SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL N,N-CYCLIC-2,4-DIHYDROXYTHIOBENZAMIDE DERIVATIVES*

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Abstract: A series of novel N,N-cyclic-2,4-dihydroxythiobenzamide derivatives is described. Test compounds were formed by the reaction of the commercially available reagents with sulfinylbis[(2,4-dihydroxyphenyl)methanethione] (STB). The chemical structures were confirmed by IR, ^{1}H NMR, ^{13}CNMR, EI-MS, and elemental analysis. For the estimation of potential activity *in vitro*, the MIC values against strains of *Candida* were determined. Antifungal properties of selected compounds under *in vitro* conditions against five phytopathogenic fungi were estimated. Furthermore, the antiproliferative activity against the HCV29T cancer cell lines has been studied. These compounds exhibited antiproliferative activity in the range of 33.98 – 10.51 µg/mL.

Keywords: 2,4-dihydroxythiobenzamide, synthesis, antifungal activity, antiproliferative activity

Cancer is a major health problem in developed countries where it is the second cause of death mainly associated with ageing of the population and lifestyle. Early diagnosis, common access to health care and developed therapies have resulted in a significant improvement of cancer survival (1). Most often, it is chemotherapy that is used in the treatment of cancer. It disrupts the way cancer cells grow and divide. However, it also affects normal cells. Chemotherapy uses cytotoxic drugs to destroy cancer cells and is used alone to treat some types of cancer. Nevertheless, it is frequently used with other treatments such as surgery, radiotherapy, hormonal therapy or other anti-cancer drugs, as targeted or biological therapies. There are many different chemotherapy drugs and new drugs are being developed all the time (2).

An important problem in the chemotherapy not solved yet is treatment of systemic mycoses. There is an increased risk of oral fungal infection during cancer therapy. Some of fungal organisms, notably *Candida albicans*, are commensal inhabitants of the oral cavity. Under normal conditions, these fungal organisms co-exist with the other microorganisms of the normal oral flora and do not cause disease. However, changes in the oral and systemic environment can result in an overgrowth of these fungal species, leading to oral fungal infection. These changes include immunosuppression (e.g., induced by drugs), imbalance in the oral flora (secondary to antibiotic therapy) and local tissue damage (chemotherapy and radiation therapy). Cancer patients receiving chemotherapy and/or radiation therapy are prone to all of the aforementioned predisposing factors and are therefore considered to be at higher risk for oral fungal infection than the general population. Systemic antifungals are poorly effective in the prevention of oral fungal infection in patients receiving cancer therapy. Similarly, currently available topical antifungal agents are less efficacious, suggesting a need for better topical agents (3, 4).

Fungicides remain vital for effective control of plant diseases, which are estimated to cause yield reductions by almost 20% in the major food crops worldwide. During the past few years, fungicide research has produced a diverse range of products (e.g., anilinopyrimidines, phenoxyquinolines, oxazolidinediones, spiroketalamines) with novel modes

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of action, which are expected to have a significant impact on disease control (5). Recently, many sulfur analogues with linear =NC(=S)- or cyclic –S-N=N- groups have been prepared, among others, tolnaftate and tolciclate (thiocarbamates) (6).

2,4-Dihydroxyphenyl derivatives of various groups of compounds with >N-C(<)S- moiety both in the linear (benzanilides and amidrazones) (7, 8) and in cyclic (4*H*-3,1-benzothiazines) systems prepared in our laboratory (9) exhibit an interesting level of antifungal activity against moulds, dermatophytes, yeast, yeastlike, and phytopathogenic fungi. Therefore, the newly prepared N,N-cyclic-2,4-dihydroxythiobenzamides have been tested for their antifungal properties.

