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Synthesis and Antimycotic Activity of *N*-azolyl-2,4-dihydroxythiobenzamides

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Abstract—*N*-pyrazole and *N*-1,2,4-triazole derivatives of 2,4-dihydroxythiobenzamide prepared from sulfinyl-bis-(2,4-dihydroxythiobenzoyl) and commercially available azole amines were tested for their antimycotic activity. The chemical structure of compounds was confirmed by IR, ^{1}H NMR, MS and elemental analysis. The MIC values against the reference strain *Candida albicans ATCC 10231*, azole-resistant clinical isolates of *Candida albicans* and non-*Candida albicans* species were determined for their potential activity in vitro. The compounds exhibited comparable or higher activity than itraconazole and fluconazole tested under the same experimental conditions. Pyrazoline derivatives showed higher activity than other analogues. The strongest fungistatic activity for *N*-(2,3-dimethyl-1-phenyl-1,2-dihydro-5-oxo-5H-pyrazol-4-yl)-2,4-dihydroxythiobenzamide was found with MIC values significantly lower than those for the studied drugs.

Introduction

Many azole drugs find application in the antimycotic therapy. The first drugs in this group were imidazole derivatives[1,2] replaced by triazole chemicals (itraconazole, fluconazole) at present. Fluconazole is used in standard therapy for the majority of *Candida* infections due to a broad spectrum of activity, excellent pharmacokinetics, and the lack of side effects.[3] These drugs inhibit a cytochrome P450 enzyme (CYP51) which catalyses demethylation of 24-methylenedihydrolanosterol in *Candida albicans*. In consequence a reduction in the intracellular level of ergosterol resulting in growth arrest of the yeast takes place.[4,5]

However, widespread use of fluconazole for extended periods is responsible for development of fluconazole-resistant isolates of *C. albicans*.[6] In addition, some non-*C. albicans* spp. tend to be less sensitive or entirely resistant to fluconazole.[3,7] The mechanism of resistance is still not fully understood. Changeable activity of sterol 14-demethylaze (CYP51, Erg11p), the target enzyme of azoles, has been observed in resistant strains of *C. albicans* presumably exerting an effect through reduced azole affinity as shown for the amino acid substitutions T315A and G464S in the molecular modelling and the mutagenesis studies.[8] Furthermore, the studies with *C. albicans* showed significant importance in fungistasis of accumulating 14α-methylergosta-8,24(28)-dien-3β,α-diol under fluconazole treatment.[9] In addition, overexpression of genes encoding intracellular efflux pumps (e.g., CDR genes) might be responsible for reduced intracellular fluconazole concentration.[10] Primary and secondary resistance to antimycotic drugs, more frequent strains of reduced sensitivity, varied resistance as well as differences in sensitivity between species and strains within the same species promote the search for new antimycotic compounds.

As follows from literature =C=S moiety is as pharmacophore of antimycotic activity, characteristic of tolnaftate or tolciclate. The compounds synthesized by us and applied in biological studies: 2,4-dihydroxythiobenzanilides and *N*-heterocyclic substituted carbothionyl analogues[11–14] similar to tolnaftate (2-naftyl-*N*-methyl-*N*-(3-tolilo)thiocarbaminiane), inhibiting epoxidase and squalene accumulation, also confirm this finding.[15]

N-derivatives of 2,4-dihydroxythiobenzamides show a wide spectrum of antifungal activity in relation to yeasts,[11] dermatophytes,[12,14] moulds[13,14] and strong inhibition action comparable with commercial antimycotic drugs.[14] Other compounds with 2,4-dihydroxy-

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thiobenzoyl moiety: derivatives of hydrazine and amidrazone also exhibit an interesting range of atimycotic activity and strong fungistatic effect.[16] At the same time, preliminary study of some 2,4-dihydroxythiobenzanilides showed their low in vitro and in vivo toxicity.[17,18] It proves to be very important for searching biologically active compounds as potential drugs including antimycotics.

Besides thiocarbonyl chromophore, in our compounds $-C_6H_3(OH)_2$ moiety seems to play a significant role. Its presence creates possibility for achieving a suitable hydrophobic-hydrophilic equilibrium, that is migration of compounds through lipoprotein membranes. It is also assumed that the substituted diphenols are capable of lytic action towards *N*-acetylglucosamine chains as well as mannose and urone polymers of cell walls. Moreover, the $-C_6H_3(OH)_2$ moiety maintaining strong reduction properties under in vivo conditions may exhibit keratolytic and contracting properties. The presence of –OH groups increases wettability of compounds with water.

