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N-Benzylsalicylthioamides as novel compounds with promising antimycotic activity

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ABSTRACT

The in vitro biological activity of *N*-benzylsalicylthioamides was evaluated against eight fungal strains by the broth microdilution method and the results were compared with those obtained with fluconazole. The compounds exhibited an in vitro antifungal activity against the fluconazole-susceptible as well as the fluconazole-resistant fungal strains. The biological activity was analyzed by quantitative structure–activity relationship (QSAR).

Fungal infections cause a wide range of diseases in humans. They are caused by microscopic fungi that can be normally found in the nature and human beings are constantly exposed to them. The establishment of a mycotic infection usually depends on the pathogenicity of a fungus and on the host resistance.[1] It is known that almost everyone comes in contact with fungi during a life period. However, the development of serious infections is largely associated with immunocompromised persons, such as HIV, elderly, cancer patients, and transplant recipients.[2–4] The incidence of systemic mycoses has increased over the last years.[5] The facts that both mammalian and fungal cells are eukaryotic and that their cell membranes contain structurally similar sterols (ergosterol in fungi vs cholesterol in mammals)[6] can be also connected with some problems in management, which can explain some serious side effects of commonly used antifungal drugs. Whereas there are many antimycotics with different chemical structures used in the therapy of superficial mycoses, that is, mycoses that infect the keratinized layers of the skin, hair, and nails, there are less efficient and specific drugs for the treatment of systemic mycoses.[7] Another problem is a growing number of opportunistic pathogens with increased resistance to antifungals, especially in the setting of immunocompromised patients.[8,9]

All these problems need a solution and the development of new antifungal compounds with a better effectiveness and a lower toxicity is the subject of scientific research.

Waisser (1998) has demonstrated that there can be some correlations between the antimycotic and antimycobacterial activities, and having found that *N*-benzylsalicylthioamides belong to the group of very active compounds against tuberculosis,[10] we tried to investigate their activities against some fungal strains. The hypothesis that these compounds can show some significant biological activity was also based on the knowledge of some drugs with a thiocarbonyl functional group that have already been introduced into practice, for example, tolnaphtate that is believed to inhibit microsomal squalene epoxidase from *Candida albicans*.[11] Also the antifungal activity of related thiosalicylanilides is known. Wagner et al. synthesized[12,13] a series of thiosalicylanilides, tested their antifungal activity and found some structure–activity relationship (SAR).[14]

Our work is focused on *N*-benzylsalicylthioamides prepared by a new method of synthesis.[10] The goal of this Letter was to study the in vitro antifungal activity of these compounds and to find some quantitative correlations between the structure and the biological activity (QSAR).

N-Benzylsalicylthioamides were prepared by microwave-assisted thionation of starting *N*-benzylsalicylamides. This synthesis, the analytical data and the evaluation of the antimycobacterial activity of these derivatives are described in full details in our previous paper.[10] An overview of the compounds under study and their calculated log *P* are summarized in Scheme 1.

In this study, the culture medium RPMI 1640 (Sevapharma, Prague, Czech Republic) buffered to pH 7.0 with 0.165 M of 3-morpholino-propane-1-sulfonic acid was used to study the antifungal

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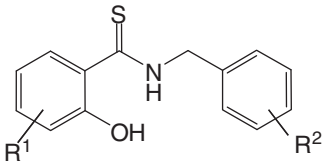

Comp.	R^1	R^2	logP	p*Ka*	Comp.	R^1	R^2	logP	p*Ka*
1	H	3,4-Cl_2	4.62	7.5	**12**	H	3-CH_3	3.99	7.5
2	5-Br	4-Br	5.16	6.9	**13**	4-CH_3	H	3.99	7.7
3	5-Cl	4-F	4.22	7.0	**14**	4-CH_3	4-CH_3	4.48	7.7
4	5-Br	3-Br	5.16	6.9	**15**	4-OCH_3	H	3.38	7.3
5	5-Cl	3,4-Cl_2	5.18	6.9	**16**	5-NO_2	4-CH_3	2.73	4.5
6	5-Br	3,4-Cl_2	5.45	6.9	**17**	3-CH_3	4-Cl	4.55	8.0
7	4-Cl	4-Br	4.89	6.7	**18**	H	3-Cl	4.06	7.6
8	H	4-*Tert*-butyl	5.21	7.5	**19**	4-OCH_3	3-Cl	3.94	7.3
9	5-Cl	H	4.06	7.0	**20**	4-CH_3	4-Cl	4.55	7.7
10	H	4-CH_3	3.99	7.5	**21**	4-CH_3	4-*Tert*-butyl	5.70	7.7
11	H	4-F	3.66	7.6					

Scheme 1. Overview of compounds under study, calculated log *P* and pK_a.

