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Original article

Dependence of fungistatic activity of 2,4-dihydroxythiobenzanilides on the structure and lipophilic nature of the compounds

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Abstract – The quantitative dependencies of in vitro fungistatic action on the physico-chemical parameters connected with the structure of 2,4-dihydroxythiobenzanilides were investigated. It was stated that the action of these compounds depends on lipophilicity determined by substitution of the N-aryl moiety and on electron properties of molecules. The lipophilicity expressed by R_{Mw} values was determined in the reversed-phase system (HPTLC). The changes in the nature of the thioamide bond were interpreted on the basis of UV and EI-MS spectra.

2,4-dihydroxythiobenzanilides / antifungal activity / dermatophytes / in vitro study / lipophilicity / structure–activity

1. Introduction

Thiobenzanilides, being analogues of benzanilides, including salicylanilides, belong to the group of compounds characterized by a wide spectrum of biological activity depending largely on the type of substitution. Most of them exhibit antimycobacterial activity [1–5], whereas much attention was paid to the studies of dependence between the structure and antituberculous activity [3, 4]. 3,4,4'-trichlorothiobenzanilide exhibits the inhibition activity in relation to the bacterium *Staphylococcus aureus* [6]. Some substitutions of thiobenzanilides promote antimycotic properties. These compounds show fungicidal effects against *Candida albicans*, *Trichophyton mentagrophytes* [7] and a number of phytopathogenic fungi [8, 9]. This group of substances exhibit also herbicidal [10, 11] and insecticidal [12] activity.

Because of significant difficulties connected with the synthesis of thiobenzanilides [13–15] the information about this group of the compounds is not complete and includes only some types of substitution. These difficulties result from instability of carbodithione or carbothione bonds as well as from limited electrophilicity of carbon atoms occurring in suitable thioesters and acid chlorides used in the synthesis. The success of these methods is limited also by the necessity of taking into consideration the requirements connected with the structure.

As biological activity depends, among others, on the appropriate hydrophilic–hydrophobic equilibrium of the molecule, synthesis of thiobenzanilides with the polyhydroxyaromatic system was worked out. It seems that such a substitution can make it possible to achieve the required characteristic of the compound. Application of salicylanilide derivatives as effective drugs for many diseases justifies the procedure. Biological activity of 2,4-dihydroxythiobenzanilides was confirmed in earlier studies against phytopathogenic fungi [16, 17].

This paper presents the changes of fungistatic activity of 33 compounds from the 2,4-dihydroxythiobenzanilide group with the modified N-aryl fragment against seven strains of dermatophytes in the in vitro conditions. Based on spectroscopic and RP-HPTLC investigations the correlation between the activity and structure including, particularly, lipophilicity was investigated.

2. Chemistry

Synthesis conditions for 2,4-dihydroxybenzthioanilides (*figure 1*) were elaborated using the information obtained

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by the EI-MS spectrometry, differential pulse polarography (DPP) and electrocapillary measurements. The proposed method allowed us to obtain a group of the compounds, which had not so far been described in literature, containing any constitutional and sterical N-aryl system and the whole process includes three relatively simple transitions [18]. The analytical data of the obtained thiobenzanilides are shown in *table I*

Table I. Analytical data obtained for thiobenzanilides.

Compound	Formula	M.p.	^{1}H-NMR	EI-MS			IR	UV
	m.w.	(°C)	(δ, ppm), [D_6] DMSO, CD_3COCD_3[a]	m/z	R^+	rel. int. (%)	$\bar{\nu}$ (cm^{-1})	λ_{max} (nm)
I	$C_{13}H_{11}NO_2S$ 245.30	181–183	11.25 (NH, s); 7.88–7.79 (q, 3H); 7.67–7.23 (m, 3H); 6.41–6.29 (m, 2H)	245 212 153 136 109 77 65	$M^{+\cdot}$ $(HO)_2C_6H_3(C{=}N)C_6H_5$ $(HO)_2C_6H_3C(=S)$ $(S{=})CNHC_6H_4$ $(HO)_2C_6H_5$ C_6H_5 C_5H_5	44.64 100.00 15.77	3 587, 3 319 (OH + NH), 1 500 NHC(=S)	295, 326
II	$C_{15}H_{15}NO_2S$ 273.33	113–114	11.28 (NH, s); 7.94 (d, H); 7.24 (q, 3H); 6.41–6.32 (m, 2H), 2.29, 2.17 (d, 6H)	273 258 240 225 153 121 91	$M^{+\cdot}$ $(HO)_2C(=S)NHC_6H_3CH_3$ $(HO)_2C_6H_3C(=N)C_6H_3(CH_3)_2$ $(HO)_2C_6H_3C(=N)C_6H_3CH_3$ $(HO)_2C_6H_3C(S)$ $(HO)_2C_6H_3C$ C_6H_5N	30.59 100.00 12.05	3 323, 3 269 (OH + NH), 2 950 CH; 1 489 NHC(=S); 1 368, 1 189 CH; 1 040 C=S; 722, 679 CH	288, 321
III	$C_{17}H_{19}NO_2S$ 301.41	139–140	11.28 (NH, s); 7.86 (d, H); 7.64 (d, 2H); 7.23 (d, 2H); 6.41–6.29 (m, 2H); 3.38 ($CH_3CH_2C\underline{H}CH_3$, s); 1.63–1.42 ($CH_3CHC\underline{H}_2CH_3$, m); 1.23–1.16 ($C\underline{H}_3CHCH_2CH_3$, d); 0.85–0.71 ($CH_3CHCH_2-C\underline{H}_3$, t)	301 268 244 153 136 91 79	$M^{+\cdot}$ $(HO)_2C_6H_3C(=N)C_6H_4C_4H_9$ $(HO)_2C_6H_3C(=S)NHC_6H_4$ $(HO)_2C_6H_3C(=S)$ $(S{=})CNHC_6H_4$ C_6H_5N, $C_6H_5(=CH_2)$ C_5H_5N	19.94 100.00 9.52	3 584, 3 318 (OH + NH), 2 872 CH; 1 515 NHC(=S); 1 225, 1 181 CH; 1 017 C(=S); 744, 728 CH	295, 302, 329
IV	$C_{13}H_{10}FNO_2S$ 263.29	99–100	11.58 (NH, s); 8.11–8.02 (d, H); 7.84–7.74 (q, H); 7.42–7.28 (m, 3H); 6.45–6.33 (m, 2H)	263 230 153 136 111 95 75	$M^{+\cdot}$ $(HO)_2C_6H_3C(=N)C_6H_4F$ $(HO)_2C_6H_3C(=S)$ $(S{=})CNHC_6H_4$ $NHC_6H_4F + H$ C_6H_4F C_6H_3	66.69 100.00 45.23	3 350 (OH + NH), 1 506 NHC(=S); 1 266, 1 224 C–F; 1 030 C=S	293, 331, 353
V	$C_{13}H_{10}FNO_2S$ 263.29	163–164	11.05 (NH, s); 8.28 (s, H); 7.98–7.60 (m, 3H); 7.36 (s, H); 6.39 (m, 2H)	263 230 153 136 121 111 95 75	$M^{+\cdot}$ $(HO)_2C_6H_3C(=N)C_6H_4F$ $(HO)_2C_6H_3C(=S)$ $(S{=})CNHC_6H_5$ $(HO)_2C_6H_3C$ $NHC_6H_4F + H$ C_6H_4F C_6H_3	50.59 100.00 30.95	3 372, 3 316, 3 239 (OH + NH), 1 494 NHC(=S); 1 194 C–F; 1 072 C=S	296, 325
VI	$C_{13}H_{10}FNO_2S$ 263.29	183–184	11.24 (NH, s); 7.89–7,66 (m, 3H); 7.30–7.15 (m, 2H); 6.42–6.30 (m, 2H)	263 230 153 136 121 111 95 75	$M^{+\cdot}$ $(HO)_2C_6H_3C(=N)C_6H_4F$ $(HO)_2C_6H_3C(=S)$ $(S{=})CNHC_6H_5$ $(HO)_2C_6H_3C$ $NHC_6H_4F + H$ C_6H_4F C_6H_3	44.04 100.00 27.97	3 584, 3 324 (OH + NH), 1 510 NHC(=S); 1 158 C–F	297, 329