Taking into account the wide application of antimycotics with azole moiety and interesting properties of 2,4-dihydroxythiobenzoyl moiety *N*-pyrazolyl- and *N*-triazolyl-2,4-dihydroxythiobenzamides were prepared as compounds with predicted antifungal activity. The paper presents the synthesis of thioamide derivatives of azoles and influence of *N*-substitution on the fungistatic properties of compounds. The studies of antifungal activity under in vitro conditions against the reference strain *C. albicans ATCC 10231*, azole-resistant clinical isolates of *C. albicans* and non-*Candida* species were carried out. Itraconazole and fluconazole were used for comparison.

Chemistry

N-pyrazoly-l and *N*-1,3,4-triazolylderivatives of 2,4-dihydroxythiobenzamide were prepared from sulfinyl-bis-(2,4-dihydroxythiobenzoyl) (SHTB)[19] and commercially available heterocyclic amines (Scheme 1). SHTB was obtained according to patent.[19] Due to the fact that the amide formation process according to the S_{N1} mechanism is greatly disturbed by spatial conditions, the mechanism of elimination-addition was considered as the most probable. Release of 2,4-dihydroxythiobenzoyl carbocations ($C_6H_3(OH)_2CS^{\oplus}$) from SHTB as a result of solvolysis and thioamide bond formation is asynchronic and occurs without the change of carbon atom hybridisation of thiocarbonyl moiety.

As tautomeric transformations are possible in heterocyclic amines, suitably modified 1,2- and 1,2,4-azole derivatives were used for the synthesis. It was found that proper position of amine group, character of other subsituents and reaction conditions provide an explicit shift of equilibrium state and stabilization of nucleophilic centres. The predicted direction of the reaction S_E is confirmed by the results of chromatographic studies, character of fragmentation in the MS spectra of compounds as well as by ^1H NMR spectra (in the area of low fields). Even in the potentially two-centre compound **3a**, synthesis is undirectional. Charge scattering due to the reaction of the substituent $-SCH_3$ limits the mesomeric effect of the group $-NH_2$ and deactivate the asometine group of the triazole ring.

The reactions with aminopyrazole run explicitly despite a relatively larger nucleophilicity of the 1,2-azole ring. In the synthesis assumptions it was stated that no essential changes of electron density should take place in 3- and 5-aminopyrazoles. Probability of mesomeric effects is diminished here by the substituents (mainly in position α) and interactions of neighbouring heteroatoms limit significantly scattering of the charge and anular isomerizations.

