Department of Pharmacology, Medical Academy[1], Department of Chemistry, University of Agriculture[2], Lublin, Poland

Evaluation of toxic activity of 2,4-dihydroxythiobenzanilides

Z. KLEINROK[1], A. NIEWIADOMY[2] and J. MATYSIAK[2]

2,4-Dihydroxythiobenzanilides represent a new group of compounds with significant fungistatic and bacteriostatic properties. The results of investigations on their cytotoxicity are also very convincing. Therefore LD_{50} doses were determined for five compounds, they ranged from 239 to 840.5 mg/kg. The results of the tests for spontaneous locomotor activity and hexabarbiturane sleeping time indicate low toxicity of the compounds tested.

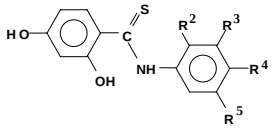
1. Introduction

2,4-Dihydroxythiobenzanilides exhibit valuable fungistatic properties. They act against dermatophytes [1], yeasts [2, 3], moulds [4] and phytopathogenic fungi [5]. In particular, strong antimycotic effects against *Epidermophyton* and *Trichophyton* strains were found with a MIC value of 1.9 μg/ml for the most active derivatives [1]. Bacteriostatic properties in relation to Gram-positive cells was also proved [6]. There is lack of detailed data concerning the mechanism of activity and toxicity of thiobenzanilides. However, anilides and thiobenzanilides, belong to the group of non-specific inhibitors of mitochondrial respiration processes. Their action is connected with the effect of cellular liberation of aromatic amines, blocking the function of succinate dehydrogenase (complex II) and sometimes with induction of the changes in ribosomes within the translation promotor [7, 8]. The selective toxicity of anilides against fungi and other microorganisms is connected with sensitivity to the action of specific membrane enzymes catalysing the reaction of amide bond cleavage. The hydrolysis reaction of most carboxyanilides leads to the liberation of aniline or its derivatives, which undergo detoxication transitions to a different extent and with a different rate [9, 10]. Basic transformation reactions consist in condensation leading to the formation of azobenzene, azoxybenzene or their analogs, in acylation of amino groups as well as in oxidation via a hydroxylamine group to nitrozo- and then to nitro-groups [11]. In the final stage owing to enzymatic transitions occurs the aromatic ring cleavage leading to the formation of simpler compounds [12]. Anilides are nontoxic in relation to homoiothermic [13–15], because after the previous hydroxylation of aromatic ring they undergo the conjunction with glucurone acid, and hydrophilic conjugates formed in this way are excreted through the urinary tract. Because thiobenzanilides exhibit strong antimycotic effects and at the same time literature lacks the data relating to toxicity of thiobenzanilides, preliminary studies concerning these questions have been carried out. Acute toxicity (LD_{50}), spontaneous locomotor activity and hexobarbital sleeping time tests were additionally performed for selected compounds.

2. Investigations, results and discussion

The structure and analytical data of the 2,4-dihydroxythiobenzanilides studied are presented in Table 1. The LD_{50} values of investigated compounds after i.p. administration to mice are given in Table 2. They indicate that all compounds are characterised by mean toxicity, though these values are differentiated depending on the kind of substitution in the aniline moiety. Compound 5 (-CF_3 in *para*-position) proves to be the most toxic and compounds **3** and **4** the least toxic. Spontaneous locomotor activity was decreased to a small extent only after administration of compounds **4** and **5**, dose 1/10 LD_{50}, for the first 60 min of

Table 1: Structure and analytical data of 2,4-dihydroxythiobenzanilides



Compd.	Substituents				m.p. (°C)	IR (cm^{-1})	^1H NMR (δ, ppm)
	R^2	R^3	R^4	R^5			
1	Cl	H	CH_3	H	161–162	2981 ν CH, 1500 ν NHC/=S/, 1393 δ_s CH, 1051 ν C–Cl	11.1247 (NH, s), 2.3333 (CH_3, s, 3 H)
2	H	H	$C_2H_5C(=O)$	H	171–172	2975, 2944, 2907 $\nu_{s,as}$ CH, 1679 ν C=O, 1510 ν NHC/=S/, 1474, 1377 δ CH, 1016 ν C=S	11.0197 (NH, s), 2.9362–2.7971 $C(=O)CH_2$, t), 1.1642–1.0227 (CH_3, k, 3 H)
3	OH	H	H	CH_3	126–127	2921 ν CH, 1308 ν_s CH	11.7569 (NH, s), 9.8580 (HOC-2, s)
4	CH_3	Cl	H	H	193–194	2968 ν CH, 1496 ν NHC/=S/, 1379 δ_s CH, 1270 ν C–Cl, 1076 ν C=S, 1019 ν C–Cl	11.4152 (NH, s), 2.2381 (CH_3, s, 3 H)
5	H	H	CF_3	H	185–186	1499 ν NHC/=S/, 1312, 1137 ν C–F, 1064 ν C=S	11.2314 (NH, s)

Table 2: Acute toxicity of investigated compounds in mice

Compd.	LD_{50} (mg/kg i.p.)	95% confidence limit
1	478.7	342.5–669.0
2	448.4	364.3–552.0
3	721.1	609.0–853.9
4	840.5	692.6–1020.0
5	239.0	192.5–296.8

observation (Table 3). The investigated compounds injected in the dose 1/10 LD_{50} did not influence the hexobarbital sleeping time (Table 4).