Compound	Formula m.w.	M.p. (°C)	^{1}H-NMR (δ, ppm), [D_6] DMSO, CD_3COCD_3[a]	EI-MS m/z	 R^+	 rel. int. (%)	IR $\bar{\nu}$ (cm^{-1})	UV λ_{max} (nm)
VII	$C_{13}H_9F_2NO_2S$ 281.28	115–116	11.46 (NH, s); 8.09–8.01 (d, H); 7.72 (q, H); 7.36–7.14 (m, 2H); 6.40 (m, 2H)	281 262 248 153 136 65	$M^{+\cdot}$ $(HO)_2C_6H_3C(=S)NHC_6H_3F$ $(HO)_2C_6H_3C(=N)C_6H_3F_2$ $(HO)_2C_6H_3C(=S)$ $(S=)CNHC_6H_5$ C_5H_5	69.64 100.00 51.78	3 410, 3 161 (OH + NH), 1 477 NHC(=S); 1 221, 1 101 C–F; 1 040 C=S	292, 330
VIII	$C_{13}H_{10}ClNO_2S$ 279.74	95–96	11.67 (NH, s); 8.17–8.08 (t, H); 7.88–7.75 (m, H); 7.64–7.37 (m, 3H); 6.40 (m, 2H)	279 246 244 153 127 121 111 77	$M^{+\cdot}$ $(HO)_2C_6H_3C(=N)C_6H_4Cl$ $(HO)_2C_6H_3C(=S)NHC_6H_4$ $(HO)_2C_6H_3C(=S)$ $NHC_6H_4Cl + H$ $(HO)_2C_6H_3C$ C_6H_4Cl C_6H_5	1.19 100.00 7.73	3 339 (OH + NH), 1 510 NHC(=S); 1 298 C · · · N; 1 266, 1 063 C–Cl; 1 034 C=S	292, 332
IX	$C_{13}H_{10}ClNO_2S$ 279.74	162–163	11.01 (NH, s); 7.99 (s, H); 7.84–7.23 (m, 4H); 6.41–6.29 (m, 2H)	279 246 244 153 136 121 111	$M^{+\cdot}$ $(HO)_2C_6H_3C(=N)C_6H_4Cl$ $(HO)_2C_6H_3C(=S)NHC_6H_4$ $(HO)_2C_6H_3C(=S)$ $C(=N)C_6H_4Cl$, $(S=)CNHC_6H_5$ $(HO)_2C_6H_3C$ C_6H_4Cl	48.50 100.00 30.71	3 291 (OH + NH), 1 511 NHC(=S); 1 230, 1 095 C–Cl, 1 070 C=S	283, 329
X	$C_{13}H_{10}ClNO_2S$ 279.74	177–178	11.13 (NH, s); 7.87–7.77 (m, 3H); 7.50–7.41 (m, 2H); 6.39 (m, 2H)	279 246 244 211 153 136 121 111	$M^{+\cdot}$ $(HO)_2C_6H_3C(=N)C_6H_4Cl$ $(HO)_2C_6H_3C(=S)NHC_6H_4$ $(HO)_2C_6H_3C(=N)C_6H_4$ $(HO)_2C_6H_3C(=S)$ $C(=N)C_6H_4Cl$, $(S=)CNHC_6H_5$ $(HO)_2C_6H_3C$ C_6H_4Cl	42.85 100.00 39.28	3 360, 3 321 (OH + NH), 1 499 NHC(=S); 1 219, 1 092 C–Cl; 1 016 C=S	298, 327
XI	$C_{13}H_9Cl_2NO_2S$ 314.19	175–176	11.72 (NH, s); 8.11 (d, H); 7.68–7.34 (m, 3H); 6.46–6.34 (m, 2H)	313 280 278 243 153 109	$M^{+\cdot}$ $(HO)_2C_6H_3C(=N)C_6H_3Cl_2$ $(HO)_2C_6H_3C(=S)NHC_6H_3Cl$ $(HO)_2C_6H_3C(=S)NHC_6H_3$ $(HO)_2C_6H_3C(=S)$ C_6H_3Cl, $(HO)_2C_6H_3$	2.90 100.00 9.82	3 675, 3 468, 3 168 (OH + NH), 1 505 NHC(=S); 1 182 C–Cl; 1 048 C=S	295, 336
XII	$C_{13}H_9Cl_2NO_2S$ 314.19	184–185	11.78 (NH, s); 8.19–8.02 (m, H); 7.63–7.46 (d-d, 2H); 7.37 (d, H); 6.46–6.34 (m, 2H)	313 280 278 243 153 139 109	$M^{+\cdot}$ $(HO)_2C_6H_3C(=N)C_6H_3Cl_2$ $(HO)_2C_6H_3C(=S)NHC_6H_3Cl$ $(HO)_2C_6H_3C(=S)NHC_6H_3$ $(HO)_2C_6H_3C(=S)$ $C_6H_4C(=O)Cl$ C_6H_3Cl, $(HO)_2C_6H_3$	6.84 100.00 8.33	3 446, 3 386 (OH + NH), 1 504 NHC(=S); 1 209, 1 089 C–Cl; 1 048 C=S	291, 338
XIII	$C_{13}H_9Cl_2NO_2S$ 314.19	163–164	11.02 (NH, s); 8.21 (s, H); 7.84–7.59 (m, 3H); 6.41–6.30 (m, 2H)	313 280 161 153 136 109 65	$M^{+\cdot}$ $(HO)_2C_6H_3C(=N)C_6H_3Cl_2$ $C_6H_3Cl_2$ $(HO)_2C_6H_3C(=S)$ $C(=N)C_6H_4Cl$, $(S=)CNHC_6H_4$ C_6H_3Cl, $(HO)_2C_6H_3$ C_5H_5	48.21 100.00 56.95	3 377, 3 240 (OH + NH), 1 504 NHC(=S); 1 223, 1 119 C–Cl; 1 033 C=S	294, 328

