N-Heterocyclic Derivatives of 2,4-Dihydroxybenzcarbothioamide as Antimycotic Agents

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N-heterocyclic derivatives of 2,4-dihydroxybenzcarbothioamide were synthesized from sulfinylbis-(2,4-dihydroxybenzenethioyl) and commercially available heterocyclic amines. The composition and chemical structures were confirmed by IR, ^{1}H NMR, EI-MS, and elemental analysis. For the estimation of potential activity in vitro the MIC values against 15 strains of dermatophytes, yeasts, and molds were determined. The strongest fungistatic potency was found for *N*-5′-(3′-oxobenzfurylidyne)-2,4-dihydroxybenzcarbothioamide in relation to all tested dermatophyte strains with MIC = 0.48−0.98 μg/mL. On the basis of the spectroscopic data the influence of N-heterocyclic substitution on antimycotic activity is discussed.

Keywords: *N-heterocyclic derivatives of 2,4-dihydroxybenzcarbothioamide; antifungal activity; in vitro study; MIC*

INTRODUCTION

During foodstuff production, higher quality requirements associated with, among others, the presence of toxic impurities such as mycotoxins as well as pesticide reidues, are often necessary. These impurities are a factor that negatively interacts with human, animal, and plant health, which, in consequence, leads to unfavorable economic results. It was found that mycotoxin pollution affects 25% of foodstuff worldwide and is responsible for many acute and protracted diseases (*1*).

Mycotoxins produced by *Aspergillus*, *Penicillium*, and *Fusarium* can cause changes with regard to protein, lipid, and carbohydrate balance. They frequently disturb nucleic acid synthesis and functions, further exhibiting mutagenic and teratogenic action (*2*, *3*).

Aflatoxins B_1 (AFB_1), G_1, B_2, and G_2, being the secondary metabolites of *Aspergillus* fungus, cause acute toxicity connected with mitochondrial DNA injury and are responsible for many other diseases. At the same time, they are considered to be one of the most serious carcinogenic factors occurring in nature and causing liver cancer (*4*, *5*).

These contaminations and their consequences may be reduced greatly due to rational application of proper fungicides. They promote the search for new compounds capable of coping with fungi within the accepted economic and natural normatives. Therefore, many compounds of either natural or synthetic origin are examined every year in the search for new structures with antimycotic activity.

The search for modern fungicides concentrates on arylamides. Many compounds of this group, such as fenhexamid (hydroxyanilide) (*6*), iprovalicarb (amino acid amide carbamate) (*7*), zoxamide (benzamide) (*8*), AC 382042 (phenoxyamide), carpropamid (cyclopropanamide), diclocymet (cyanoacetamide) (*9*), or MON 65500 (hindered silyl amide) (*10*), are the object of great interest of many world agrochemical firms. Aryl- and heteroaryl-substituted thioamides are also a group of biologically active compounds including antimycotic properties. Recently, many sulfur analogues with linear ═NC(═S)− or cyclic −SN═N− groups have been prepared, including, among others, tolnaftate and tolciclate (thiocarbamates) (*11*) or acibenzolar-*S*-methyl (benzothiadiazole) (*12*).

A very promising group of compounds with a wide spectrum of biological activity (*13*−*17*) including antimycotic activity (*18*) are thiobenzanilides. In our laboratory the synthesis of 2,4-dihydroxythiobenzanilide derivatives substituted in the N-aromatic ring has been carried out. They exhibit essential fungistatic properties in relation to molds, yeasts, dermatophytes (*19*−*22*), and pathogenic fungi (*23*). Especially strong inhibitory action against *Epidermophyton floccosum* strains has been found with MIC = 1.9 μg/mL for some kinds of substitution (*22*). Preliminary study of 2,4-dihydroxythiobenzanilides showed their low in vitro and in vivo toxicities (manuscript in preparation). Other compounds with the 2,4-dihydroxybenzenecarbothioacyl moiety, derivatives of hydrazine and amidrazone, also show strong antimycotic effect (*24*, *25*). Therefore, we decided to carry on the research to prepare new derivatives with this moiety as potential antimycotic agents. As heterocyclic compounds exhibit significant pharmacological properties including fungistatic ones, the group of 2,4-dihydroxybenzcarbothioamide N-heterocyclic derivatives as leading structures has been synthesized. To compare the antifungal potencies of the compounds, the minimum inhibitory concentration (MIC) values were determined.