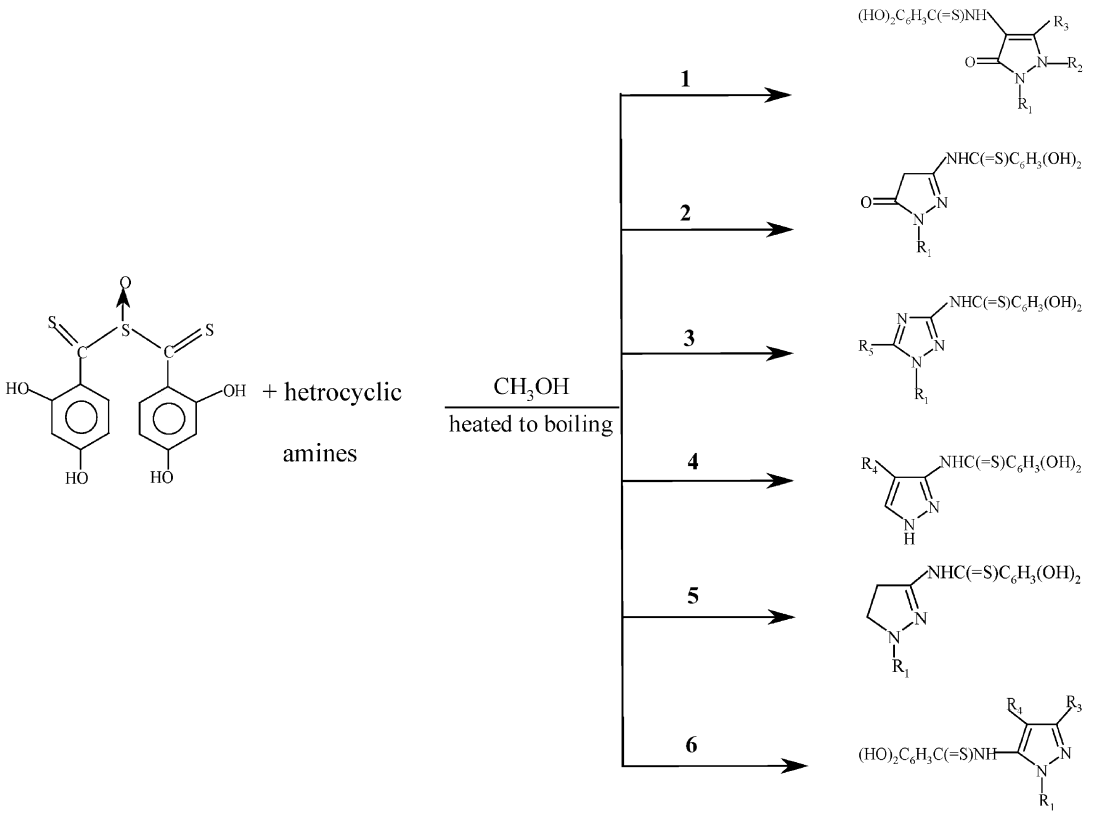
Results and Discussion

The synthetic pathway for the compounds under consideration is illustrated in Scheme 1. The structures of derivatives are presented in Table 1. The analytical data of chemicals were in agreement with the proposed structures. The purity of compounds was confirmed by HPLC chromatography in the reversed-phase system RP-18, methanol–water. Log k_w values for methanol–water 8:2 v/v mobile phase were given.

The inhibition actions of compounds were estimated by the minimal inhibitory concentration values (Tables 1 and 2). MIC values of compounds against the reference strain *C. albicans ATCC 10231* and six clinical isolates of non-*C. albicans* species were determined. The mean MIC values against 10 clinical isolates of *Candida albicans* and against six non-*C. albicans* isolates were calculated (Tables 1 and 2). A similar procedure for fluconazole and itraconazole, as the reference substances was applied. Taking into account structure of tested compounds, drug-resistance (including fluconazole and itraconazole) *Candida* clinical isolates were used.

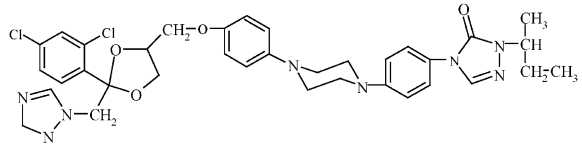
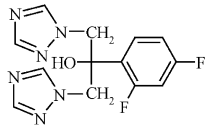
MIC values against the reference strain *C. albicans ATCC 10231* and the mean MIC for 10 clinical isolates are in the range 25–200 μg/mL and 12.5–170 μg/mL, respectively. The analogues MIC values for the drugs are about 200 μg/mL (Table 1). The mean MIC values for the six clinical isolates of non-*C. albicans* strains are in the range 21.9–166.7 μg/mL, however, for itraconazole and fluconazole tested under the same experimental conditions they are 91.7 and 120.8 μg/mL respectively (Table 2). Sensitivity for individual isolates is differentiated. The lowest MIC values against *C. glabrata* were found, particularly for compound **6c** (MIC = 6.25 μg/mL) (Table 2).

The data indicate that all compounds exhibit comparable or better activity towards the fungi under investigation than commonly applied azole drugs tested under the same experimental conditions. Taking into account the MIC values of fluconazole for the resistant strains (MIC ≥ 64 μg/mL) and dose-dependent susceptibility



Scheme 1. Synthesis scheme of *N*-azolyl-2,4-dihydroxythiobenzamides.