Benzanilide (N-phenylbenzamide) and thiobenzanilide derivatives possess a broad spectrum of biological activities. They have been found to exhibit antimalarial, antibacterial and antifungal properties (10-13). Derivatives of N-(2-hydroxyphenyl)benzamide have been studied for the last few years as the possible metabolites of the antibacterial active benzo[d]oxazole derivatives (14). Benzamides have promising anti-inflammatory and analgesic properties (15, 16) and anti-convulsant activity (17). What is more, novel inhibitors representing a diverse range of chemical scaffolds have been screened as potent and selective inhibitors of plasmodium falciparum dihydroorotate dehydrogenase, making the enzyme a strong candidate for the development of new antimalarial compounds (18). Benzamides have weak cholinesterase inhibitory properties but protect cholinesterase *in vitro* from stronger inhibitors like dichlorvos (19). Some novel benzamides were found to have high affinity for dopamine D3 (20, 21) and have been reported to inhibit the c-Met tyrosine kinase receptor, which is a potentially important target for the treatment of cancer (22, 23).

2,4-Dihydroxybenzanilides obtained by our team are characterized by inhibitory effect against dermatophytes (24), yeasts (25) and phytopathogenic fungi (26).

At present, the synthesis of thiobenzamides with sulfinylbis[(2,4-dihydroxyphenyl)methanethione] (STB) and the secondary cyclic amines has been carried out in our laboratory. The paper presents the preparation of N,N-cyclic-2,4-dihydroxythiobenzamides, their *in vitro* antifungal activity against *Candida* and phytopathogenic fungi. The antiproliferative activity of selected compounds to cell line of human bladder tumor (HCV29T) was also evaluated.

EXPERIMENTAL

Chemistry

The IR spectra were measured with a Perkin-Elmer FT-IR 1725X spectrophotometer (in KBr). The spectra were made in the range of 600-4000 cm⁻¹. ¹H NMR and ¹³C NMR spectra were recorded in DMSO-d₆ on Varian Mercury 400 or Bruker DRX 500 spectrometers. Chemical shifts (δ, ppm) were described in relation to tetramethylsilane (TMS). The spectra MS (EI, 70 eV) were recorded using the apparatus AMD-604. Elemental analyses (C, H, N) were performed with the use of Perkin-Elmer 2400 instrument and were found to be in good agreement ($\pm$ 0.4%) with the calculated values. The melting points (m.p.) were determined using a Büchi B-540 (Flawil, Switzerland) melting point apparatus.

The purity of the compounds was examined by HPLC (Knauer, Berlin, Germany) with a dual pump, a 20 μL simple injection valve and a UV-visible detector (330 nm). The Hypersil Gold C18 (1.9 μm, 100 × 2.1 mm) column was used as the stationary phase. The mobile phase included different contents of MeOH and acetate buffer (pH 4, 20 nM) as the aqueous phase. The flow rate was 0.5 mL/min at room temperature. The retention time of an unretained solute (t_o) was determined by the injection of a small amount of acetone dissolved in water. The log k values for 70% of MeOH (v/v) in the mobile phase are presented. The log k values were calculated as log k = log(t_R - t_o)/t_o, where: t_R - the retention time of a solute, t_o - the retention time of an unretained solute.

Synthesis of N,N-cyclic-2,4-dihydroxythiobenzamide derivatives

4-(2,4-Dihydroxybenzencarbothiol)piperazine-1-carboxylate ethyl (1)

STB (0.02 mol) and piperazine-1-carboxylate ethyl (Alfa Aesar) (0.02 mol) were added to MeOH (80 mL) and heated to boiling (3 h). The mixture was hot filtered and water (50 mL) was added to the filtrate. The removed compound was washed by water and recrystallized from MeOH (20 mL) and then from benzene (50 mL).