Compound	Formula	M.p.	^{1}H-NMR	EI-MS			IR	UV
	m.w.	(°C)	(δ, ppm), [D_6] DMSO, CD_3COCD_3[a]	m/z	R^+	rel. int. (%)	$\bar{\nu}$ (cm^{-1})	λ_{max} (nm)
XIV	$C_{13}H_9FClNO_2S$ 297.74	105–106	11.11 (NH, s); 8.09 (d, H); 7.87–7.35 (m, 3H); 6.42–6.30 (m, 2H)	297	$M^{+\cdot}$	46.42	3 377, 3 171 (OH + NH), 1 500 NHC(=S); 1 235 C–Cl; 1 203 C–F	292, 329, 375
				264	$(HO)_2C_6H_3C(=N)C_6H_3FCl$	100.00		
				261	$(HO)_2C_6H_3C(=S)NC_6H_3F$			
				229	$(HO)_2C_6H_3C(=N)C_6H_3F$			
				153	$(HO)_2C_6H_3C(=S)$	50.00		
				136	$C(=N)C_6H_3Cl$, $(S=)CNHC_6H_4$			
				109	$(HO)_2C_6H_3$, C_6H_3Cl			
XV	$C_{13}H_{10}BrNO_2S$ 342.18	110–111	11.60 (NH, s); 8.10 (d, H); 7.77–7.45 (m, 2H); 7.39–7.18 (q, 2H); 6.46–6.34 (m, 2H)	323	$M^{+\cdot}$	10.11	3 344 (OH + NH), 1 504 NHC(=S); 1 050 C=S; 1 031 C–Br	288, 292, 336
				290	$(HO)_2C_6H_3C(=N)C_6H_4Br$	100.00		
				244	$(HO)_2C_6H_3C(=S)NHC_6H_4$			
				183	$C(=N)C_6H_4Br$			
				173	$NHC_6H_4Br + H$			
				155	C_6H_4Br			
				153	$(HO)_2C_6H_3C(=S)$	6.54		
				121	$(HO)_2C_6H_3C$			
XVI	$C_{13}H_{10}INO_2S$ 371.20	198–199	10.83 (NH, s); 7.85–7.74 (m, 3H); 7.54–7.35 (m, 2H); 6.43 (m, 2H)[a]	371	$M^{+\cdot}$	53.57	3 318, 3 279 (OH + NH), 1 510 NHC(=S); 1 010 C=S; 683, 658 C–I	284, 329
				338	$(HO)_2C_6H_3C(=N)C_6H_4I$	100.00		
				243	$(HO)_2C_6H_3C(=S)NHC_6H_4$			
				211	$(HO)_2C_6H_3C(=N)C_6H_4$			
				153	$(HO)_2C_6H_3C(=S)$	26.08		
				136	$(S=)CNHC_6H_4$			
				121	$(HO)_2C_6H_3C$			
				109	$(HO)_2C_6H_3$			
XVII	$C_{14}H_{12}FNO_2S$ 277.32	146–147	11.45 (NH, wide and broad, s); 8.05–8.00 (t, H); 7.96–6.98 (m, 3H, owing to conjunction the number of signals does not correspond to number of protons in the system)	277	$M^{+\cdot}$	22.61	3 400, 3 187 (OH + NH), 1 516 NHC(=S); 1 493 C–F; 1 441 CH; 1 275 C–F; 1 035 C=S	293, 334
				262	$(HO)_2C_6H_3C(=S)NHC_6H_3F$	100.00		
				244	$(HO)_2C_6H_3C(=N)C_6H_3(CH_3)F$			
				153	$(HO)_2C_6H_3C(=S)$	30.05		
				136	$(S=)CNHC_6H_4$			
				109	$NHC_6H_3CH_3 + H$, $(HO)_2C_6H_3C$			
				107	$NC_6H_3CH_3$			
XVIII	$C_{14}H_{11}ClNO_2S$ 293.78	193–194	11.41 (NH, s); 7.93 (d, H); 7.48–7.35 (q, H); 7.27 (d, 2H); 6.43–6.31 (m, 2H); 2.23 (CH_3, s)	293	$M^{+\cdot}$	12.20	3 411, 3 213 (OH + NH), 1 496 NHC(=S); 1 403, 1 379 CH; 1 270, 1 225 C–Cl; 1 076 C=S; 1 019 C–Cl	284, 333, 365
				278	$(HO)_2C_6H_3C(=S)NHC_6H_3Cl$	100.00		
				260	$(HO)_2C_6H_3C(=N)C_6H_3(CH_3)Cl$			
				257	$(HO)_2C_6H_3C(=S)NHC_6H_3(CH_3)$			
				225	$(HO)_2C_6H_3C(=N)C_6H_3(CH_3)$			
				210	$(HO)_2C_6H_3C(=N)C_6H_3$			
				153	$(HO)_2C_6H_3C(=S)$	18.75		
				90	C_7H_6			
XIX	$C_{14}H_{11}ClNO_2S$ 293.78	161–162	11.12 (NH, s); 7.94–7.76 (t, 2H); 7.56 (d, H); 7.36 (d, H); 6.41–6.30 (m, 2H); 2.23 (CH_3, s)	293	$M^{+\cdot}$	43.45	3 370 (OH + NH), 1 500 NHC(=S); 1 393 CH; 1 119 C–Cl; 1 051 C=S	293, 330, 365
				277	$(HO)_2C_6H_3C(=N)NHC_6H_3Cl$			
				260	$(HO)_2C_6H_3C(=S)C_6H_3(CH_3)Cl$	100.00		
				225	$(HO)_2C_6H_3C(=N)C_6H_3CH_3$			
				153	$(HO)_2C_6H_3C(=S)$	22.21		
				136	$(S=)CNHC_6H_4$			
				89	C_7H_5			
XX	$C_{14}H_{10}F_3NO_2S$ 313.30	164–165	11.07 (NH, s); 7.97–7.76 (m, 2H); 7.52–7.25 (m, 2H); 7.22–6.93 (m, H); 6.39 (m, 2H)	313	$M^{+\cdot}$	12.50	3 452, 3 339, 3 209 (OH + NH), 1 509 NHC(=S); 1 319 C–F; 1 274 C–F; 1 037 C=S	300, 330
				280	$(HO)_2C_6H_3C(=N)C_6H_4CF_3$	100.00		
				244	$(HO)_2C_6H_3C(=S)NHC_6H_4$			
				212	$(HO)_2C_6H_3C(=N)C_6H_4$			
				161	$HNC_6H_4CF_3 + H$			
				153	$(HO)_2C_6H_3C(=S)$	23.21		
				136	$(S=)CNHC_6H_4$			
				69	CF_3			

