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

The nature of interaction of 4′-[(N-benzoyl) aminomethanocarboxy]-2,4-dihydroxybenzcarbothioamide with blood lymphocytes

J. Matysiak[1], B. Kwiatkowska-Drabik[2], J. Wojcierowski[2], and A. Niewiadomy[1]

Compounds of the thiobenzanilide group show an interesting spectrum of biological activity, depending on their structure, especially antimycotic and antimycobacterial properties [1–3]. At the same time the majority of anilide type derivatives are characterised by low toxicity to higher organisms [4–6]. In studies of the fungistatic compounds, the synthesis conditions for a new group of thiobenzanilides with a *meta*-substituted dihydroxybenzthioacyl moiety: 2,4-dihydroxythiobenzanilides modified in the aniline ring have been determined [7].

It has been stated that this type of substitution guarantees the maintenance of a proper hydrophilic-hydrophobic equilibrium, determining the ability of the compounds to penetrate membrane cells and their activity against fungi and bacteria [8–10]. The compounds obtained show a relatively wide range of fungistatic action. Depending on the type of modification of the N-aryl fragment these compounds can act against phytopathogenic fungi [8] and pathogenic pathogens. Even at concentrations as low as 1.98 μg/ml some of these compounds act against the dermatophytes [11]. Higher concentrations (MIC ca. 7.8 μg/ml) are required against yeasts [12], while the highest resistance is shown by moulds (MIC ca.15.6 μg/ml) [13]. Microbiological tests also show that these compounds act mainly against Gram positive cells; however, depending on the nature of these compounds (including the type of modification of derivatives) their activity is very heterogeneous and varies over a relatively wide range e.g. from 3.9 to 500 μg/ml [14].

Considering that we have at our disposal sufficient fungistatic drugs acting either locally or generally against mycosis induced by dermatophytes and yeasts, while the possibilities for pharmacotherapy of systematic mycoses are significantly smaller, we have examined the action of these compounds against the blood lymphocytes.

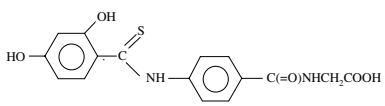
From the 60 compounds of the 2,4-dihydroxythiobenzanilide group with different modifications aniline moiety which were prepared in our laboratory and tested for antimycotic activity against twenty strains of dermatophytes, yeasts, moulds and phytopathogenic fungi, 4′-[(N-benzoyl) aminomethanocarboxy]-2,4-dihydroxybenzcarbothioamide was selected for further investigations. This was because of the significantly lower cytotoxicity of this derivative in relation to the group compounds, studied as confirmed by the following tests: highest tolerable dose (HTD), neutral red uptake (NRU) and kenacid blue (KB) [15].

4′-[(*N*-benzoyl)aminomethanocarboxy]-2,4-dihydroxybenzcarbothioamide was obtained by the reaction according to the patent [7]. The analytical data of the compound were in agreement with the proposed structure. Purity was confirmed by HPLC and HPTLC chromatography using a reversed-phase system (RP-8, RP-18, methanol-water). The results of the tested compound's interaction with blood lymphocytes, as expressed by the three following factors: cell vitality, blastic index and mitotic index, are given in the Table.

The results obtained show that the tested compound at dose of 10 μg/ml (above approximately three times the amount necessary for inhibition of the growth of some microorganisms *in vitro*) does not exhibit toxic action against lymphocytes. This was generally confirmed by all tests irrespective of time. An effect of this compound on the mitotic index was observed only after 96 h of incubation. At a dose of 100 μg/ml the tolerance is significantly lower. The compound exerts the greatest influence on mitotic index (about 50%) independent of incubation time. Nevertheless for any combination the decoding of chromosomes was not observed, permiting the relative safety of intrasystem introduction of this compound to be predicted.

In vitro toxicity tests: the highest tolerable dose (HTD), neutral red uptake (NRU) and kenacid blue (KB) tests on the Feline Kidney cells of line CCC clone 81 for 2,4-dihydroxythiobenzanilides showed significantly lower cytotoxicity of this group of compounds than of thiuram and imaverol (commonly used fungicides) which were studied as the reference system [15]. Particularly low toxicity was found for 4′-[(N-benzoyl)aminomethanocarboxy]-2,4-dihydroxybenzcarbothioamide, several times smaller for the NRU and KB tests and several scores smaller for the HTD test than imaverol.