Yield: 76%; m.p.: 195-197°C; log k = -0.401. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 9.74 (s, 1 H, HO-C2), 9.56 (s 1 H, HO-C4), 7.00 (d, 1 H, *J* = 8.6 Hz, H-C6), 6.28 (s, 1 H, H-C3), 6.19 (m, 1 H, H-C5), 4.06 (q, 2 H, *J* = 7.2 Hz, CH₂), 3.71 (m, 4 H, H-C (piperazine)), 3.15 (m, 4 H, H-C (piperazine)), 1.19 (t, 3 H, *J* = 7.1 Hz, CH₃); ¹³C NMR (125 MHz, DMSO-d₆, δ, ppm): 169.9 (C=S), 159.0 (C=O),

154.5, 151.8, 130.5, 121.3, 106.6, 101.8, 60.9 (CH$_2$), 50.33 (C piperazine), 48.5 (C piperazine), 43.2 (C piperazine), 40.0 (C piperazine), 14.4 (CH$_3$); IR (KBr, cm^{-1}): 3220 (OH), 1669 (C=O), 1613 (C=C), 1594 (C=C), 1493, 1435 (C-H), 1386, 1350, 1305, 1285, 1249, 1217 (C-OH), 1190, 1132, 1111 (C=S), 1083, 1056, 1032, 1017, 998, 981, 939, 872, 847, 803, 766, 748, 723; EI-MS (m/z, %): 310 (M$^+$, 100), 281 (4), 277 (5), 264 (5), 194 (22), 157 (19), 153 (45), 141 (10), 136 (5), 97 (4), 85 (4), 81 (3), 69 (9), 56 (9). Analysis: calcd. for C$_{14}$H$_{18}$N$_2$O$_4$S (310.37): C 54.18, H 5.85, N 9.03%; found: C 54.21, H 5.89, N 9.07%.

(4-Benzylpiperazin-1-yl)(2,4-dihydroxyphenyl)methanethione (2)

STB (0.02 mol) and 1-benzylpiperazine (Alfa Aesar) (0.02 mol) were added to MeOH (50 mL) and heated to boiling (3 h). The mixture was hot filtered and water (100 mL) was added to the filtrate. The formed product was dissolved in a solution of Na$_2$CO$_3$ (5%) (50 mL) and filtered. The filtrate was then treated with a solution of HCl (10%) until acidic. The removed compound was washed by water and recrystallized from MeOH (50 mL).

Yield: 69%; m.p.: 78-80°C; log k = -0.073. ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 9.69 (s, 1 H, HO-C2), 9.54 (s, 1 H, HO-C4), 8.58 (m, 1 H, H-C Ar), 7.78 (m, 1 H, H-C Ar), 7.38 (m, 2 H, H-C Ar), 6.97 (m, 2 H, H-C Ar), 6.26 (d, 1 H, J = 2.3 Hz, H-C3), 6.23 (dd, 1 H, J = 8.3 and 2.3 Hz, H-C5), 3.59 (s, 2 H, CH$_2$), 2.68 (m, 4 H, H-C piperazine), 2.33 (m, 4 H, H-C piperazine); IR (KBr, cm^{-1}): 3029 (OH, C Ar-H), 2916 (CH), 2809 (CH), 1617, 1594 (C=C), 1484, 1441 (C-H), 1293, 1213 (C-OH), 1113 (C=S), 1066, 1033, 1002, 978, 844, 798, 775, 746, 701; EI-MS (m/z, %): 328 (M$^+$, 32), 295 (12), 219 (4), 194 (8), 175 (6), 159 (32), 153 (16), 146 (36), 134 (21), 117 (5), 91 (100), 65 (5), 42 (17). Analysis: calcd. for C$_{18}$H$_{20}$N$_2$O$_2$S (328.48): C 65.83, H 6.14, N 8.53%; found: C 65.98, H 6.16, N 8.57%.

(2,4-Dihydroxyphenyl)[4-(2-methoxyphenyl)piperazin-1-yl]methanethione (3)

STB (0.01 mol) and 1-(2-methoxyphenyl)piperazine (Alfa Aesar) (0.01 mol) were added to MeOH (50 mL) and heated to boiling (3 h). The mixture was hot filtered and the filtrate was left at room temperature (24 h). The filtrate was concentrated to dryness and recrystallized from aqueous (4 : 1) methanol (50 mL).