Table 1. MIC values of compounds and drugs (itraconazole, fluconazole) against the reference strain *Candida albicans ATCC 10231* and mean MIC values against ten *Candida albicans* strains isolated from the patients on the Sabouraud's medium after 24 h of incubation (μg/mL)

Substance	–R					*Candida albicans ATCC 10231* ($n=1$) MIC [μg/mL]	*Candida albicans* isolates ($n=10$) MIC [μg/mL]	SD[a]	SE[b]
	$-R_1$	$-R_2$	$-R_3$	$-R_4$	$-R_5$				
1	$-C_6H_5$	$-CH_3$	$-CH_3$			25	12.5	0	0
2a	$-C_6H_5$					50	18.1	9.05	2.86
2b	$-(2,4,6)C_6H_2Cl_3$					25	50	0	0
3a	–H				$-SCH_3$	100	100	0	0
3b	$-CH_3$				$-SCH_3$	50	25	15.1	10.8
4	H			$-CO_2C_2H_5$		200	135	57.98	18.3
5	$-C_6H_5$			–H	–H	100	50	0	0
6a	$-CH_3$		$-CH_3$	–H		200	160	51.6	16.3
6b	$-C_6H_5$		$-CH_2CN$	–CN		100	170	63.24	20
6c	$-CH_3$		$-C_6H_5$	–H		50	25	0	0
6d	$-C_6H_5$		–H	$-C(=O)NH_2$		50	47.5	7.9	22.5
Itraconazole						200	182.5	55.33	17.5
Fluconazole						200	200	0	0

[a]Standard deviation.
[b]Average deviation.

Table 2. MIC values of compounds and drugs (itraconazole, fluconazole) against non-*Candida albicans* species on the Sabouraud's medium after 24 h of incubation (μg/mL)

Strains	Substance MIC [μg/mL]												
	Itraconazole	Fluconazole	**1**	**2a**	**2b**	**3a**	**3b**	**4**	**5**	**6a**	**6b**	**6c**	**6d**
Candida krusei	100	200	50	50	50	50	100	200	100	200	50	25	100
Candida tropicalis I	50	25	25	50	50	50	50	200	100	200	50	25	50
Candida tropicalis II	50	50	25	50	50	50	50	200	100	200	50	25	50
Candida paratropicalis	200	200	25	50	50	50	50	200	100	200	50	25	25
Candida species	100	50	12.5	50	50	100	200	100	25	100	100	25	50
Candida glabrata	50	200	12.5	12.5	50	200	50	100	25	100	25	6.25	12.5
6 isolates of non-*Candida albicans* (mean MIC values)	91.7	120.8	23.1	43.8	50	83.3	83.3	150	75	166.7	54.2	21.9	47.9
SD[a]	58.5	87.2	16.3	15.3	0	60.6	60.6	54.8	38.7	51.6	24.6	7.7	30.0
SE[b]	41.7	79.2	8.3	10.4	0	44.4	44.4	50	33.3	44.4	15.3	5.2	19.4

[a]Standard deviation.
[b]Average deviation.

(MIC 16–32 μg/mL) under the experimental conditions all isolates proved to be resistant to the drug used which resulted from the assumption of isolate selection.[20] Applying the same criterion for the obtained compounds as for azole derivatives, it was found that the isolates of *C. albican*s showed susceptibility to one compound (compound **1**), dose-dependent susceptibility to three compounds (compounds **2a**, **3b**, **6c**) and resistance to the others (Table 1). Using the same sensitivity range for non-*C. albicans* strains, sensitivity of *C. glabrata* was found to compounds **1**, **2a**, **6c**, **6d** and non-identified non-*Candida* species to compound **1** again (Table 2).

As for the structure of compounds and antimycotic properties a significant effect of azole ring can be observed. The data show that thioamide derivatives of the structures with a pyrazoline ring exhibit the greatest activity towards the studied fungi (**1**, **2a**, **2b**). Their activity compared with the standards is a few times larger and most probably depends on the degree of ring saturation. The less saturated structure (**1**), showed the greatest activity for both *C. albicans* and non-*C. albicans* species. The transitions from thioamidophenoazone structures to enole forms are likely to take place. This isomerization is confirmed by the parameters of ^{1}H NMR spectra and IR (appearance of enole groups). Tautomeric migrations probably diminish ring stresses caused by C=O groups and lead to forms of stronger amphophilic character.

Activity of triazole derivatives (**3a**, **3b**) is average but higher for the structures with the methyl substituent at N-1 than that for the unsubstituted analogue.

Among pyrazole derivatives (groups **4**–**6**) activity is differentiated, depending on the degree of ring saturation as well as on number, kind and arrangement of substituents. Compound **6c** exhibits the highest fungistatic activity while compound **6a** the weakest one. Saturated system (5) is characterized by a relatively low activity.