Table: Effect of 4′-[(N-benzoyl)aminomethanocarboxy]-2,4-dihydroxybenzcarbothioamide on cell vitality, on blastic index and on mitotic index



Contribution of the compound Type of culture		Cell vitality Culture time (h)				Blastic index Culture time (h)			Mitotic index Culture time (h)	
(μg/ml)		24	48	72	96	48	72	96	72	96
Control	m	93.77	85.95	81.45	80.27	21.33	62.44	72.94	2.58	3.06
	s	4.44	7.75	13.41	11.16	7.14	16.87	18.36	0.29	0.45
10	m	90.09	84.88	80.70	79.61	20.24	63.34	70.88	2.23	2.05
	s	10.11	10.42	11.45	12.74	4.16	19.25	19.23	0.49	0.94
100	m	79.47	70.76	64.49	63.45	15.33	42.30	67.43	1.10	1.53
	s	16.62	14.37	15.01	17.23	8.13	20.12	15.24	0.42	0.09

m - mean values (%), s - standard deviation (%)

There are numerous reports on the antimycotic and antimycobacterial activities of thiobenzanilides, but information about their toxicity is poor. Only hepatotoxicity has been investigated, finding that 4′-methoxy derivatives were the least toxic of the group of substitutions studied. However 4-methoxy derivatives did not show any hepatotoxicity under identical experimental conditions. Other authors report that thiobenzanilides have low toxicity for warm-blooded animals [3].

Taking into consideration their wide spectrum of antimycotic activity, strong inhibition effect against some strains and at the same time relatively low toxicity, this group of compounds seems to be very interesting. Therefore further investigations to determine the toxicological properties of 2,4-dihydroxythiobenzanilides including acute toxicity will be carried out.

Experimental

1. Tested compound

4′-[(*N*-Benzoyl)aminomethanocarboxy]-2,4-dihydroxybenzcarbothioamide was obtained by the reaction of sulphinyl-bis-2,4-dihydroxybenzenethioyl [7] with 4-aminohippuric acid.

0.01 Mol bis-(2,4-dihydroxybenzenecarbothioyl)thionyl and 0.025 mol of 4-aminohippuric acid (Merck KGaA, Darmstadt, Germany) were heated to boiling (1 h) in methanol (75 cm^3). The mixture was filtered and the filtrate was left to stand at room temperature. The separated compound was crystallized in methanol (40 cm^3) to give yellow needles (89%).

Anal. ($C_{16}H_{14}N_2O_5S$) C, H, N; M = 346.36; m. p.141–142 °C; UV (λ_{max}, nm): 210, 234, 298, 338; ^{1}H NMR, DMSO-d_6 (δ, ppm): 11.66 (s, 1 H, HOC-4), 11.00 (s, 1 H, C(=S)NH), 9.23 (s, 1 H, HOC-2), 8.23 (t, 1 H, C(=O)NH), 8.05–7.75 (m, 5 H, HC-2′,3′,5′,6′,3), 6.45 (s, 2 H, HC-5,6), 4.16 (d, 2 H, CH_2); IR (cm^{-1}): 3407, 3209 (OH + NH), 1755 C=O, 1549 C=C, 1508 NHC(=S), 1460 C=C, 1437 =N···C(-S)-, 1396 CH, 1365 C–OH (COOH), 1309 C–O (COOH), 1123 CH (CH_2COOH), 1020 C=S, 991, 958, 875, 847 C_{Ar}H; MS (EI, m/z): 345 (M^+-1), 328, 327, 270, 244, 208, 153, 137, 121.

This compound was tested on cultures of peripheral blood lymphocytes obtained from 7 healthy patients.

2. Cultures determination

The lymphocytes obtained by the sedimentation method were suspended in a culture medium consisting of RPMI background, 20% of calf serum, 2 mM of glutamine (Serum and Vaccine Works, Lublin, Poland) and LF-7 (Biomed, Serum and Vaccine Works, Krakow, Poland) as a stimulating agent. In order to actieve sterility of the culture the following mixtures have been used in successive trials:

– mixture of antibiotics – 100 units of penicilin, 0.1 g of streptomycin and 25 units of mycostatin (Polfa, Tarchomin, Poland)
– 10 and 100 μg/ml doses of tested compounds

The cultures were grown for 96 h, and the following factors were then determined:

a) cell vitality after 24, 48, 72 and 96 h from the number of living cells per 1000 visualised.
b) blastic index after 48, 72 and 96 h calculated from the number of blasts corresponding to 1000 cells.
c) mitotic index after 72 and 96 h.

The cultures were previously treated with colcemide (1 h, concentration of 0.2 μg/ml) (Gifco, Holland) and terminated in a routine way, and then the number of mythosis corresponding to 1000 cells was calculated. The results of individual determinations expressed as mean values and corresponding standard deviations are listed in the Table.

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Professor Andrzej Niewiadomy
Department of Chemistry
Agriculture University
20-950 Lublin
Poland
JMATY@agros.AR.Lublin.PL