Yield: 63%; m.p.: 165-167°C; log k = -0.496. ^{1}H NMR (500 MHz, DMSO-d$_6$, δ, ppm): 9.73 (s, 1 H, HO-C2), 9.50 (s, 1 H, HO-C4), 8.31 (s, 1 H, H-C

Ar), 7.02-6.85 (m, 4 H, H-C Ar), 6.28 (d, 1 H, J = 2.2 Hz, H-C3), 6.23 (dd, 1 H, J = 8.4 and 2.2 Hz, H-C5), 3.78 (s, 3 H, CH$_3$), 3,60 (m, 4 H, H-C(piperazine), 3.2-3.1 (m, 4 H, H-C(piperazine); IR (KBr, cm^{-1}): 3246 (OH), 2955 (CH), 2833 (CH), 1617 (C=C), 1500 (C=C), 1439 (C-H), 1386, 1241 (C-OH), 1153, 1057, 1025, 976, 919, 845, 809, 751; EI-MS (m/z, %): 344 (M$^+$, 53), 328 (7), 311 (10), 298 (5), 258 (5), 224 (3), 200 (9), 191 (11), 175 (75), 162 (100), 153 (45), 150 (32), 147 (7), 131 (58), 120 (15), 117 (11), 106 (14), 97 (7), 91 (7), 81 (5), 77 (12), 69 (6), 65 (10), 56 (18), 51 (6), 45 (6), 41 (5), 39 (9). Analysis: calcd. for C$_{18}$H$_{20}$N$_2$O$_3$S (344.43): C 62.77, H 5.85, N 8.13%; found: C 62.81, H 5.88, N 8.08%.

(2,4-Dihydroxyphenyl)[4-((2E)-phenylprop-2-ene-1-yl)piperazin-1-yl]methanethione (4)

STB (0.01 mol) and 1-[(2E)-3-phenylprop-2-en-1-yl]piperazine (Alfa Aesar) (0.01 mol) were added to MeOH (40 mL) and heated to boiling (3 h). The mixture was hot filtered and the removed compound was washed by water and recrystallized from aqueous (2 : 1) methanol (45 mL).

Yield: 69%; m.p.: 110-115°C; log k = -0.492. ^{1}H NMR (500 MHz, DMSO-d$_6$, δ, ppm): 9.67 (s, 1 H, HO-C2), 9.51 (s, 1 H, HO-C4), 7.44 (d, 2 H, J = 8.7 Hz, H-C Ar), 7.33 (m, 2 H, H-C Ar), 7.23 (m, 1 H, H-C Ar), 6.98 (d, 1 H, J = 8.4 Hz, H-C Ar), 6.56 (d, 1 H, J = 16 Hz, H-C), 6.39 (m, 1 H, H-C), 6.26 (d, 1 H, J = 2.2 Hz, H-C3), 6.22 (dd, 1 H, J = 8.1 and 2.2 Hz, H-C5), 3.01 (m, 4 H, H-C piperazine), 3.18 (d, 2 H, J = 6.6 Hz, CH$_2$), 2.62 (m, 4 H, H-C piperazine); IR (KBr, cm^{-1}): 3024 (OH), 2921 (CH), 2818 (CH), 1614 (C=C), 1436, 1230 (C-OH), 1129 (C=S), 973, 843, 806, 785, 744; EI-MS (m/z, %): 354 (M$^+$, 10), 338 (3), 321 (7), 185 (17), 172 (21), 153 (56), 137 (9), 117 (100), 94 (5), 91 (13), 69 (7), 56 (14), 44 (15), 39 (5). Analysis: calcd. for C$_{20}$H$_{22}$N$_2$O$_2$S (354.47): C 67.77, H 6.26, N 7.90; found: C 67.68, H 6.29, N 7.85%.