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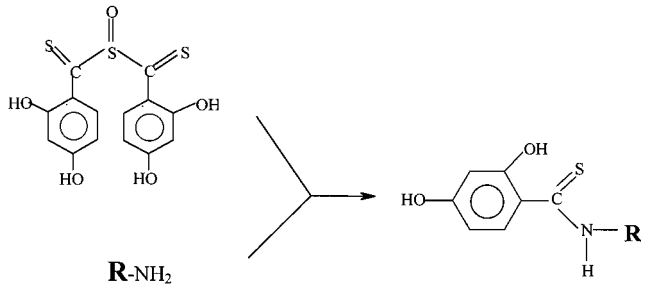


Figure 1. General synthetic route to N-heterocyclic derivatives of 2,4-dihydroxybenzcarbothioamide.

The investigations were carried out under in vitro conditions for 15 strains of potentially pathogenic fungi. On the basis of the spectroscopic investigations the influence of N-heterocyclic substitution on the distribution of electron density in the carbamoyl moiety and antimycotic activity has been studied.

MATERIALS AND METHODS

The synthetic pathway for the compounds described is illustrated in Figure 1. Sulfinylbis(2,4-dihydroxybenzenethioyl) as the starting materials was prepared according to a patent (*26*). The chemicals were purchased from Merck Co. or Fluka Ltd. and used without further purification.

Synthesis of Compounds I—X. Sulfinylbis(2,4-dihydroxybenzenethioyl) (0.01 mol) and the corresponding heterocyclic amine (0.025 mol) were heated until boiling in methanol (**VII** in pyridine; **VIII** in ethanol). The mixture was filtered during boiling (compounds **I**, **III**, and **V**−**X**) or after cooling (compounds **II** and **IV**). The filtrate was left at room temperature for 24 h (**I**, **III**, **VI**−**VIII**, and **X**) or concentrated to obtain a small volume (**II**, **IV**, **V**, and **IX**). The separated compound was filtered and recrystallized in methanol solution (**I**, **II**, **IV**, **V**, and **VII**−**X**) or water (**III** and **VI**).

Analytical Investigations. Melting point measurements on a Boetius apparatus are given uncorrected. Elemental analysis was performed to determine C, H, and N contents (Perkin-Elmer 2400 analyzer). The results were acceptable in accordance with the calculated values (0.7% for C, 1.0% for N, and 1.2% for H).

EI-MS spectra were recorded with an AMD-604 mass spectrometer (electron ionization at 70 eV). The parameters of basic band and characteristic fragmentation ions corresponding to the products of the primary fragmentations and to the structure relatively close to that of the tested compound are given. Uncharged fragments eliminated during even- and odd-electron fragmentation were identified on the basis of mass differences between M^+ ions and the products of primary fragmentation (or significant connection between them) as well as on the basis of mass difference between the parent and polar ions. Table 2 also presents line intensities (percent) of 153 *m*/*z* cations (2,4-dihydroxybenzcarbothionyl) evolved owing to the fragmentation cleavage of the thioamide bond.

^{1}H NMR spectra were recorded with an FT-NMR Tesla BS 567 A spectrometer (100 MHz) in relation to TMS. The spectra of compounds were registered mainly to confirm the structure and if possible to determine the chemical shift of thioamide proton. At the same time the detailed interpretation of individual conjunctions and determination of the constant values have been omitted, drawing attention to the changes of the spectral nature due to the presence of substituents in the successive derivative system.

The oscillation spectra were recorded with a Perkin-Elmer apparatus (in KBr). In Table 2 the frequencies of stretching vibrations in the equilibrium states of the amidothione system typical for frequencies (for *cis* conformation) are also listed.

Calculations of energy and the dipole moment of compounds for the two tautomeric forms -C(═S)NH− ↔ −C(SH)⋯N− were carried out with an Allcheme 2000 program. The semiempirical AM1 method and geometry optimalization were used.