Compound	Formula	M.p.	^{1}H-NMR	EI-MS			IR	UV
	m.w.	(°C)	(δ, ppm), [D_6] DMSO, CD_3COCD_3[a]	m/z	R^+	rel. int. (%)	$\bar{\nu}$ (cm^{-1})	λ_{max} (nm)
XXI	$C_{14}H_{13}NO_3S$ 275.33	169–170	10.65 (NH, s); 8.15–7.94 (m; 2H); 7.14 (m, 3H); 6.45 (m, 2H), 3.86 (OCH_3, s)[a]	275 260 244 242 227 153 123 108 92	$M^{+\cdot}$ $(HO)_2C_6H_3C(=S)NHC_6H_4O$ $(HO)_2C_6H_3C(=S)NHC_6H_4$ $(HO)_2C_6H_3C(=N)C_6H_4OCH_3$ $(HO)_2C_6H_3C(=N)C_6H_4$ $(HO)_2C_6H_3C(=S)$ $HNC_6H_4OCH_3$ + H NHC_6H_4O + H [$(HO)C_6H_3$–(CO + H°)]	17.85 100.00 8.52 	3 304 (OH + NH), 1 491 NHC(=S); 1 458 CH; 1 242 C–O; 1 185 C–O–C; 1 113 CH; 1 046 C=S; 1 020 C–O–C	300, 334
XXII	$C_{14}H_{13}NO_3S$ 275.33	193–194	11.35 (NH, s); 7.84 (d, H); 7.60 (d, 2H); 6.96 (d, 2H), 6.37 (m, 2H); 3.77 (OCH_3, s)	275 242 227 153 136 108 92	$M^{+\cdot}$ $(HO)_2C_6H_3C(=N)C_6H_4OCH_3$ $(HO)_2C_6H_3C(=N)C_6H_4O$ $(HO)_2C_6H_3C(=S)$ $(S=)CNHC_6H_4$ $HNC_6H_4OCH_3$ + H [$(HO)C_6H_3$–(CO + H°)]	35.71 100.00 11.90 	3 410, 3 176 (OH + NH), 1 499 NHC(=S); 1 244 C–O; 1 212 C–O–C; 1 029 C=S; 1 019 C–O–C	299, 328
XXIII	$C_{13}H_{11}NO_3S$ 261.30	177–178	9.12 (NH, s); 7.84–7.74 (m, H); 7.19 (m, 3H); 6.82 (d, H); 6.45 (m, 2H)[a]	261 228 170 153 137 109 65 53 39	$M^{+\cdot}$ $(HO)_2C_6H_3C(=N)C_6H_4OH$ $(HO)_2C_6H_3C(=S)OH$ $(HO)_2C_6H_3C(=S)$ $(HO)_2C_6H_3C(=O)$ HNC_6H_4OH + H, $(HO)_2C_6H_3$ C_5H_5 C_4H_5 C_3H_3	61.90 100.00 30.95 	3 274 (OH + NH), 1 505 NHC(=S); 1 230 C–O (δ OH); 1 112 OH; 1 053 C=S;	300, 328
XXIV	$C_{14}H_{13}NO_3S$ 275.33	213–214	11.13 (NH, s); 7.90 (d, H); 7.07 (d, H); 6.69–6,59 (s, d 2H); 6.42–6.30 (m, 2H); 2.12 (CH_3, s)	275 260 242 153 137 107 78 77	$M^{+\cdot}$ $(HO)_2C_6H_3C(=S)OH$ $(HO)_2C_6H_3C(=N)C_6H_4OH(CH_3)$ $(HO)_2C_6H_3C(=S)$ $(HO)_2C_6H_3C(=O)$ $C_7H_6(OH)$ C_5H_4N C_6H_5	41.36 100.00 32.64 	3 320, 3 192 (OH + NH), 1 513 NHC(=S), 1 397, 1 366 CH; 1 232 C–O, 1 122 OH, 1 011 C=S	289, 325
XXV	$C_{14}H_{11}NO_4S$ 289.31	213–214	13.00–12.00 (wide signal of exchangeable protons from COOH group); 11.07 (NH, s); 7.97 (s; 2H); 7.86–7.77 (t, 3H); 6.42–6.30 (m, 2H)	289 272 256 244 153 92 65	$M^{+\cdot}$ $(HO)_2C_6H_3C(=S)NHC_6H_4C(=O)$ $(HO)_2C_6H_3C(=N)C_6H_4CO_2H$ $(HO)_2C_6H_3C(=S)NHC_6H_4$ $(HO)_2C_6H_3C(=S)$ [$(HO)C_6H_3$-(CO + H°)] C_6H_5	11.90 100.00 25.58 	3 187, 3 079 (OH + NH), 1 693 C=O; 1 511 NHC(=S), 1 440 OH, 1 344, 1 223 C–O, 1 018 C=S	298, 338
XXVI	$C_{14}H_{11}NO_5S$ 305.31	219–220	11.07 (NH, s); 11.00–9.00 (broad band of exchangeable protons from OH and COOH groups, 3H); 7.86–7.69 (m, 3H); 6.43–6.32 (m, 2H)	305 289 272 261 254 244 228 153 137 109 91 79 53 45 39	$M^{+\cdot}$ $(HO)_2C_6H_3C(=S)NHC_6H_3(OH)C(=O)$ $(HO)_2C_6H_3C(=N)C_6H_3(OH)CO_2H$ $(HO)_2C_6H_3C(=S)NHC_6H_3(OH)$ $(HO)_2C_6H_3C(=N)C_6H_3CO_2H$ $(HO)_2C_6H_3C(=S)NHC_6H_3$ $(HO)_2C_6H_3C(=N)C_6H_3OH$ $(HO)_2C_6H_3C(=S)$ $(HO)_2C_6H_3C(=O)$ $(HO)_2C_6H_3$ C_7H_7, C_6H_2OH C_6H_7 C_4H_5 CO_2H C_3H_3	57.00 100.00 	3 580, 3 295, 3 073 (OH + NH), 1 672 C=O; 1 510 NHC(=S), 1 447 C–OH, 1 223, 1 208 C–O, 1 086 C=S	296, 323