(2,4-Dihydroxyphenyl)(5-methoxy-1H-indol-1-yl)methanethione (5)

STB (0.0075 mol) and 2-methoxy-1H-indole (Alfa Aesar) (0.0075 mol) were added to MeOH (50 mL) and heated to boiling (3 h). The mixture was hot filtered and water (100 mL) was added to the filtrate. The removed compound was washed by water and recrystallized from aqueous (2 : 1) methanol (75 mL).

Yield: 66%; m.p.: 165-168°C; log k = -0.001. ^{1}H NMR (500 MHz, DMSO-d$_6$, δ, ppm): 12.74 (s, 1 H, HO-C2), 10.39 (s, 1 H, HO-C4), 8.07 (d, 1 H, J

= 3.1 Hz, H-C indole 2), 7.86 (d, 1 H, J = 8.7 Hz, H-C indole 7), 7.71 (d, 1 H, J = 2.4 Hz, H-C indole 4), 7.45 (d, 1 H, J = 8.8 Hz, H-C6), 6.92 (dd, 1 H, J = 8.8 and 2.5 Hz, H-C indole 6), 6.68 (m, 1 H, H-C indole 3) 6.47 (dd, 1 H, J = 8.7 and 2.3 Hz, H-C5), 6.41 (d, 1 H, J = 2.3 Hz, H-C3), 3.83 (s, 3 H, CH$_3$); IR (KBr, cm^{-1}): 3312 (OH), 2942 (CH), 2833 (CH), 1624 (C=C), 1591 (C=C), 1559 (C=C), 1511 (C=C), 1471, 1436 (C-H), 1381, 1343, 1298, 1267, 1202 (C-OH), 1162, 1126 (C=S), 1071, 1022, 981, 922, 887, 836, 800, 784, 770, 746; EI-MS (m/z, %): 299 (M$^+$, 4), 297 (4), 283 (61), 266 (5), 174 (6), 147 (100), 142 (6), 137 (5), 132 (25). Analysis: calcd. for C$_{16}$H$_{13}$NO$_3$S (299.34): C 64.20, H 4.38, N 4.68%; found: C 64.73, H 4.40, N 4.66%.

(2,4-Dihydroxyphenyl)(1*H*-imidazo[4,5-*b*]pyridin-1-yl)methanethione (6)

STB (0.01 mol) and 1*H*-imidazo[4,5-b]pyridine (Alfa Aesar) (0.01 mol) were added to MeOH (50 mL) and heated to boiling (3 h). The mixture was hot filtered and water (100 mL) was added to the filtrate. The removed compound was washed by water and recrystallized from aqueous (2:1) methanol (60 mL).

Yield: 61%; m.p.: 172-175°C; log k = -0.702. ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 11.89 (s, 1 H, HO-C2), 10.26 (s, 1 H, HO-C4), 8.49 (s, 1 H, H-C2'), 8.38 (m, 1 H, H-C5'), 8.05 (m, 1 H, H-C7'), 7.50 (d, 1 H, J = 8.8 Hz, H-C6), 6.69 (m, 1 H, H-C6'), 6.43 (d, 1 H, H-C3), 6.35 (m, 1 H, H-C5); IR (KBr, cm^{-1}): 3271 (OH), 3099 (C(Ar)-H), 2947 (CH), 1679 (C=N), 1616 (C=C), 1512 (C=C), 1459, 1391, 1334, 1262, 1164 (C-OH), 1129 (C=S), 1060, 1008, 971, 950, 886, 845, 810, 780; EI-MS (m/z, %): 270 (22), 184 (8), 167 (9), 153 (7), 135 (6), 119 (100), 92 (39), 77 (4), 65 (11), 57 (6), 41 (8), 38 (7). Analysis: calcd. for C$_{13}$H$_9$N$_3$O$_2$S (271.29): C 57.55; H 3.34; N 15.49%; found: C 57.82; H 3.35; N 15.46%.