Biological Assay. Using the dilution method the MIC of individual compounds for 15 strains of dermatophytes, molds, and yeasts has been determined. These were either reference strains of known sensitivity to antifungal drugs or the strains isolated directly from the clinical material. Microorganisms were multiplied on the slants developed from the Muller−Hinton agar containing 4% glucose (pH 5.6) and from the analogous Muller−Hinton broth. The tested compounds were dissolved in methanol. Different amounts of solutions were added to the accurately measured, dissolved, and cooled to 45 °C agar medium and then mixed and emptied onto Petri plates. The medium of more and more decreasing concentration, ranging from 1000 to 0.003 μg/mL, was obtained. The medium containing 0.5 mL of the substance had also 5% of methanol. After solidification, the plates were dried, and after the 0.02 mL culture [10^4 colony-forming units (cfu) of fungi] had been sprayed, the plates were incubated for 2−10 days at 22 °C. At the same time sensitivity of the strains to methanol was determined. The activity of ketoconazole and batrafen against all fungi was also estimated. The presented results were obtained from three independent measurements.

RESULTS AND DISCUSSION

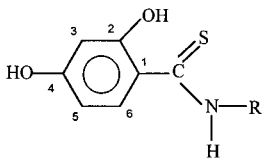
The structures of thiobezamides **I**−**X** and theoretically calculated energy and dipole moments for the two tautomeric forms −C(═S)NH− ↔ −C(SH)⋯N− are presented in Table 1. The analytical data of compounds are summed in Table 2. They were in agreement with the proposed structures. The results of in vitro screening against 15 strains of potentially pathogenic fungi are given in Table 3.

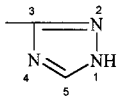
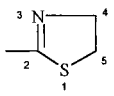
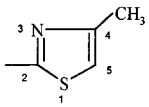
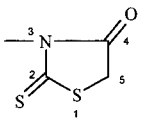
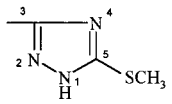
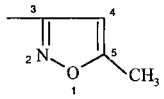
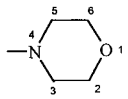
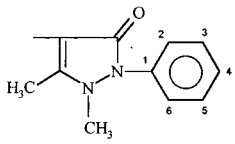
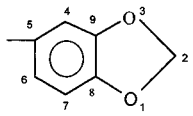
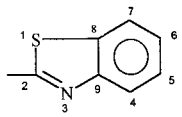
Theoretically calculated values of the energies for both tautomeric forms indicate preferences for the imido−thiol form due to the more favorable energetic level (Table 1) (*27*, *28*), which is confirmed by the presence of the band ═N⋯C(SH)− in the IR spectrum (Table 2). The character of changes of C═S bonds strength constants is confirmed by the shifts of valency group bands into the area of lower frequencies in the IR spectra (Table 2). This may refer to the stability of boundary structures and hydrogen bond bridges, which is also conditioned by the position of N-ring heteroatoms interacting with the hydrogen atom of imido−thiol forms. With the increase of the number of heteroatoms and the length of bonds in the stretched five-membered rings, the number of substituents changes the barrier of energetic inversion, which is 5.32 kJ·mol^{-1} for compound **V** and 24.83 kJ·mol^{-1} for compound **IV** (Table 1).

In MS spectra the intensity of peaks corresponding to the ions *m*/*z* 153 and the residues after desulfhydration $[M - SH^\circ]^+$ makes it possible to determine the conformation equilibrium state as well as the C−N bond polarization degree and the size of the stabilizing charge on the carbon atom of thiocarbonyl moiety (Table 2).

Analysis of the ^{1}H NMR spectra indicates that at the measurement temperature in all of the compounds characterized by a low energetic barrier of tautomeric rearrangement −C(═S)NH ↔ −C(SH)⋯N− (not exceeding 5−10 kJ·mol^{-1}) (Table 1), the averaged spectra are observed (isomers are not uncovered) (Table 2). In some cases differences of chemical shifts of uncovered and covered protons in the heteroaromatic rings can be a basis for establishing a qualitative criterion of N-amide substituent aromatic property. In most cases combina-

Table 1. Chemical Structures of N-Heterocyclic 2,4-Dihydroxybenzcarbothioamides, Energy and Dipole Moment for Two Tautomeric Forms −C(=S)NH− ↔ −C(SH)⋯N−[a]