In other cases choice of kind, localization and number of substituents is difficult. It is possible that changes of activity can correspond to shifts of equilibrium states from thioamide to thiolimine: –C(=S)-NH ↔ –C(SH)=N– similar to other *N*-aryl (heterocyclic) thioamides.[21] Though in individual compounds ability for rearrangement is differentiated and depends on electron deficit on nitrogen atoms, it leads to more stable energetic forms in every case as confirmed by theoretical computer calculations.[13,14] Broadening of electron density on heteroatoms in iminothiol states corresponds to the changes of lipophilicity and dipole moment which probably changes arrangement and makes migration of compounds through membrane structures easier. This characteristic continuity of changes of biological activity and retention parameters in the reversed phase system (RP-18, HPLC; RP-8, HPTLC, methanol-water and acetonitrile mobile phase) was confirmed for many compounds investigated chromatographically by us.[11–13,21,22]

Recently prepared compounds with the azole ring, often structural analogues of commonly applied antimycotic substances as a rule exhibit smaller activity towards drugs. Menozzi and co-workers obtained compounds of very smaller antimycotic activity synthesizing the analogues of bifonazole with a phenylisoxazolyl or phenylpyrimidinyl moiety.[23] A similar effect was found for [α-(1,5-disubstituted 1H-pyrazolyl-4-yl)benzyl]azoles, as analogues of the same drug.[24] Series of azole-containing 1,2,3,4-tetrahydroisoquinoline isomers showed lower activity against yeasts than the drugs used for comparison. Activity in relation to moulds was at the same level for some derivatives.[25] Azole derivatives of 1,4-benzothiazine show also weaker fungistatic properties than fluconazole.[26] However, sulfur analogues (alkylthio and alkylsulfonyl) of the compound designed as SM-8668 (azole derivative) exhibit several times higher activity than that of same drug.[27]

Therefore *N*-azolyl-2,4-dihydroxythiobenzamides, particularly pirazoline derivatives seem to be an interesting group of compounds. This justifies our search for antimycotic substances in the group of 2,4-dihydroxythiobenzoylaminoderivatives or suitable cyclic analogues with $(HO)_2C_6H_3$– moiety.

Experimental

Chemistry

Melting point measurements were made using a Boetius apparatus. Elemental analysis was performed in order to determine C, H and N contents (Perkin-Elmer 2400 analyser). Analyses (C, N, H) were within ±0.4% of the theoretical values.

^{1}H NMR spectra were made using a Varian 200 or a Bruker 500 spectrometer, standard TMS, solutions in DMSO-d_6, shift δ (ppm). The spectra were interpreted with reference to the proton connected with heteroatoms. Position of these signals defining the interaction strength of induction systems determines indirectly the ability of compounds for tautomeric rearrangement, associated molecules formation and change of lipophilicity.

The oscillation spectra were recorded with a Perkin-Elmer FT-IR 1725X spectrophotometer (in KBr). The spectra were made in the range of 600–4000 cm^{-1}. The spectra MS (EI-70 eV) were recorded using a AMD-604 spectrometer. The spectra of compound **6d** were taken by means of the ionisation method LSIMA using the resolving power HR for M^+, $M+H^+$ and $M+Na^+$.

The purity of the compounds was checked by liquid chromatograph (Knauer) with a dual pump, a 20 μL simple injection valve and UV–visible detector (320 nm). Hypersil BDS C18 (5 μm, 150×4.6 mm) column was used as the stationary phase. The mobile phase consisted of different content of methanol and 10 mM acetate buffer (pH 4) as the aqueous phase. The flow rate was 0.5 mL/min at room temperature. The column dead time was determined by the injection of small amount of acetone dissolved in water.

***N*-(2,3-dimethyl-1-phenyl-1,2-dihydro-5-oxo-5H-pyrazol-4-yl)-2,4-dihydroxythiobenzamide (1).** This compound was described earlier.[14]

***N*-(1-phenyl-2-pyrazolin-5-on-3-yl)-2,4-dihydroxythiobenzamide (2a).** 0.025 mol 3-amino-1-phenyl-2-pyrazolin-5-one (Lancaster) and 0.01 mol SHTB were put into 50 mL methanol and heated to boiling (3 h). The mixture was hot filtered and the filtrate was concentrated to dry. The removed compound was fractionally crystallized from methanol (50 mL).