Antiproliferative assay *in vitro*

The following established *in vitro* human cell line was used in this study: HCV29T (bladder cancer) from the Fibiger Institute, Copenhagen, Denmark. Twenty-four hours before the addition of the tested agent, the cells were plated in 96-well plates (Sarstedt Inc.) at a density of 10^4 cells/well. Cell line was maintained in the opti-MEM medium supplement with 2 mM glutamine (Gibco), streptomycin (50 μg/mL), penicillin (50 U/mL) (Polfa, Tarchomin), and 5% fetal calf serum (Gibco). The cells were incubated at 37°C in a humid atmosphere saturated with 5% CO$_2$. The solutions of compounds

(1 mg/mL) were prepared *ex tempore* by dissolving the substance in 100 μL of DMSO followed by addition of 900 μL of tissue culture medium. Afterwards, the compounds were diluted in the culture medium to final concentrations ranging from 0.1 to 100 μg/mL. The solvent (DMSO) used at the highest concentration in the test did not reveal any cytotoxic activity. Cisplatin was used as a test reference agent. The cytotoxicity assay was performed after 72 h exposure of the cultured cells at concentrations of tested agents ranging from 0.1 to 100 μg/mL. The SRB test was used to measure inhibition of cell proliferation *in vitro* (28). The cells attached to the plastic were fixed with cold 50% TCA (trichloroacetic acid, Sigma-Aldrich Chemie GmbH) added on the top of the culture medium in each well. The plates were incubated at 4°C for 1 h and then washed 5 times with tap water.

The background optical density was measured in the wells filled with the medium, without the cells. The cellular material fixed with TCA was stained with 0.4% sulforhodamine B (SRB, Sigma-Aldrich Chemie GmbH) dissolved in 1% acetic acid (POCh) for 30 min. The unbound dye was removed by rinsing (4 times) with 1% acetic acid, and the protein-bound dye was extracted with 10 mM unbuffered Tris base (tris(hydroxymethyl)aminomethane, POCh) for determination of optical density (at 540 nm) in a computer-interfaced, 96-well microtiter plate reader Uniskan II (Labsystems). The compounds were tested in triplicate for each experiment, and the experiments were repeated at least 3 times.

Antifungal activity of the compounds against fungi *Candida*

Fifty strains of *Candida albicans* taken from the mouth cavity of patients suffering from tumor diseases were used as the selective material. Itraconazole and fluconazole were administered for prophylaxis or due to the symptoms of candidosis. The isolates resistant to a number of drugs (5-fluorocytosine, ketoconazole, amphotericin B, itraconazole, miconazole, and fluconazole), as shown by Fungitest^R, were chosen for the dilution method testing of the compounds. The drug resistance was 56.7%; the resistance to itraconazole and fluconazole were 87.5% and 82.5%, respectively. In addition, the strain was used for comparison with the American Type Culture Collection (University Boulevard, Manassas, VA) *C. albicans* (ATCC 10231).
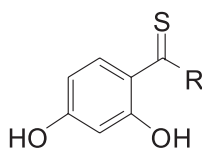
Six isolates of non-*albicans Candida* (two isolates of *C. tropicalis*, one of each of *C. glabrata, C.*

krusei, C. paratropicalis, and *C. tropicalis*) (n = 6) were tested. The drug resistance by Fungitest[R] was also determined for them. The yeast isolates were identified to the species level by the conventional morphological and biochemical methods by the Candi Select (Bio-Rad), Fungiscreen 4H (Bio-Rad), and Auxacolor (Bio-Rad) tests. The compounds were dissolved in 1% DMSO. The susceptibility testing was achieved by the agar dilution method. MIC values were determined by the agar dilution procedure according to the National Committee for Clinical Laboratory Standards (NCCLS) reference document M27 (29). The Sabouraud's medium (Bio-Rad) was used. Starting inocula were adjusted by the spectrophotometric densitometry (Bio-Merieux) to 1×10^{-5} CFU/mL. The concentrations of compounds were from 6.25 to 200 μg/mL. 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 the fungal growth. Itraconazole (Pliva, Kraków, Poland) and fluconazole (Janssen-Cilag) tested under the same experimental conditions were used as the references. MIC values expressed as the average value from 10 measurements for *C. albicans* and from 6 for non-*albicans Candida* (Table 2).