No.	R	name of compounds	energy (kJ·mol^{-1})		dipole moment (D)	
			-C(=S)NH-	-C(SH)⋯N-	-C(=S)NH-	-C(SH)⋯N-
I		N-(1H-1,2,4-triazol-3yl)-2,4-dihydroxybenzcarbothioamide	66.52 35.23	59.965 26.014	1.0014 4.8801	3.5312 3.7011
II		N-(2-thiazolin-2-yl)-2,4-dihydroxybenzcarbothioamide	33.933 46.128	27.811 37.965	0.8711 5.8290	2.1448 3.1612
III		N-(4-methylthiazol-2-yl)-2,4-dihydroxybenzcarbothioamide	37.532 37.513	31.233 31.104	1.3771 0.1579	3.5680 4.3573
IV		N-(thiazolidyne-2-thion-4'-on-3-yl)-2,4-dihydroxybenzcarbothioamide	40.313	15.476	3.2010	4.1171
V		N-(5-methylthio-1H-1,2,4-triazol-3-yl)-2,4-dihydroxybenzcarbothioamide	70.145	64.825	1.8786	2.3072
VI		N-(5-methylisoxazol-3-yl)-2,4-dihydroxybenzcarbothioamide	44.73	37.862	1.9958	3.9738
VII		N-(morpholin-4-yl)-2,4-dihydroxybenzcarbothioamide	18.901	13.184	4.1227	0.9059
VIII		N-(2,3-dimethyl-1-phenyl-3-pyrazoline-5-on-4-yl)-2,4-dihydroxybenzcarbothioamide	77.571 32.559	71.124 24.413	5.0626 2.6917	7.6383 3.4508
IX		N-(benzodioxolan-5-yl)-2,4-dihydroxybenzcarbothioamide	52.316	43.535	6.7518	2.0738
X		N-(benzothiazol-2-yl)-2,4-dihydroxybenzcarbothioamide	48.396 48.377	42.251 42.265	1.3504 1.8970	1.6457 2.2813

[a] For compounds **I**−**III**, **VIII**, and **X** structure optimalization indicates various ring rotations

tion of a few effects (especially those cooperating) is responsible for significant changes of chemical shifts due to the cooperating mesomeric effect and multiple bond anisotropy. Irregular changes of electron density are observed in compounds **I**−**IV** and **VII**. The diamagnetic uncovering effect of N and S atoms of heterorings on protons α overlapping with the decrease of electron density in this position causes band shifts toward lower fields.

In vitro activities of compounds against potentially pathogenic fungi were compared. The broth dilution method for estimation of MIC values (minimal inhibi-