Table 1
Antifungal activity

Strains	Compounds (MIC (μmol/L) 24/48 h)											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Flu**
Candida albicans ATCC 44859	15.62/ 31.25	15.62/ 31.25	31.25/ 125	15.62/ 31.25	31.25/ 31.25	15.62/ 31.25	15.62/ 31.25	>500/ >500	62.5/ 125	62.5/ 125	62.5/ 125	1(±1)/ 2(±1)
Candida tropicalis 156	31.25/ 31.25	31.25/ 31.25	62.5/ 125	15.62/ 62.5	31.25/ 62.5	31.25/ 31.25	31.25/ 31.25	>500/ >500	62.5/ 125	125/125	125/250	3(±1)/ 5(±2)
Candida krusei E28	15.62/ 31.25	3.9/ 15.62	7.81/ 15.62	3.9/7.81	3.9/ 15.62	7.81/ 15.62	3.9/ 15.62	>500/ >500	15.62/ 31.25	62.5/ 125	62.5/ 125	>50/>50
Candida glabrata 20/I	31.25/ 31.25	31.25/ 125	62.5/ 125	7.81/ 31.25	31.25/ 31.25	31.25/ 62.5	31.25/ 31.25	>500/ >500	15.62/ 62.5	125/125	125/250	22(±6)/ >50
Trichosporon asahii 1188	15.62/ 15.62	3.9/7.81	7.81/ 15.62	15.62/ 31.25	3.9/7.81	3.9/7.81	7.81/ 7.81	250/250	31.25/ 31.25	31.25/ 62.5	31.25/ 62.5	4(±2)/ 9(±3)
Aspergillus fumigatus 231	15.62/ 31.25	15.62/ 15.62	31.25/ 15.62	7.81/ 15.62	3.9/ 15.62	7.81/ 7.81	7.81/ 7.81	250/ >500	31.25/ 31.25	125/250	62.5/ 62.5	>50/>50
Absidia corymbifera 272	7.81/ 15.62	3.9/3.9	15.62/ 15.62	3.9/3.9	3.9/7.81	3.9/3.9	0.98/ 1.95	15.62/ 62.5	15.62/ 15.62	15.62/ 31.25	15.62/ 31.25	>50/>50
Trichophyton mentagrophytes 445*	7.81/ 15.62	15.62/ 31.25	15.62/ 15.62	1.95/ 1.95	0.98/ 0.98	0.98/ 0.98	0.98/ 1.95	15.62/ 15.62	1.95/3.9	31.25/ 15.62	7.81/ 31.25	17(±6)/ 26(±1)

Flu: fluconazole (standard).
* MIC evaluated after 72 and 120 h.

activity. The organisms examined included the following strains: *C. albicans* ATCC 44859, *C. tropicalis* 156, *C. krusei* E28, *C. glabrata* 20/I, *Trichosporon asahii* 1188, *Aspergillus fumigatus* 231, *Absidia corymbifera* 272, and *Trichophyton mentagrophytes* 445. The minimum inhibitory concentration (MIC) was determined after 24 and 48 h of static incubation at 35 °C (72 and 120 h of incubation in the case of *T. mentagrophytes*) and the value of MIC of fluconazole was included for the sake of comparison. The values of antifungal activity are summarized in Tables 1 and 2.

5-Bromo-*N*-(4-brombenzyl)salicylthioamide with the activity within the range of 0.98–31.25 μmol/L seems to be the most prospective compound. But also the compounds **2, 4–6** exhibited high activity in the range of 0.98–62.5 μmol/L. In most cases, the activities of these compounds are comparable to or better than that of fluconazole. Regrettably, none of the compounds has shown a better antifungal activity against *C. albicans* and *C. tropicalis* than fluconazole. However, we must be careful before making any definitive conclusions because primary resistance or decreased susceptibility to fluconazole should be taken into consideration in the case of evaluation of the antifungal potential of the compounds tested against *A. fumigatus*, *A. corymbifera*, *C. krusei*, and *C. glabrata*.

The SPARC 4.5 program was used to calculate pK_a values (Scheme 1). The calculated pK_a values of the most active compounds (**2, 4–7**) were found in the range of 6.7–6.9 and considering the incubation conditions (35 °C, pH 7.00) it would be expected to make 54–66% of the deprotonated form of the active compounds. The pK_a range 6.7–6.9 seems to be the optimum for the antifungal activity since the less active compounds had lower or higher calculated pK_a than the more active antifungal derivatives.