Activity of the compounds against phytopathogenic fungi

The test *in vitro* estimated inhibition of mycelium in the agar culture medium caused by the compound under investigation. The bioindicators: *Alternaria alternata, Botrytis cinerea, Rhizoctonia solani, Fusarium culmorum* and *Phytophthora cactorum* were used in the test. The solutions (suspensions) were prepared at the concentrations needed to obtain 200 and 20 μg/mL of the studied substance after dilution with the agar culture medium (PDA). Petri scale pans were used, into which the agar culture medium and the studied substance were poured. When the culture medium set, the infectious material of the tested fungus, in the form of agar disks overgrown with mycelium, was placed at three sites on its surface. After 3-5 days, depending on the mycelium culture, the linear growth of the mycelium colony was measured. The compound's action was determined as the percentage of mycelium growth inhibition compared with the control using the Abbott equation. Carbendazim and procymidon were used as the reference substances and tested under the same experimental conditions. The results are given in the four-degree scale, determined as the percentage of mycelium growth inhibition compared with the control (Table 3).

Table 1. Structure and antiproliferative activity of N,N-cyclic-2,4-dihydroxythiobenzamide derivatives against the cell of human HCV29T line expressed as IC_{50} (μg/mL).[a]

No.	R:	ID_{50} [μg/mL]	No.	R:	ID_{50} [μg/mL]
1		21.77 ± 6.00	4		24.28 ± 6.19
2		-[b]	5		10.51 ± 4.14
3		-[b]	6		33.98 ± 1.04
	Cisplatin	0.93 ± 0.29			

[a]ID_{50}: indicates the compound concentration that inhibits the proliferation rate of tumor cells by 50% as compared to the control untreated cells. The values are the means ± SD of nine independent experiments. [b]Negative in the studied concentrations.

Table 2. Antifungal activity against *C. albicans* and *non-C. albicans* strains expressed in average MIC values [μg/mL].[a]

Compound	*C. albicans* ATCC 10231[b]	*C. albicans*[c] (n = 10)	*non-Candida albicans*[d] (n = 6)
1	170.0 ± 48.3	170.0 ± 48.3	-[e]
2	200.0 ± 0.0	190.0 ± 31.6	145.8 ± 84.3
3	200.0 ± 0.0	200.0 ± 0.0	166.7 ± 51.6
5	100.0 ± 0.0	60.0 ± 21.1	66.7 ± 37.6
6	200.0 ± 0.0	190.0 ± 31.6	-[e]
Itraconazole	200.0 ± 0.0	47.5 ± 7.9	27.5 ± 7.9
Fluconazole	200.0 ± 0.0	27.5 ± 7.9	12.5 ± 0.0

[a] MIC – the minimal inhibitory concentration caused full inhibition of growth in relation to the control [μg/mL]. [b] MIC ± SD (means ± standard deviation of three independent experiments). [c] MIC ± SD (MIC values expressed as the average value from 10 isolates ± standard deviation). [d] MIC ± SD (MIC values expressed as the average value from 6 isolates ± standard deviation). [e] Test was not performed.

Table 3. Antifungal activity of compounds against phytopathogenic fungi.[a]

Compound	Estimation of mycelium growth inhibition [mg/×L]									
	A. alternata		*B. cinerea*		*R. solani*		*F. culmorum*		*P. cactorum*	
	200	20	200	20	200	20	200	20	200	20
2	2	1	2	1	2	1	2	1	2	1
3	2	1	1	0	1	0	2	1	1	0
Carbendazim	-[b]	-[b]	-[b]	-[b]	3	3	3	3	3	3
Procymidon	2	2	3	3	-[b]	-[b]	-[b]	-[b]	-[b]	-[b]

[a] The results are given in the four-degree scale determined in percent of mycelium growth inhibition compared with the control: 0 = 0-20%; 1 = 21-40%; 2 = 41-60%; 3 = 61-80%; 4 = 81-100%. [b] Test was not performed.