Table 2. Analytical Data of 2,4-Dihydroxybenzcarbothioamides

compd	formula, MW	mp (°C)	^{1}H NMR (δ), [D_6]DMSO, CD_3COCD_3[a]	EI-MS *m/z*	EI-MS R^+	EI-MS rel in (%)	IR $\bar{\nu}$ (cm^{-1})
I	$C_9H_8N_4O_2S$ 238.26	253−255	14.00−11.00 (NH, OH, m, 2H), 10.18 (HOC-4, s), 8.20 (m, HC-3, HC-5′, 2H), 6.41−6.30 (m, HC-5,6, 2H)	238	$M^{+\bullet}$	100	3413, 3120 (OH + NH), 2581 (C−N), 1589 (C═C), 1499 [NHC(═S)], 1461 [cis ═N┈C(SH)−], 1372 (C═C), 1330 (C−N), 1269 (C−O), 1211 (C═N ring), 1129 ($C_{Ar}H$), 1042 (C═S)
				219	$[M - NH_3]^+$	30.05	
				209	$[M - HCN]^+$	81.20	
				203	$[M - SH^\circ]^+$		
				167	$(HO)_2C_6H_5C(=S)N$		
				153	$(HO)_2C_6H_3C(=S)$		
				136	$(HO)_2C_6H_5C(=N)$		
				127	$C(=S)NHC_2H_2N_3$		
				85	$NH_2C_2H_2N_3$		
				69	$C_2H_3N_3$		
				53	C_4H_5		
II	$C_{10}H_{10}N_2O_2S_2$ 254.27	160−161	10.76 (HOC-2, s), 9.33 (NH, s), 9.21 (HOC-4, s), 8.12 (HCC-3, m), 7.88−6.84 (H_2C-4′, m, 2H), 6.42−6.34 (HC-5, 6, m, 2H), 3.54−3.45 (NCH_2, m, 2H), 3.36−3.17 (SCH_2, m, 2H)	254	$M^{+\bullet}$	3.25	3377 (OH + NH), 1617 (C═C), 1506 (C═C), 1457 [NHC(═S)], 1426 [cis ═N┈C(SH)−], 1344 (C═C), 1310 (C−H), 1235 (C−O), 1168 ($C_{Ar}H$), 1129 (CH), 1019 (C═S)
				224	$[M - C_2H_2N]^+$	31.40	
				221	$[M - SH^\circ]^+$	100	
				195	$[M - C_2H_2S]^+$		
				167	$(HO)_2C_6H_3C(=S)N$		
				153	$(HO)_2C_6H_3C(=S)$		
				135	$(HO)_2C_6H_3C(=N)$		
				110	NC_3H_4NS		
				108	$(HO)_2C_6H_2$		
				96	C_3H_4NS		
				69	C_2H_3S		
				44	CS		
III	$C_{11}H_{10}N_2O_2S_2$ 266.35	114−115	13.71 (HOC-2, s), 11.83 (NH, s), 10.07 (HOC-4, s), 8.30−8.21 (HOC-3, d), 6.79 (HC-5′, s), 6.38−6.30 (HC-5, 6, m, 2H), 3.41 (CH_3, s, 3H)	266	$M^{+\bullet}$	5.15	3241 (OH, NH), 2874 (CH, CH_3), 1602 (C═C), 1521 [NHC(═S)], 1457 [cis ═N┈C(SH)−], 1384 ($C_{Ar}H$), 1329 (CH), 1205 (C−O), 1124 ($C_{Ar}H$), 1004 (C═S)
				250	$[M - NH_2]^+$	100	
				233	$[M - SH^\circ]^+$		
				184	$[M - C_3HNS]^+$		
				168	$(HO)_2C_6H_3C(=S)NH$		
				153	$(HO)_2C_6H_3C(=S)$		
				137	$(HO)_2C_6H_3CH(=NH)$		
				114	$NH_2C_2HNSCH_3$		
				98	C_2HNSCH_3		
				71	$[C_2HNS(CH_3) - HCN]$		
				69	C_3HS		
				55	$[C_2HNS - HCN]$		
				45	CH═S		
				39	C_3H_3		