Compound	Formula	M.p.	^{1}H-NMR	EI-MS			IR	UV
	m.w.	(°C)	(δ, ppm), [D_6] DMSO, CD_3COCD_3[a]	m/z	R^+	rel. int. (%)	$\bar{\nu}$ (cm^{-1})	λ_{max} (nm)
XXVII	$C_{15}H_{13}NO_4S$ 304.34	187–188	11.53 (NH, s); 8.13–7.88; 7.74–7.55; 7.47–7.38 (m, 5H); 6.45–6.35 (m, 2H); 3.78 (OCH_3, s)	303 270 245 244 170 153 108 92 77 65 51	$M^{+\cdot}$ $(HO)_2C_6H_3C(=N)C_6H_4CO_2CH_3$ $(HO)_2C_6H_3C(=S)NHC_6H_4$ $(HO)_2C_6H_3C(=S)NHC_6H_4$ $(HO)_2C_6H_3C(=S)OH$ $(HO)_2C_6H_3C(=S)$ $(HO)_2C_6H_2C$ $[(HO)_2C_6H_3$-$(CO + H^o)]$ C_6H_5 C_5H_5 C_4H_3	11.60 100.00 7.44	3 376, 3 293 (OH + NH), 1 664 C=O; 1 514 NHC(=S), 1 454 $CH(COOCH_3)$, 1 277, 1 202 C–O ($COOCH_3$), 1 145, 1 127 CH, 1 054 C=S, 975 CH	295, 339
XXVIII	$C_{15}H_{13}NO_3S$ 284.34	236–237	11.03 (NH, s); 7.98 (m, 4H); 7.86 (d, 2H); 6.38 (m, 2H)	287 254 244 153 136 120 75 53 51 43	$M^{+\cdot}$ $(HO)_2C_6H_3C(=N)C_6H_4C(=O)CH_3$ $(HO)_2C_6H_3C(=S)NHC_6H_4$ $(HO)_2C_6H_3C(=S)$ $NHC_6H_4C(=O)CH_3$ + H $(O=)C_6H_4=C(=O)$ C_5H_5 C_4H_5 C_4H_3 $C(=O)CH_3$	37.50 100.00 26.19	3 200 (OH + NH), 2 588 CH, 1 657 C=O, 1 495 NHC(=S), 1 406 $C(=O)CH_2CH_3$, 1 340, 1 307 CH, 1 119, 1 109 $C(=O)CH_3$, 1 016 C=S	294, 346
XXIX	$C_{16}H_{15}NO_3S$ 301.37	171–172	11.02 (NH, s); 7.99 (s, H); 7.87–7.64 (q, 2H); 6.58 (d, 2H); 6.39–6.31 (m, 2H); 2.93–2,79 ($C(=O)CH_2$, t); 1.16–1.02 (CH_3, q)	301 268 244 211 153 137 121 120 109 77 76 65 57	$M^{+\cdot}$ $(HO)_2C_6H_3C(=N)C_6H_4C(=O)CH_2CH_3$ $(HO)_2C_6H_3C(=S)NHC_6H_4$ $(HO)_2C_6H_3C(=N)C_6H_4$ $(HO)_2C_6H_3C(=S)$ $(HO)_2C_6H_3C(=O)$ $(HO)_2C_6H_3C$ $(O=)C_6H_4=C(=O)$ $(OH)_2C_6H_3$ C_6H_5 C_6H_4 C_5H_5 $CH_3CH_2C(=O)$	38.09 100.00 24.10	3 428, 3 357, 3 294 (OH + NH), 2 975, 2 944, 2 907 CH, 1 718, 1 679, 1 662 C=O; 1 510 NHC(=S), 1 407 $C(=O)CH_2CH_3$, 1 373 CH, 1 177 $C(=O)CH_2CH_3$, 1 016 C=S	296, 334
XXX	$C_{14}H_{10}NO_2S$ 270.31	235–236	10.94 (NH, s); 8.08 (d, H); 7.91–7.73 (m, 4H), 6.41–6.30 (m, 2H)	270 237 211 153 51 39	$M^{+\cdot}$ $(HO)_2C_6H_3C(=N)C_6H_4CN$ $(HO)_2C_6H_3C(=N)C_6H_4$ $(HO)_2C_6H_3C(=S)$ C_4H_3 C_3H_3	49.10 100.00 40.17	3 324, 3 095, 3 043 (OH + NH), 2 564, 2 350, 2 232 C≡N, 1 515 NHC(=S), 1 021 C=S, 810 C≡N	286, 339, 364
XXXI	$C_{13}H_{10}N_2O_5S$ 306.30	257–258	10.53 (NH, s); 8.33–8.24 (q, 2H); 8.02–7.80 (m, 2H); 6.48 (m, 2H)[a]	289 273 272 226 91 79	$(HO)_2C_6H_3C(=S)NHC_6H_4O$ $(HO)_2C_6H_3C(=S)NHC_6H_4NO_2$ $(HO)_2C_6H_3C(=N)C_6H_4(O)NO_2$ $(HO)_2C_6H_3C(=N)C_6H_4O$ C_7H_4 C_6H_7	 100.00	3 392, 3 105 (OH + NH), 1 510 NHC(=S), 1 263 NO_2; 1 241 C–O, 1 072 OH, 1 040 C=S, 844 C–NO_2	290, 327
XXXII	$C_{14}H_{12}N_2O_2S$ 288.33	245–246	11.11 (NH, s); 7.95–7.64 (m, 4H); 7.88 (s, H); 7.36 ($C(=O)NH_2$, s); 6.42–6.30 (m, 2H)	288 270 255 153 137 121 120 77 65	$M^{+\cdot}$ $(HO)_2C_6H_3C(=S)NHC_6H_4CN$ $(HO)_2C_6H_3C(=N)C_6H_4C(=O)NH_2$ $(HO)_2C_6H_3C(=S)$ $NHC_6H_4C(=O)NH_2$, $(S=)CNHC_6H_4$ + H $(HO)_2C_6H_3C$ $(O=)C_6H_4=C(=O)$ C_6H_5 C_5H_5	41.96 100.00 23.80	3 387, 3 328, 3 197 (OH + NH), 1 652 C=O, 1 515 NHC(=S), 1 424 $C(=O)NH_2$; 1 196 C–NH_2; 1 016 C=S	300, 338

Compound	Formula	M.p.	^{1}H-NMR	EI-MS			IR	UV
	m.w.	(°C)	(δ, ppm), [D_6] DMSO, CD_3COCD_3[a]	m/z	R^+	rel. int. (%)	$\bar{\nu}$ (cm^{-1})	λ_{max} (nm)
XXXIII	$C_{16}H_{14}N_2O_5S$ 346.35	141–142	11.00 (NH(C=S), s); 8.23 (C(=O)NH, t); 8.04–7.75 (m, 5H); 6.44 (m, 2H); 4.16 ($NHCH_2C$=O, d)	345	$M^{+\cdot}$	9.52	3 407, 3 209 (OH + NH), 1 755 C=O; 1 734, 1 704 C(=O)NH, 1 508 NHC(=S), 1 396 CH; 1 365 OH(COOH); 1 309 C–O(COOH); 1 125 $CH(CH_2COOH)$; 1 020 C=S	398, 338
				328	$(HO)_2C_6H_3C(=S)NHC_6H_4C(=O)NHCH_2C(=O)$			
				327	$(HO)_2C_6H_3C(=S)NHC_6H_4C(=N)CH_2COOH$			
				270	$(HO)_2C_6H_3C(=S)NHC_6H_4C(=O)$			
				244	$(HO)_2C_6H_3C(=S)NHC_6H_4$			
				153	$(HO)_2C_6H_3C(=S)$	29.76		
				121	$NHC_6H_4C(=O)$, $(OH)_2C_6H_3C$			
				120	$(N=)C_6H_4C(=O)$	100.00		
				92	$C(=O)NHCH_2CO_2H$			
				77	C_6H_5			

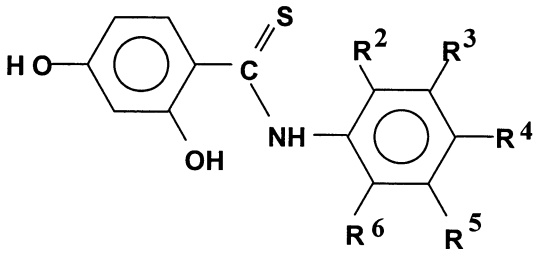

Figure 1. Structure of thiobenzanilides.

3. Results

The results of in vitro screening of 2,4-dihydroxythiobenzanilides against seven varieties of dermatophytes are compared in *table II*. The compounds studied show fungicidal potency within the range of 1.9–1 000 μg/mL. The strongest fungicidal activity is observed for some dihalogeno derivatives against *E. floccosum* I, *E. floccosum* II, *T. interdigitale, T. galline and T. rubrum* for which MIC = 1.9 μg/mL.