Biological studies were done in the Department of Pesticides Application, IPO, Warszawa, with the SPR/FA$_2$/11 procedures (certificate GLP Compliance No. G 013).

RESULTS AND DISCUSSION

Compounds **1-6** were formed by the reaction of the commercially available secondary cyclic amines with sulfinylbis[(2,4-dihydroxyphenyl) methanethione] (STB) in MeOH under reflux (3 h). The starting reagent STB was prepared from 2,4-dihydroxybenzenecarbodithioic acid and SOCl$_2$ in ethoxyethane (27). The yields of processes were in the range of 61-76%. Purity of the obtained compounds was checked by the reversed phase (RP-18) HPLC chromatography using the MeOH : H$_2$O mixture as a mobile phase. The structures of the obtained compounds are shown in Table 1. All these derivatives were characterized by spectral and elemental analysis data which confirmed their structures.

In the ^{1}H NMR spectra, the resonance signals of hydroxyl groups protons are usually registered as broad singlets in the range ca. 11.89 and 10.26 ppm. The protons of the piperazine are detected as two multiplies at 3.71 ppm and 3.15 ppm. The IR spectra show two strong bands in the region about 3312-3024 and 1241-1164 cm^{-1}, corresponding to ν (O-H) The signals of molecular ions M$^+$ (EI) are visible in the spectra of all compounds.

The antiproliferative activity of synthesized compounds has been evaluated against the cell of human HCV29T line (Table 1). Cisplatin was used as a reference drug. The cytotoxic activity *in vitro* was expressed as ID$_{50}$ (μg/mL), the concentration of compound that inhibits proliferation rate of the tumor cells by 50%, as compared to the control untreated cells. The results of substance screening are summarized in Table 1. Antiproliferative activity *in vitro* of the presented compounds is varied - compound **5** proved to be the most active.

Antifungal activity of compounds was expressed as average MIC values against several clinical isolates of *C. albicans* (Table 2). Analyzing the results summarized in Table 2 it can be concluded that the activity of the compounds is varied and

depends significantly of the structure. The data of inhibitory effects indicate that, depending on the type of substitution, the obtained compounds are characterized by differentiated activities expressed in the MIC values ranging from 60 to 200 µg/mL. The strongest fungistatic activity is observed for studied compound **5** against *C. albicans* (MIC 60.0 µg/mL). Some derivatives were also tested for their activity against non-*albicans Candida*. The compounds are characterized by higher antifungal effects than those against the isolates of *C. albicans*. However, the compounds studied exhibit lower activity against *C. albicans* and non-*C. albicans* compared with standard substances. Our preliminary results may suggest some directions for further synthesis.

The results of *in vitro* screening against five strains of phytopathogenic fungi (*A. alternata, B. cinerea, R. solani, F. culmorum, and P. cactorum*) under the *in vivo* conditions are given in Table 3. Sarfun 500SC (carbendazim) and Sumilex 500SC (procymidone) were used as reference systems. In the laboratory studies at the concentration of 200 µg/mL, compound **2** revealed fungistatic action (at the level 41-60%) against all bioindicators. Compound **3** showed a particular inhibitory action, inhibiting growth of two pathogens – *A. alternata* and *F. culmorum*. This pathogens seem to be particularly susceptible to tested compounds.

CONCLUSION

Novel N,N-cyclic-2,4-dihydroxythiobenzamide derivatives were formed by the reaction of the commercially available reagents with STB. The compounds show antiproliferative activity *in vitro* against the HCV29T cancer cell lines. They are also characterized by antifungal properties against *Candida, non-Candida* and phytopathogenic fungi. Following on from these results, one may synthesize a larger series of thiobenzamide derivatives in order to further investigate these findings.

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