IV	$C_{10}H_8N_2O_3S_3$ 300.38	204−205	11.46 (HOC-2, s, H), 11.08 (HOC-4′ enolic form, s, H), 10.40 (NH, s), 10.06 (HOC-4, s, H), 8.00−7.86 (HC-5′, HC-3, d−d, 2H), 6.97 (HC-5′, s, H), 6.53−6.36 (HC-5, 6, m, 2H)	300	$M^{+\bullet}$	100	3463, 3412 (OH + NH), 2974 ($C_{Ar}H$), 2574 (C−N), 1719 (C═O), 1618 (C═C), 1491 [C(═S)NNHC(═S), 1468, 1442 [NHC(═S)], [cis ═N┈C(SH)], 1388 (C═C), 1324, 1280 (C−O), 1199, 1177, 1122 (C_{Ar}-H), 1089 C═S (ring), 1057 (C═S)
				282	$[M - H_2O]^+$	12.40	
				267	$[M\text{-}SH^\circ]^+$	36.90	
				239	$[M - CH_4NS]^+$		
				191	$C_3H_2ONS_2NHCS$		
				167	$(HO)_2C_6H_3C(=S)$		
				153	$(HO)_2C_6H_3CS$		
				135	$(HO)C_6H_3CN$		
				115	C_3HNS_2		
				69	C_3H_3S		
				59	$CS - NH(C_2H_2S)$		
				45	CSH		
				39	C_3H_3		
V	$C_{10}H_{10}N_4O_2S_2$ 282.35	234−235	12.28 (HOC-1, NH), 10.35 (HOC-4, s), 8.16−8.06 (HC-3, m), 6.47−6.35 (HC-5,6, m), 3.86 (SCH_3, s, 3H), 2.56 (NH, heterocyclic ring)	282	$M^{+\bullet}$	14.80	3224, 3105 (OH + NH), 1580 (C═C), 1459 [NHC(═S)], 1374 [cis ═N┈C(SH)-], 1335 (C═C, C═N), 1283 [CH(SCH_3)], 1223 (C−O), 1122 ($C_{Ar}H$), 1069 (C═S)
				265	$[M - NH_3]^+$	100	
				249	$[M - SH^\circ]^+$		
				235	$[M - SCH_3]^+$		
				153	$(HO)_2C_6H_3C(=S)$		
				136	$(HO)_2C_6H_3CH(=N)$		
				114	$C_2H_2N_3(SCH_3)$		
				108	$C_6H_2(OH)_2$		
				97	$C_2N_2(SCH)$		
				69	$C_2N_3H_3$		
				53	C_4H_5		
				45	HOC═O		
				39	C_3H_3		
VI	$C_{11}H_{10}N_2O_3S$ 250.28	133−134	11.85 (H-C-2, s), 11.59 (NH, s), 10.22 (HOC-4, s), 8.03−7.95 (HC-3, d), 6.54 (HC-4′, s), 6.52−6.35 (HC-5,6, m, 2H), 4.15 (CH_3, 3H)	250	$M^{+\bullet}$	96.00	3594 (OH + NH), 3306 (OH), 3068, 2975,2791 ($C_{Ar}H$ CH), 1722 (NC═NO−), 1619 (C═C), 1520 [NHC(═S)], 1489 [cis ═N┈C(SH)−], 1438 (C═N), 1421 [CH(CH_3)], 1353 (C═C), 1314 (C═N), 1276 (C═N, CON), 1242 (CO), 1182, 1163, 1122 ($C_{Ar}H$), 1052, 1041 (C═S)
				235	$[M - CH_3]^+$	3.85	
				221	$[M - C_3H_3]^+$	100	
				208	$[M - C_2H_2O]^+$		
				167	$(HO)_2C_6H_3C(=S)N$		
				153	$(HO)_2C_6H_3(=S)$		
				136	$(HO)_2C_6H_3C{=}NH$		
				108	$(HO)_2C_6H_2$		
				69	C_3H_2NO		
				54	C_2H_2CO		
				52	C_4H_4		
				43	HCNO		
				39	C_3H_3		