Mp 176–178 °C; HPLC: log $k=-0.777$; EI–MS (m/z): 327 (M^+, 3215), 310, 208, 175 (100), 160, 153, 136, 110, 91, 77; ^{1}H NMR (DMSO-d_6), δ (ppm): 10.48 (d, NH, 1H); IR (cm^{-1}): 2943, 1680, 1592, 1498, 1452 ν N⎓C(SH), 1338, 1312, 1242, 1175, 1154, 1087, 1030 ν C=S. Anal. calcd for $C_{16}H_{13}N_3O_3S$: C, 58.64; H, 3.97; N, 12.84. Found: C, 58.77; H, 3.85; N, 12.73.

***N*-[1-(2,4,6-trichlorophenyl)-2-pyrazolin-5-on-3-yl]-2,4-dihydroxythiobenzamide (2b).** 0.025 mol 3-amino-1-(2,4,6-trichlorophenyl)-2-pyrazolin-5-on (Aldrich) and 0.01 mol SHTB were put into 50 mL methanol and heated to boiling (3 h). The mixture was hot filtered and the filtrate was concentrated to dry. The removed compound was fractionally crystallized from diluted (3:1) methanol (60 mL).

Mp 180–181 °C; HPLC: log $k=-0.728$; EI–MS (m/z): 431 (19.35), 234, 221, 208 (100), 197, 195, 167, 153, 136, 108, 99, 72; ^{1}H NMR (DMSO-d_6), δ (ppm): 12.76 (s, NH, 1H); IR (cm^{-1}): 1619, 1554, 1502, 1462 ν N⎓C(SH), 1354, 1293, 1242, 1123, 1014 ν C=S. Anal. calcd for $C_{16}H_{10}Cl_3N_3O_3S$: C, 44.57; H, 2.32; N, 9.76. Found: C, 44.43; H, 2.51; N, 9.54.

***N*-(5-methylthio(1H-1,2,4-triazol-3-yl))-2,4-dihydroxythiobenzamide (3a).** This compound was described earlier.[14]

***N*-(1-methyl-5-methylthio-1,2,4-triazol-3-yl)-2,4-dihydroxythiobenzamide (3b).** 0.025 mol 3-amino-1-methyl-5-methylthio-1,2,4-triazole (Lancaster) and 0.01 mol SHTB were put into 50 mL methanol and heated to boiling (3 h). The mixture was hot filtered, 100 mL of water were added to the filtrate. The removed compound was crystallized from diluted (3:1) methanol (60 mL).

Mp 266–268 °C; HPLC: log $k=-0.641$; EI–MS (m/z): 296 (M^+, 100), 281, 263, 249, 240, 161, 153, 144, 111, 88, 69, 43, 36; ^{1}H NMR (DMSO-d_6), δ (ppm): 10.28 (s, NH, 1H), 3.48 (s, SCH_3, 3H), 2.51 (m, NCH_3, 3H); IR (cm^{-1}): 1610, 1578, 1535, 1469 ν N⎓C(SH), 1407, 1337, 1297, 1259, 1200, 1140, 1116, 1074 ν C=S. Anal. calcd for $C_{11}H_{12}N_4O_2S_2$: C, 44.54; H, 4.05; N, 18.90. Found: C, 44.77; H, 3.92; N, 19.03.

***N*-(ethyl pyrazol-4-carboxylate-3-yl)-2,4-dihydroxythiobenzamide (4).** 0.025 mol ethyl 3-aminopyrazole-4-carboxylate (Aldrich) and 0.01 mol SHTB were put into 50 mL methanol and heated to boiling (3 h). The mixture was hot filtered and the filtrate was left for 24 h (at room temperature). The removed compound was crystallized from diluted (3:1) methanol (60 mL).