Table III shows the number and percentage (%) of strains sensitive to the antifungal agents at various concentrations. Starting with a conventionally determined break point for the susceptibility to topical antifungals, i.e. 15–25 μg/mL and exceptionally up to 100 μg/mL [19] in the corresponding concentrations, 11 and 30 compounds inhibit all seven dermatophyte species. For compound **III**, 100% of the tested fungi were also sensitive at a concentration of < 5 μg/mL. Compound **XXVI** was fully inactive (MIC ≥ 250 μg/mL) against all dertatophytes (*table III*).

Lipophilicity of the compounds was determined by RP-HPTLC on the basis of the relationship between the R_M values and composition of the mobile phase [20, 21], expressed by the following equation:

$$R_M = b\varphi + R_{Mw} \quad (1)$$

where b is the constant characterising a given system and φ is the volume percent of the organic modifier. The obtained linear relationship allowed us to extrapolate the data obtained for water–methanol mixtures to the water as the mobile phase. The R_{Mw} values in most cases correlate with log D and with biological activity [22, 23]. The results of HPTLC investigations were presented in the form of the regression equations describing the relation between R_M and the volume fraction of an organic modifier for a particular solute (*table II*).

To determine the probable mechanism of the action of these compounds and to derive the QSAR equations, MIC values characterising a given compound with the surface activity expressed indirectly by the R_{Mw} parameters were correlated (*figure 2*). Parabolic dependencies for all strains studied were obtained, described by the equations:

Epidermophyton floccosum I

$$\log MIC = 0.077 (R_{Mw})^2 - 0.753 R_{Mw} + 2.502 \quad (n = 31, r = 0.700) \quad (2)$$

Epidermophyton floccosum II

$$\log MIC = 0.059 (R_{Mw})^2 - 0.665 R_{Mw} - 2.293 \quad (n = 31, r = 0.734) \quad (3)$$

Microsporum gypseum

$$\log MIC = 0.059 (R_{Mw})^2 - 0.662 R_{Mw} + 2.938 \quad (n = 31, r = 0.714) \quad (4)$$

Trichophyton interdigitale

$$\log MIC = 0.088 (R_{Mw})^2 - 0.826 R_{Mw} + 2.675 \quad (n = 31, r = 0.716) \quad (5)$$

Trichophyton rubrum

$$\log MIC = 0.070 (R_{Mw})^2 - 0.720 R_{Mw} - 2.637 \quad (n = 31, r = 0.721) \quad (6)$$

Trichophyton mentagrophytes

Table II. HPTLC parameters of thiobenzanilides and biological activity of these compounds against dermatophytes.

Compound	Substituents	$R_M = b\varphi + R_{Mw}$		MIC (μg/mL)						
		b	R_{Mw}	*Epidermophyton floccosum* I	*Epidermophyton floccosum* II	*Microsporum gypseum*	*Trichophyton galline*	*Trichophyton interdigitale*	*Trichophyton mentagrophytes*	*Trichophyton rubrum*
I	R^2–R^6 = H	–0.033	2.26	15.7	7.8	31.3	7.8	7.8	15.7	15.7
II	R^2, R^4 = $-CH_3$	–0.046	3.57	31.3	15.7	62.5	31.3	15.7	15.7	15.7
III	R^4 = sec $-C_4H_9$	–0.064	5.36	3.9	1.9	3.9	1.9	3.9	3.9	3.9
IV	R^2 = -F	–0.042	2.93	15.7	15.7	62.5	31.3	15.7	7.8	31.3
V	R^3 = -F	–0.056	4.34	3.9	3.9	7.8	1.9	3.9	3.9	3.9
VI	R^4 = -F	–0.041	2.94	15.7	15.7	31.3	31.3	31.3	31.3	31.3
VII	R^2, R^4 = -F	–0.038	2.85	7.8	3.9	31.3	15.7	15.7	15.7	15.7
VIII	R^2 = -Cl	–0.037	2.74	31.3	31.3	62.5	31.3	15.7	31.3	31.3
IX	R^3 = -Cl	–0.047	3.66	15.83	3.9	31.3	15.7	31.3	31.3	31.3
X	R^4 = -Cl	–0.050	3.91	7.8	3.9	31.3	7.8	7.8	7.8	7.8
XI	R^2, R^4 = -Cl	–0.057	4.57	3.9	1.9	7.8	3.9	3.9	3.9	3.9
XII	R^2, R^5 = -Cl	–0.056	4.47	3.9	3.9	31.3	7.8	3.9	7.8	3.9
XIII	R^3, R^4 = -Cl	–0.060	4.92	1.9	1.9	7.8	1.9	1.9	3.9	1.9
XIV	R^3 = -Cl, R^4 = -F	–0.061	4.73	3.9	1.9	7.8	7.8	7.8	7.8	7.8
XV	R^2 = -Br	–0.051	3.75	3.9	3.9	31.3	7.8	7.8	7.8	7.8
XVI	R^4 = -I	–0.056	4.40	7.8	7.8	31.3	7.8	3.9	7.8	7.8
XVII	R^3 = $-CH_3$, R^5 = -F	–0.051	3.74	31.3	7.8	31.3	31.3	31.3	31.3	31.3
XVIII	R^4 = $-CH_3$, R^3 = -Cl	–0.052	3.94	3.9	1.9	7.8	1.9	3.9	7. 8	7.8
XIX	R^2 = -Cl, R^4 = $-CH_3$	–0.059	4.65	3.9	1.9	15.7	7.8	3.9	7.8	3.9
XX	R^2 = $-CF_3$	–0.042	3.04	15.7	7.8	31.3	15.7	15.7	15.7	15.7
XXI	R^2 = $-OCH_3$	–0.040	2.72	3.9	3.9	15.7	3.9	7.8	7.8	7.8
XXII	R^4 = $-OCH_3$	–0.034	2.32	3.9	7.8	62.5	7.8	7.8	7.8	7.8
XXIII	R^3 = -OH	–0.021	1.02	31.3	31.3	62.5	31.3	15.7	62.5	31.3
XXIV	R^2 = $-CH_3$, R^4 = -OH	–0.030	1.59	62.5	15.7	62.5	62.5	62.5	62.5	62.5
XXV	R^4 = $-CO_2H$	–0.050	3.73	31.3	62.5	500	62.5	31.3	31.3	31.3
XXVI	R^3 = $-CO_2H$, R^4 = -OH	–0.026	1.44	250	250	1 000	250	250	250	250
XXVII	R^2 = $-CO_2CH_3$	–0.051	3.70	1.9	1.9	3.9	7.8	7.8	3.9	7.8
XXVIII	R^4 = $-C(=O)CH_3$	–0.038	2.62	3.9	3.9	62.5	7.8	7.8	7.8	31.3
XXIX	R^4 = $-C(=O)CH_2CH_3$	–0.052	3.81	3.9	3.9	31.3	3.9	3.9	15.7	15.7
XXX	R^4 = -CN	–0.034	3.07	7.8	3.9	31.3	7.8	7.8	7.8	7.8
XXXI	R^2 = -OH, R^4 = $-NO_2$	–0.061	5.11	1.46	3.9	15.7	1.9	3.9	3.9	1.9
XXXII	R^4 = $-C(=O)NH_2$	–0.026	1.41	3.9	3.9	62.5	7.8	3.9	62.5	31.3
XXXIII	R^4 = $-CONHCH_2CO_2H$	–0.025	1.23	15.7	15.7	125	15.7	62.5	31.3	31.3

$$\log MIC = 0.100 (R_{Mw})^2 - 0.914 R_{Mw} + 2.910 \quad (n = 31, r = 0.731) \quad (7)$$

The analogous dependence but with a poorer correlation was obtained for *Trichophyton galline*. Two compounds are characterised by a great deviation from the derived equations for all studied strains and were not taken into consideration in the regression. Namely, compound **XXXII** of low lipophilicity exhibits a higher activity than was expected from the obtained equations. A contrary effect is observed in the case of compound **XXV**.