In vitro toxicity tests: the highest tolerable dose (HTD), the neutral red (NR) and Kenacid blue (KB) performed on the Feline Kidney cells of line CCC clone 81 exhibited low toxicity of 2,4-dihydroxythiobenzanilides which was much lower than thiuram and imaverol (commonly used fungicidal) studied under the same experiment conditions as reference system [16]. Scarce literature reports indicate also a low level of thiobenzanilides hepatotoxicity due to their substitution pattern. For 4-methoxy derivatives no hepatotoxicity was found [17]. Other authors report that thiobenzanilides are toxic for warm-blooded animals but to a small extent [18].

The smallest toxicity was observed for the compounds with the free *para*-position in the N-aromatic ring (compounds **3**, **4**) which may be caused by a metabolism similar to that observed in rats after administration of benzanilides (including salicylanilides) i.e. hydroxylation in the *para*-position and conjugation [11]. Such an analogy is justified considering the significant structural similarity of the compounds under discussion. The 4′-position on the aniline ring has been shown to be important in this respect for many other similar compounds [11].

Due to the promising preliminary results presented here, the investigations should be continued. This includes extension of toxicity tests, studies on metabolism and mechanism of action as well as search for new, optimal structures (high activity, low toxicity). These can be obtained through modification of both the aniline and thioacyl moiety.

3. Experimental

3.1. Tested compounds

The tested compounds (Table 1) were obtained in the reaction of sulphinyl-bis-2,4-dihydroxybenzenethioyl with the appropriate amine. Sulphinyl-bis-2,4-dihydroxybenzenethioyl as the starting material was prepared according to a patent [19]. Oscillating spectra (IR) were registered in the range of 4000–500 cm^{-1} by means of a Perkin-Elmer apparatus (compound in KBr) (Table 1). ^1H NMR spectra were registered by means of a FT-NMR Tesla BS 567 A (100 MHz), solvent: DMSO-d_6, standard: TMS (Table 1).

Table 4: Influence of the compounds investigated on hexobarbital sleeping time (75 mg/kg i.p.) in mice ($\bar{x} \pm SE$)

Compd.	Sleeping time (min)
1	24.7 ± 2.5
2	28.5 ± 2.9
3	29.5 ± 4.9
4	25.9 ± 3.4
5	28.4 ± 2.5

3.2. Biological investigations

The experiments were carried out on male Albino-Swiss mice weighing 20–25 g. The investigated compounds were administered intraperitoneally (i.p.) as a suspension in 3% Tween® 80 in the constant volume of 10 ml/kg. The compounds were injected in doses equivalent to 1/10 of their LD_{50}. For the compounds whose LD_{50} was greater than 2000 mg/kg the administered dose was 200 mg/kg. The control animals received the equivalent volume of solvent. Each experimental group consisted of 8 mice. The animals were housed in a vivarium at 20 ± 2 °C with natural light/dark cycle and had free access to food and water. All experiments took place between 10 and 12 a.m.

The following pharmacological tests were performed:

Acute toxicity was assessed by the Litchfield and Wilcoxon methods [20] and presented as LD_{5O} calculated from the death rate of mice after 24 h.

Spontaneous locomotor activity was measured in actometers. After injection of the investigated compounds animals were placed in the actometers for 2 h. The number of impulses was recorded after 60 and 120 min.

Hexobarbital sleeping time: Sodium hexobarbital 75 mg/kg i.p., was given 30 min after the test compounds. The sleeping time was counted from the time of loss to recovery of the righting reflex.

Statistics

The obtained results were presented as mean and evaluated statistically using the Student's t-test.

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Table 3: Influence of the investigated compounds on spontaneous locomotor activity in mice ($\bar{x} \pm SE$)

Compd.	Time (min)	Locomotor activity	Total distance (cm)	Horizonid activity
Control	0–60	3078.1 ± 440.3	658.9 ± 128.6	261.9 ± 57.2
	60–120	1710.4 ± 342.7	324.9 ± 98.9	143.2 ± 62.5
1	0–60	3656.3 ± 1238.6	1854.1 ± 1435.6	128.9 ± 59.2
	60–120	2288.6 ± 630.2	847.2 ± 656.9	55.5 ± 26.2
2	0–60	2207.4 ± 394.7	534.0 ± 116.4	178.6 ± 66.6
	60–120	1039.4 ± 211.9	146.9 ± 55.0	56.1 ± 27.3
3	0–60	4739.6 ± 2337.3	2079.3 ± 1568.8	555.3 ± 379.2
	60–120	3342.8 ± 2343.1	2358.8 ± 2272.1	326.0 ± 311.0
4	0–60	2142.2* ± 223.1*	1194.6 ± 733.6	281.4 ± 1447.9
	60–120	2409.1 ± 729.8	1050.3 ± 590.8	361.0 ± 190.0
5	0–60	2317.4** ± 537.1**	807.3 ± 424.7	134.7 ± 32.9

* $p < 0.05$ vs. control
** $p < 0.01$ vs. control

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Prof. Andrzej Niewiadomy
University of Agriculture
Department of Chemistry
20-950 Lublin
Akademicka 15
Poland