Mp 215–216 °C; HPLC: log $k=-0.467$; EI–MS (m/z): 307 (M^+, 40.96), 261, 203, 192, 172, 153 (100), 137, 110, 97, 69,53, 39; ^{1}H NMR (DMSO-d_6), δ (ppm): 10.75 (s, NH, 1H), 4.18 (q, OCH_2, 2H), 1.43 (t, CH_3, 3H); IR (cm^{-1}): 1706, 11671, 1591, 1524, 1496, 1461 ν N⎓C(SH), 1376, 1221, 1051 ν C=S. Anal. calcd for $C_{15}H_{13}N_3O_4S$: C, 50.76; H, 4.23; N, 13.67. Found: C, 50.93; H, 4.08; N, 13.57

***N*-(4,5-dihydro-1-phenyl-pyrazol-3-yl)-2,4-dihydroxythiobenzamide (5).** 0.025 mol 3-amino-4,5-dihydro-1-phenylpyrazole (Lancaster) and 0.01 mol SHTB were put into 50 mL methanol and heated to boiling (3 h). The mixture was hot filtered. The filtrate was left for 24 h (at room temperature). The removed compound was crystallized from diluted (3:1) methanol (60 mL).

Mp 95–97 °C; HPLC: log $k=-0.605$; EI–MS (m/z): 313 (M^+; 24.13), 295, 278, 256, 243 (100), 224, 208, 191, 184, 169 159, 137, 124, 108, 93, 80, 77, 64, 51; ^{1}H NMR (DMSO-d_6), δ (ppm): 11.43 (s, NH, 1H); IR (cm^{-1}):

1680, 1591, 1490 ν N⎓C(SH), 1391, 1322, 1260, 1024 ν C=S. Anal. calcd for $C_{16}H_{15}N_3O_2S$: C, 61.26; H, 4.79; N, 13.41. Found: C, 61.03; H, 4.99; N, 13.25.

***N*-(1,3-dimethylpyrazol-5-yl)-2,4-dihydroxythiobenzamide (6a).** 0.025 mol 5-amino-1,3-dimethylpyrazole (Lancaster) and 0.01 mol SHTB were put into 50 mL methanol and heated to boiling (3 h). The mixture was hot filtered and 100 mL of water were added to the filtrate. The removed compound was crystallized from diluted (3:1) methanol (60 mL).

Mp 210–211 °C; HPLC: log $k = -0.607$; EI–MS (m/z): 184, 153 (100), 142, 111, 97, 87, 57, 45, 399 (lack of M^+ pick); ^{1}H NMR (DMSO-d_6), δ (ppm): 10.89 (s, NH, 1H), 3.52 (s, NCH_3, 3H), 1.8 (s, CH_3, 3H); IR (cm^{-1}): 1617, 1563, 1526, 1471 ν N⎓C(SH), 1435, 1420, 1401, 1386, 1363, 1243, 1216, 1078 ν C=S. Anal. calcd for $C_{12}H_{13}N_3O_2S$: C, 54.68; H, 4.94; N, 15.96. Found: C, 54.51; H, 5.06; N, 15.79.

***N*-(4-cyano-3-cyanomethyl-1-phenylpyrazol-5-yl)-2,4-dihydroxythiobenzamide (6b).** 0.025 mol 5-amino-4-cyano-3-cyanomethyl-1-phenylpyrazole (Lancaster) and 0.01 mol SHTB were put into 50 mL methanol and heated to boiling (3 h). The mixture was hot filtered and 100 mL of water were added to the filtrate. The removed compound was crystallized from diluted (2:1) methanol (60 mL).

Mp 145–147 °C; HPLC: log $k = -0.241$; EI–MS (m/z): 223 (100), 184, 153, 91, 77 (lack of molecular ion M^+); ^{1}H NMR (DMSO-d_6), δ (ppm):11.85 (s, NH, 1H), 4.19 (t, CH_2CN, 2H); IR (cm^{-1}): 1625, 1588, 1540, 1511, 1469 ν N⎓C(SH), 1445, 1407, 1343, 1208, 1114, 1049 ν C=S. Anal. calcd for $C_{19}H_{13}N_5O_2S$: C, 60.73; H, 3.46; N, 18.66. Found: C, 60.92; H, 3.28; N, 18.95.

***N*-(1-methyl-3-phenyl-pyrazol-5-yl)-2,4-dihydroxythiobenzamide (6c).** 0.025 mol 3-amino-1-methyl-3-phenylpyrazole (Lancaster) and 0.01 mol SHTB were put into 50 mL methanol and heated to boiling (3 h). The mixture was hot filtered and 100 mL of water were added to the filtrate. The removed compound was crystallized from diluted (2:1) methanol (75 mL).