4. Discussion

Searching for the correlation between the structure and activity of individual compounds it has been stated that, independently of generally accepted conditions connected with their lipophility, obtaining more complete data is possible if the nature of intra- and intermolecular interactions is established. This refers either to searching for the relationships between the change in the nature of substitution in the -C(=S)NH- moiety and R_{Mw} values or

Table III. Number and percentage of strains sensitive to 2,4-dihydroxythiobenzanilides at various concentrations[a].

Compound	Minimum inhibition concentration in μg/mL					
	< 5		< 20		< 100	
	number	percentage	number	percentage	number	percentage
I	3	43	7	100	7	100
II	0	0	4	57	7	100
III	7	100	7	100	7	100
IV	0	0	4	57	7	100
V	6	86	7	100	7	100
VI	0	0	2	29	7	100
VII	1	14	6	86	7	100
VIII	0	0	1	14	7	100
IX	1	14	3	43	7	100
X	1	14	6	86	7	100
XI	6	86	7	100	7	100
XII	4	57	6	86	7	100
XIII	6	86	7	100	7	100
XIV	2	29	7	100	7	100
XV	2	29	6	86	7	100
XVI	0	0	6	86	7	100
XVII	0	0	1	14	7	100
XVIII	4	57	7	100	7	100
XIX	4	57	7	100	7	100
XX	0	0	6	86	7	100
XXI	3	43	7	100	7	100
XXII	1	14	6	86	7	100
XXIII	0	0	1	14	7	100
XXIV	0	0	1	14	7	100
XXV	0	0	0	0	6	86
XXVI	0	0	0	0	0	0
XXVII	4	57	7	100	7	100
XXVIII	2	29	5	71	7	100
XXIX	4	57	6	86	7	100
XXX	1	14	6	86	7	100
XXXI	6	86	7	100	7	100
XXXII	3	43	4	57	7	100
XXXIII	0	0	3	43	1	14

[a] 15–25 μg/mL is break point conventionally determined for susceptibility to the topical antifungals (exceptionally up to 100 μg/mL).

to application of UV-VIS and MS spectra for explanation of discrepancies.

From the dependencies observed in the individual groups it can be shown that a significant increase of lipophility of mono- and dialkyl derivatives is accompanied by chypschromic shifts and by the changes in intensity of the bands corresponding to π–π* (K) transitions in relation to the parent compound (*tables I* and *II*). Generally, a higher lipophilicity is the factor which promotes activity of the compounds against the dermatophytes, although compound **II** (R^2, R^4 = $-CH_3$) is characterised by an exceptionally lower activity (*table II*). The observed facts are connected with a differential transfer of electrons into the thiocarbonyl carbon atom, which may be confirmed also by the intensity of 153 m/z cation line determining probability of their stabilisation after disintegration of M^+ ions (*table I*).

Among monofluoro derivatives, compound **V** is the most active. This can result from either its highest lipophilicity, represented by the R_{Mw} value, or specific interactions of a fluorine atom localised in the *meta* position, which is confirmed by the parameters of electron spectra. As for the two other isomers, a higher activity, according to the predicted mechanism of action, is exhibited by the compound **IV**, which correlates with localisation of the bands in the UV-VIS spectra (shift of the signal corresponding to the amide proton) and with intensity of the lines corresponding to the cation frag-

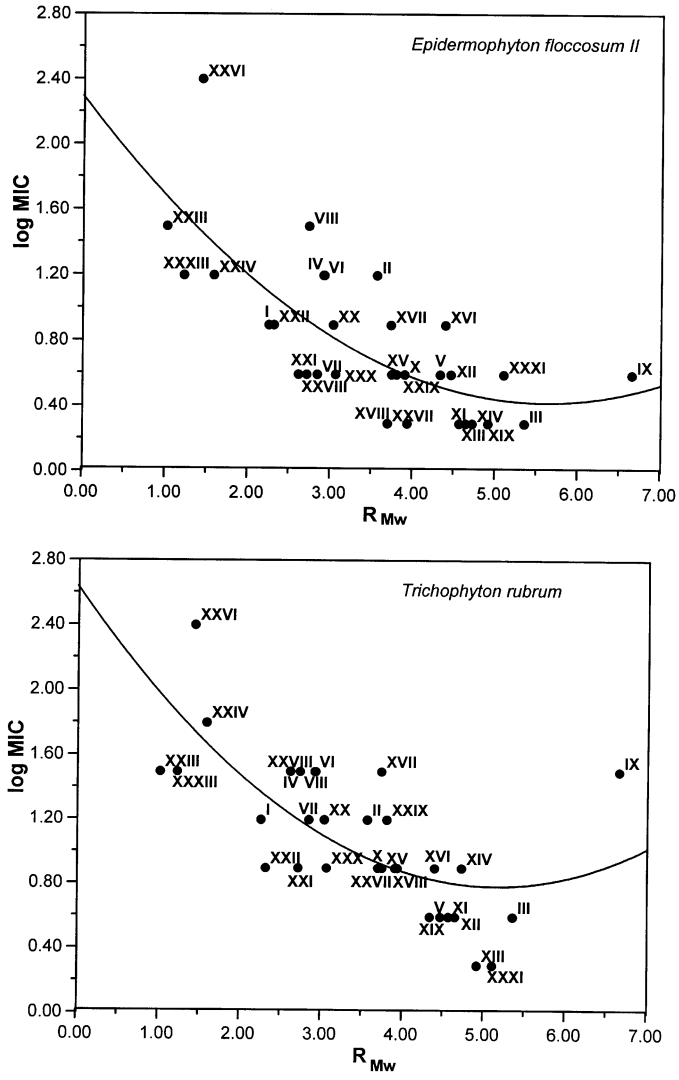

Figure 2. Relationships between the fungistatic activity of 2,4-dihydroxythiobenzanilides (log MIC) and the lipophilicity parameter (R_{Mw}) for *Epidermophyton floccosum II* and *Trichophyton rubrum.*

mentation (153 m/z). Localisation of two fluorine atoms (R^2, R^4 = -F) reduces the lipophilicity of compound **VII** in relation to monosubstituted derivatives, but a specifically higher activity results probably from stronger polarisation of a C ⋯ N bond (*table I*).

