited antiedematous effects: in preventive (3a) and therapeutic (3b) application. Other compounds (1a, 1b, 2c, 4, amtisole), exhibiting high protective effects in hypobaric hypoxia, showed no sufficient antiedematous effect.

The best antiedematous effects were shown by substances 2 and 3. The presence of bromine atom in their structure was virtually inessential for antiedematous activity.

Hence, thiazolo[5,4-b]indole derivatives exhibited pronounced antihypoxic and antiedematous activities (particularly 2a, 2b, and 3a). No relationship between high antihypoxic (hypobaric hypoxia) and antiedematous activities were detected in other compounds. Structures 2 and 3 are good candidates for

TABLE 2. Therapeutic Effects of Reference Antihypoxants after 24 h on Mice and Rats

Group (mice)	PC	Survival, %	Group (rats)	PC	Survival, %	K_p
Control, phosgene, 5.3 mg×min/liter	16.4±1.0	40	Control, phosgene, 7.0 mg×min/liter	14.3±1.1	17	
Bemitil, 25 mg/kg	18.6±1.0	10	Bemitil, 25 mg/kg	16.3±1.3	17	1.00
Gutimin, 25 mg/kg	18.9±0.8	10	Gutimin, 25 mg/kg	18.7±2.3	0	0.84
Amtisole, 25 mg/kg	15.4±0.5	10	Amtisole, 25 mg/kg	15.3±0.8	33	1.14

TABLE 3. K_p of Compounds 1-4 in Hypoxia and Lung Edema, LD_{50} Values

Compounds	LD_{50}, mg/kg	K_p			
		hypobaric hypoxia	hemic hypoxia	lung edema (prevention)	lung edema (therapy)
1a	>1000	1.89	1.00	0.83	
1b	309±29	1.63	1.00	1.04	1.04
1c	1950±180	1.17	1.00	1.25	0.83
2a	1420±110	1.89	1.80	1.67	
2b	842±142	1.33	1.50	1.46	1.17
2c	3890±370	1.83	1.00	1.00	1.20
3a	>1000	1.67	1.00	1.40	1.20
3b	>1000	1.67	1.00	1.00	1.34
4	>1000	1.50	1.00	0.83	
Amtisole	336	1.89	1.00	1.14	

the search and creation of antiedematous preparations for the treatment of toxic edema of the lung.

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