Mp 224–225 °C; HPLC: log $k = -0.403$; EI–MS (m/z): 323 (M^+, 17.15), 293, 205, 184, 173 (100), 137, 130, 117, 102, 97, 77, 69, 57, 51; ^{1}H NMR (DMSO-d_6), δ (ppm): 10.88 (s, NH, 1H), 3.66 (s, NCH_3, 3H); IR (cm^{-1}): 1613, 1505, 1466 ν N⎓C(SH), 1450, 1417, 1360, 1237, 1215, 1187, 1154, 1121, 1013 ν C=S. Anal. calcd for $C_{17}H_{15}N_3O_2S$: C, 62.69; H, 4.61; N, 12.91. Found: C, 62.85; H, 4.43; N, 12.76.

***N*-(1-phenylpyrazol-5-yl-4-carboxamide)-2,4-dihydroxythiobenzamide (6d).** 0.025 mol 5-amino-1-phenylpyrazole-4-carboxamide (Lancaster) and 0.01 mol SHTB were put into 50 mL methanol and heated to boiling (3 h). The mixture was hot filtered and the filtrate was concentrated to dry. The removed compound was fractionally crystallized from diluted (2:1) methanol (75 mL).

Mp 124–126 °C; HPLC: log $k = -0.218$; MS (m/z): 427.2 $[2M^+ + Na]^+$, 405.2 $[2M^+ + H]^+$ (100), 355, 303.2, 279,3, 257, 225 $[M + Na]^+$, 217, 203.1 $[M + H]^+$ (100%), 185, 153, 96, 87, 65; ^{1}H NMR (DMSO-d_6), δ (ppm): 10.57 (wide pick, 3H); IR (cm^{-1}): 1656, 1625, 1554, 1502, 1471, 1460 ν N⎓C(SH), 1434, 1341, 1264, 1226, 1213, 1153, 1121, 1055 ν C=S. Anal. calcd for $C_{17}H_{14}N_4O_3S$: C, 57.56; H, 3.95; N,15.81. Found: C, 57.42; H, 3.88; N, 15.95.

Biological investigations

To determine the antifungal activity of compounds they were tested against *Candida* species. Ten azole-resistant fresh clinical isolates of *C. albicans* were used. 40 strains of *C. albicans* taken from the mouth cavity ontocenosis of patients suffering from tumor diseases were used as the selective material. Itraconazole and fluconazole were administered for prophylactic or due to the symptoms of candidase. Ten isolates resistant to drugs (5-fluorocytosine, ketoconazole, amphotericin B, itraconazole, miconazole, fluconazole) as shown by Fungitest® were chosen for dilution-method testing of the compounds. The drug-resistance was 56.7% but to itraconazole and fluconazole 87.5% and 82.5%, respectively. Additionally, the isolate from the American Type Culture Collection (University Blvd., Manassas), *C. albicans ATCC 10231* was used as the quality control strain. Six isolates of non-*C. albicans* (two isolates of *C. tropicalis*, *C. glabrata, C. krusei, C. paratropicalis, C. tropicalis)* were tested for which drug-resistance by Fungitest® was also determined. Itraconnazole and fluconazole were used as the reference system.

The yeast isolates were identified to the species level by conventional morphological and biochemical methods by the Candi*Select* (Bio-Rad), Fungiscreen 4H (Bio-Rad), Auxacolor (Bio-Rad) tests.

The compounds were dissolved in 1% DMSO. The susceptibility testing was performed by the agar dilution method. For yeasts MICs were determined by the agar dilution procedure according to the National Committee for Clinical Laboratory Standards (NCCLS) reference document M27.[28] The Sabouraud's medium- SB (Bio-Rad) was used. Starting inocula were adjusted by the spectrophotometric method densitometre (BioMerieux) to 1×10^5 CFU/mL. The concentrations of compounds were ranging from 6.25 to 200 g/L. The plates were incubated at 37 °C and read after 24 h of incubation. A solvent control was included in each set of assays; the DMSO solution at the maximum final concentration of 1% had no effect on fungal growth. Each measurement was repeated three times. Itraconazole (Pliva, Krakow, Poland) and fluconazole (Janssen-Cilag) tested under the same experimental conditions were used as the reference system. The investigations were carried out in the Mycological Laboratory of General Nursing Department, Medical Academy, Białystok.

The Student t-test (two-tailed) was used to compare the mean MIC values. Significance was defined as a p value

of 0.05. These analyses were performed using a personal computer with a commercially available statistics program Statistica 5.0.

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