There is also evidence that the effect resulting from localisation of chlorine atoms bonded to the ring, weakening of the electron interactions of substituents and changes of the dipole moment of compound **X** are accompanied by the increase of its lipophilicity and biological activity (*table II*). Lipophilic properties (including activity) of isomer **VIII** are the weakest. It is connected with the strong influence of electron interactions and steric substituents localised in the vicinal position in relation to the amide system, and probably with the increase of the charge localised on the chlorine atom (fragmentation elimination of $[M–Cl]^+ \rightarrow 244$ m/z type represents the main band in the MS spectrum of this compound). Typical for this case is also the strongest decrease of energy of electron transitions in the amide system of this compound, which may indicate a significant dissipation of electron density adopted in this case mainly by thiocarbonyl carbon atom (the lowest within this group of isomers, intensity of the line corresponding to the elimination of the cations 153 m/z). The observed changes may result from non-typical orientation of the substituent localised at the nitrogen atom (limiting the ability for formation of imide structures) as well as from its conformational tendencies and from the course of desulphydration of M^+ ions.

All dihalogeno derivatives are characterised by high lipophility (R_{Mw} = 4.47–4.52) and therefore by high biological activity (MIC = 1.9–7.8). The highest activity in this case is exhibited by isomer **XIII** (R^3, R^4 = -Cl) and the changes observed for other isomers correspond to the relative decrease of lipophilicity and positive charge localised on the thiocarbonyl carbon atom. These differences should be associated with dipole moments considering the limited conformational tendency determined by special interactions of chlorine atoms in compounds **XI** and **XIII** and with unprofitable distribution of charge density in the amide bond of the compound **XIV** (broadening of electrons is confirmed by localisation of π–π* bands in conjugated =C(=S) groups). In the series of halogenoalkyl derivatives, slightly different dependencies have been observed. In spite of the fact that compound **XVII** (R^2 = -CH_3, R^5 = -F) is characterised by relatively high lipophility (R_{Mw} = 3.74), great accumulation of electron density on the nitrogen atom and rather non-typical interaction of the -CH_3 group (base band corresponds to demethylation of the M^+ ion) do not increase the biological activity.

The derivatives with -OCH_3 substituents are characterised by relatively differentiated lipophilicity and corresponding biological activity. Also in this series, a significant effect of localisation of substituents can be observed. From the analytical data (*tables I* and *II*) it results that the -OCH_3 group is localised in the *ortho* position (**XXI**) which shows the ability for retraction of electrons but it is different when the same group is localised in the *para* position (**XXII**). For compound **XXII**, intensification of

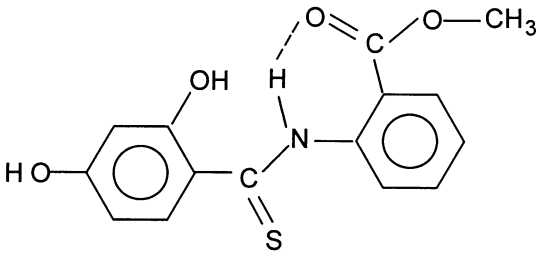

Figure 3. Effect of the stabilisation of the ester group after bonding of the amide proton in compound **XXVII**.

the effect is accompanied by both phase affinity and a relative decrease of activity [24].

Similarly, lipophilicity of compound **XXVII** (R^2 = $-CO_2CH_3$) is smaller than expected which shows, however, a significant activity against the mentioned fungi. It seems that in this case apparent discrepancies may result from the concentration of electron density on substituents ($[M–CO_2CH_3]^+ \rightarrow 244$ m/z is in this case the main band in MS spectrum) and from probability of conjunction of three electron pairs of oxygen atoms with a multiple bond leading to formation of boundary structures characterised by higher polarity (*figure 3*). The increase in oxygen atom basicity probably facilitates isomeric stabilisation of the oxo-ion formed after bonding a strongly acidic proton with the amide system.

Specific dependencies appear as a consequence of acyl substituent interactions. Structurally similar compounds **XXVIII** and **XXIX** are parameters, which differ in hyperconjugation induced polarity, because the steric changes in the thiocarbamoyl group of compound **XXIX** (R^4 = $-COCH_2CH_3$) limit the complanarity and the effect of =C(=O) group conduction.

The R_{Mw} values determined for the group of compounds under consideration describe well the properties resulting from the structure of the compounds and show a good correlation with the biological activity. In most cases the R_{Mw} values are additive and reflect not only the contribution of standard fragments, but also all molecular changes determining the rate of equilibrium establishment in local processes of phase partition, probably also in the fungi cells. From the physico-chemical parameters indicating the changes of lipophilicity (determining the ability for diffusion and local concentration of the compounds) and the microbiostatic activity of the compounds it results that the attainment of the intended purpose requires an appropriate substituent localised in the appropriate position. The obtained results permit the conclusion that the substituents localised in the vicinal position in relation to the thiocarbamyl group limit, usually independently of the electron effect, the structural equilibrium transition and moreover, induce the changes of local dipole moments and molecule orientation (especially water molecules) in the vicinity of this group.

From the properties of the 2,4-dihydroxythiobenzanilides, determined using chromatographic methods, it is possible to select the compounds most useful for biological investigations.

5. Experimental protocols

5.1. Analytical investigations

Elemental analysis was performed in order to determine C, H and N contents (Perkin-Elmer-2400 analyser). EI-MS spectra were recorded with an AMD-604 mass spectrometer (electron ionisation at 70 eV). The parameters of basic band and characteristic fragmentation ions corresponding to the products of the primary fragmentation and to the structure relatively close to that of the tested compound were 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 I* also presents line intensities (%) of 153 m/z cations 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. Because of the determined mechanism of synthesis, the spectra of the compounds were registered mainly in order to confirm the structure and if possible to determine the chemical shift of the thioamide proton. At the same time the detailed interpretation of individual conjunctions and determination of the constant values has been omitted, drawing attention to the changes of the spectra 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 I* the frequencies of stretching vibrations in equilibrium states of the amidothione system typical for frequencies (for *cis* comformation) are listed [25]. UV-VIS spectra were registered by means of a UV-160 A Shimadzu spectrophotometer (solutions in ethanol). The interpretation of the obtained data was limited to the evaluation of the nature of the transition occurring in the thiocarbamyl system.

5.2. Chromatographic measurements

TLC was performed on 10×10 cm pre-coated HPTLC plates of RP-8, F_{254S} (E. Merck); 1 µL samples of the solutes (0.5 mg/mL in methanol) were spotted with a Desaga AS 30 Applicator. The chromatograms were developed over a distance of 9.5 cm in horizontal 'sandwich' chambers of CAMAG for TLC. The chambers were saturated with the organic solvent vapour for 20 min. In the studies with the 2,4-dihydroxythiobenzanilides the water–methanol mixtures were used as the mobile phases. The concentration of the organic modifier in a mobile phase ranged from 50–85%. All TLC measurements were performed at 21 °C. Spots were visualised under UV light at 254 nm.

5.3. Investigation of antifungal activity

Using the dilution method the minimal inhibition concentration (MIC) of individual compounds against seven fungi species (dermatophytes) was determined. These were either reference strains of known sensitivity to antifungal drugs or the strains isolated directly from the clinical material. Micro-organisms 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 into Petri dishes. The medium of more and more decreasing concentration ranging from 0.9–1 000 µg/mL was obtained. The medium containing 0.5 mL of the substance also had 5% of methanol. After solidification the plates were dried, and after spraying the 0.02 mL culture (10^4 cfu of fungi) the plates were incubated for 2–10 days at 22 °C. At the same time the sensitivity of the strains to methanol was determined. The presented results were obtained from three independent measurements.

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