Table 2. (Continued)

compd	formula, MW	mp (°C)	^{1}H NMR (δ), [D_6] DMSO, CD_3COCD_3[a]	EI-MS *m/z*	EI-MS R^+	EI-MS rel in (%)	IR $\bar{\nu}$ (cm^{-1})
VII	$C_{11}H_{14}N_2O_3S$ 254.31	155−156	11.44 (HOC-2, s), 10.08 (NH, s), 9.85 (HOC-4, s), 7.75 (HC-3, s), 6.39−6.27 (HC-5,6, m, 2H), 3.68 (H_2C-2′,6′, s, 4H), 2.52 (H_2C-3′,5′, s, 4H)	254	$M^{+\bullet}$	4.10	3473, 3259 (OH + NH), 3016 (C_{Ar}H), 2934 (CH), 2845 (CH), 1624 (C=C), 1508 [NHC(=S)], 1472 [cis =N···C(SH)−], 1438 (CH), 1368 (C=C), 1327 (NHN), 1243 (CO), 1155 (C_{Ar}H), 11.20 (COC), 1048 (C=S)
				237	$[M - NH_3]^+$	55.50	
				221	$[M - SH^\circ]^+$	100	
				211	$[M - C_2H_3O]^+$		
				194	$C_{10}H_{12}O_3N$		
				168	$(HO)_2C_6H_3C(=S)NH$		
				153	$(HO)_2C_6H_3C(=S)$		
				136	$(HO)_2C_6H_3C=NH$		
				108	$(HO)_2C_6H_2$		
				86	C_4H_8NO		
				58	C_2H_4NO		
				53	C_3H_3N		
				52	C_4H_4		
				41	C_2H_2N		
				39	C_3H_3		
VIII	$C_{18}H_{17}N_3O_3S$ 357.42	158−159	12.33 (HOC-2, s), 10.37 (NH, s), 9.35 (HOC-4, s), 7.93, 7.84 (HC-3, d), 7.46 (HC-2′,3′,4′,5′,6′, m), 6. 35 (HC-5, 6, m), 2.16 (H_3CC, s, 3H), 2.05 (H_3CN, t, 3H)[1]	355	$M^{+\bullet}$	19.67	3307, 3192 (OH + NH), 2939, 2827 (C_{Ar}H, CH), 1612 (C=O), 1569, 1526 (C=C), 1497 NHC(=S), 1473 [cis =N···C(SH)−], 1459 (CH), 1435, 1397 (C=N), 1335 (C−H), 1218 (C−O), 1178, 1124 (C_{Ar}H), 1061, 1022 (C=S)
				340	$[M - CH_3]^+$	31.14	
				322	$[M - SH^\circ]^+$	100	
				203	$C_6H_5(C_5H_6N_2O)NH_2$		
				153	$(HO)_2C_6H_4C(=S)$		
				137	$(HO)_2C_6H_3CHNH$		
				110	$C_5H_6N_2O$		
				84	$C_3H_4N_2O$		
				77	C_6H_5		
				56	C_3H_6N		
				53	C_4H_5		
IX	$C_{14}H_{11}NO_4S$ 289.31	155−156	11.54 (HOC-2, s), 11.32 (NH, s), 10.08 (HOC-4, s), 7.87−7.78 (HC-3, d, H), 7.41−7.40 (HC-4′, d, H), 7.14−6.90 (HC-6′,7′, m, 2H), 6.41−6.29 (HC-5,6, m, 2H), 6.13−6.07 (H_2C, s, 2H)	289	$M^{+\bullet}$	68.80	3416 (OH + NH), 2900 (C_{Ar}H), 2689 [CH(CH_3), 1621 (C=C), 1592 NHC(=S), 1501 [cis =N···C(SH)−], 1351 (C=C), 1242 (CO), 1118 (C_{Ar}H), 1038 (C=S)
				256	$[M - SH^\circ]^+$	24.40	
				230	$[M - HCN]^+$		
				226	$[M - OCH_2]^+$		
				153	$(HO)_2C_6H_3C(=S)$		
				137	$(HO)_2C_6H_3CH=NH$, $C_7H_7O_2N$		
				108	$(HO)_2C_6H_2$		
				79	C_6H_7		
				65	C_5H_5		
				52	C_4H_4		
				39	C_3H_3		
X	$C_{14}H_{10}N_2O_2S_2$ 302.21	233−234	13.56 (HOC-2, s), 10.64 (NH, s), 10.32 (HOC-4, s), 8.35−8.26 (HC-3, d, H), 8.07−8.05 (HC-4′, d, H), 7.73−7.34 (HC-5′,6′,7′, m, 3H), 6.47−6.29 (HC-5, 6, m, 2H)	302	$M^{+\bullet}$	10.78	3171, 3063 (OH + NH), 1678, 1595 (C=C), 1529 [NHC(=S)], 1454 [cis =N···C(SH)−], 1387 (C=C), 1330 (C=N), 1240 (CO), 1207 (CN), 1165, 1128 (C_{Ar}H), 1072 (SC=N ring), 1017 (C=S)
				269	$[M - SH^\circ]^+$	100	
				243	$[M - HCN]^+$		
				167	$(HO)_2C_6H_3C(=S)N$		
				153	$(HO)_2C_6H_3C(=S)$		
				150	$C_7H_6N_2S$		
				137	$(HO)_2C_6H_3CH(=NH)$		
				109	C_6H_5S		
				91	$C_5H_4=C=NH$		
				69	C_3HS		
				45	CSH		
				44	CS		
				39	C_3H_3		

Table 3. In Vitro Antimycotic Activity of 2,4-Dihydroxybenzcarbothioamides (I−X) and Standards: Batrafen (B) and Ketoconazole (K) Expressed by MIC Values

		MIC (μg/mL)											
no.	strain	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	B	K
1	*Penicillium* sp.	62.5	31.25	62.5	15.6	31.25	500	31.25	500	31.25	>31.25	31.25	15.6
2	*Aspergillus fumigatus*	>125	15.6	125	31.25	125	500	31.25	>500	31.25	>31.25	31.25	>31.25
3	*Aspergillus niger*	>125	15.6	125	31.25	125	500	31.25	>500	31.25	>31.25	31.25	>31.25
4	*Scopulariopsis brevicaulis*	125	31.25	62.5	31.25	62.5	250	31.25	500	31.25	>31.25	31.25	31.25
5	*Candida albicans*	125	31.25	62.5	15.6	31.25	500	15.6	500	15.6	31.25	15.6	62.5
6	*Candida albicans* ATCC 10231	62.5	15.6	62.5	31.25	31.25	500	15.6	500	7.8	31.25	15.6	62.5
7	*Cryptococcus neoformans*	62.5	31.25	62.5	31.25	15.6	500	31.25	500	7.8	>31.25	15.6	3.9
8	*Geotrichum candidum*	62.5	15.6	125	31.25	62.5	500	15.6	>500	31.25	>31.25	31.25	3.9
9	*Trichosporon* sp.	62.6	15.6	62.5	31.25	62.5	500	7.8	125	15.6	31.25	15.6	3.9
10	*Epidermophyton floccosum*	62.5	7.8	3.9	0.98	7.8	62.5	0.98	15.6	0.49	15.6	15.6	0.003
11	*Microsporum gypseum*	31.25	15.6	7.8	0.98	31.25	62.5	3.9	250	0.98	15.6	31.25	0.12
12	*Trichophyton gallinae*	62.5	3.9	15.6	0.98	3.9	62.5	0.98	31.25	0.98	3.9	15.6	0.48
13	*Trichophyton interdigitale*	62.5	7.8	7.8	0.98	7.8	62.5	0.98	125	0.98	7.8	31.25	0.24
14	*Trichophyton mentagrophytes*	31.25	7.8	7.8	0.98	15.6	62.5	3.9	62.5	0.98	15.6	31.25	0.12
15	*Trichophyton rubrum*	31.25	7.8	7.8	0.98	3.9	62.5	1.95	62.5	0.98	3.9	31.25	0.48

tory concentration causing full inhibition of growth) was applied to evaluate the antimycotic activity. The series of four strains of molds, five yeasts, and six dermatophytes were tested. The MIC values against dermatophytes were ≥0.49 μg/mL, against yeasts, ≥7.8 μg/mL, and against molds, 31.25 μg/mL (Table 3). This indicates

significantly greater sensitivity of dermatophytes to the tested compounds compared with the other studied fungi. Similar tendencies were observed during the study of the fungistatic activity of other compounds with a 2,4-dihydroxybenzenecarbothioacyl moiety (*21*, *22*, *24*, *25*). From the analysis of the compounds' structure it can be stated that depending on the N-substitution type, the derivatives were characterized by quite different fungistatic potency within the range of 0.49−1000 μg/mL. The strongest fungistatic effect was observed for compounds **IX**, especially against dermatophytes (MIC $\leq$ 0.98 μg/mL). Compounds **IV** and **VII** exhibited also potent activity; however, derivatives **I** and **VIII** were characterized by the poorest fungistatic activities, with MIC $\geq$ 31.25 μg/mL.

As biological tests are relative measures, batrafen and ketoconazole were used as the reference system. The MIC values of these antimycotic drugs were compared with the MIC values of the compounds studied (Table 3). It can be generally stated that the level of inhibition action of the most active compounds, **IV**, **VII**, and **IX**, against molds and yeasts can be compared with the standards, but significant differences in activity were observed against dermatophytes. These compounds were characterized by much lower levels of MICs compared with batrafen but comparable (*Trichophyton gallinae*) or higher toward ketoconazole.

The relationship between the structures and antimycotic activity of the studied compounds can refer mainly to the electron properties of the heterocyclic ring and its effect on the electron density of the −C(=S)NH− moiety. The electron-acceptor character intensifies the tendency for privileged imido−thiol structure formation (lower internal energy), which is accompanied by the lipophilicity increase (**IV**, **V**, and **IX**). The hydrophobic character of the substituent (**X**) also promotes high activity. The level of the internal energy of individual compounds is an important factor, which makes it possible to explain statistically some deviations between the character of electron interactions, the polarization of bonds, and the course of fragmentation, particularly of unstable parent ions. Great differences in the activities of compounds **I** and **V** result from the presence of compound **V** of the second pharmacophoric system ($-SCH_3$) bound with the carbon atom having a large electron deficiency. The same trend can be observed also in the case of binding of the system −C(=S)NH− with the ring (compounds **I**−**III** and **X**). Similar comparison of the activity−structure relationship to the high barrier of tautomeric inversion in the case of compound **IV** indicates indirectly the function of =C=S groups in the molecular interactions.

The presented analysis of N-heterocyclic 2,4-dihydroxybenzcarbothioamides indicates a general state of N-substitution effect on antimycotic activity. This will enable orientation of successive synthesis, most probably, toward the most effective compounds. However, selected derivatives of the highest activity can be subjected to structural modifications taking into account different limitations caused by the type of heterocyclic ring.

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