Table 2
Antifungal activity

Strains	Compounds (MIC (μmol/L) 24/48 h)										
	12	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	21	Flu
Candida albicans ATCC 44859	125/250	62.5/125	125/250	62.5/250	500/>500	62.5/125	125/125	125/250	>500/>500	>500/>500	1(±1)/2(±1)
Candida tropicalis 156	125/250	125/250	125/500	250/250	>500/>500	125/250	125/125	125/250	>500/>500	>500/>500	3(±1)/5(±2)
Candida krusei E28	125/250	62.5/125	62.5/125	250/250	>500/>500	>500/>500	62.5/125	125/125	>500/>500	>500/>500	>50/>50
Candida glabrata 20/I	125/250	125/250	125/500	500/500	>500/>500	>500/>500	125/250	250/500	>500/>500	>500/>500	22(±6)/>50
Trichosporon asahii 1188	31.25/62.5	62.5/62.5	31.25/62.5	250/250	500/500	62.5/62.5	31.25/31.25	31.25/62.5	>500/>500	>500/>500	4(±2)/9(±3)
Aspergillus fumigatus 231	62.5/62.5	62.5/125	125/500	250/500	500/500	62.5/125	62.5/62.5	62.5/125	>500/>500	>500/>500	>50/>50
Absidia corymbifera 272	62.5/15.62	7.81/31.25	7.81/31.25	62.5/250	500/500	31.25/31.25	31.25/31.25	7.81/7.81	>500/>500	>500/>500	>50/>50
Trichophyton mentagrophytes 445*	15.62/15.62	31.25/31.25	15.62/15.62	62.5/62.5	62.5/62.5	15.62/15.62	7.81/15.62	15.62/15.62	>500/>500	>500/>500	17(±6)/26(±1)

Flu: fluconazole (standard).
* MIC evaluated after 72 and 120 h.

Table 3
Results of QSAR calculations

Eq.	Strain; incubation time	Results of QSAR calculations
1	*Candida albicans* ATCC 44859; 24 h	Log MIC = −0.440 (±0.109) log *P* −0.145 (±0.161) *I* +0.021 (±0.233) *σ* +3.699 (±0.422) *R* = 0.83 *s* = 0.259 *F* = 10.45 *n* = 18
2	*Candida albicans* ATCC 44859; 48 h	Log MIC = −0.392 (±0.118) log *P* −0.135 (±0.131) *I* −0.267 (±0.304) *σ* +3.801(±0.497) *R* = 0.87 *s* = 0.200 *F* = 13.91 *n* = 17
3	*Candida tropicalis* 156; 24 h	Log MIC = −0.318 (±0.086) log *P* −0.097 (±0.097) *I* −0.476 (±0.224) *σ* +3.360 (±0.365) *R* = 0.92 *s* = 0.148 *F* = 24.18 *n* = 17
4	*Candida tropicalis* 156; 48 h	Log MIC = −0.292 (±0.155) log *P* −0.153 (±0.173) *I* −0.478 (±0.401) *σ* +3.498 (±0.655) *R* = 0.80 *s* = 0.264 *F* = 7.71 *n* = 17
5	*Candida krusei* E28; 24 h	Log MIC = −0.444 (±0.127) log *P* −0.190 (±0.144) *I* −1.232 (±0.346) *σ* +3.588 (±0.537) *R* = 0.95 *s* = 0.210 *F* = 41.07 *n* = 16
6	*Candida krusei* E28; 48 h	Log MIC = −0.331 (±0.113) log *P* −0.249 (±0.129) *I* −0.913 (±0.309) *σ* +3.418 (±0.480) *R* = 0.95 *s* = 0.188 *F* = 33.19 *n* = 16
7	*Candida glabrata* 20/I; 24 h	Log MIC = −0.230 (±0.149) log *P* −0.090 (±0.170) *I* −1.088 (±0.407) *σ* +2.998 (±0.632) *R* = 0.88 *s* = 0.247 *F* = 14.26 *n* = 16
8	*Candida glabrata* 20/I; 48 h	Log MIC = −0.244 (±0.183) log *P* −0.116 (±0.208) *I* −0.798 (±0.499) *σ* +3.327 (±0.776) *R* = 0.80 *s* = 0.304 *F* = 7.15 *n* = 16

The Hansch approach was carried out to study the relationship between the structure of *N*-benzylsalicylthioamides and their antifungal activity. The QSAR study included the results obtained both after 24 and 48 h of incubation (72/120 h for *T. mentagrophytes*). All regression calculations (Eqs. **1**–**16** in Tables 3 and 4) were set up using the MULTIREG program for Microsoft Excel. The logarithms of the partition coefficient (log *P*) were calculated using ChemBioDraw 11.0 and the values are presented in Scheme 1, the biological activity data in Tables 1 and 2 and the values of *σ* Hammett constants were taken from the literature.[15]

The parameters with the greatest influence on the biological activity include lipophilicity (log *P*), the indicator parameter *I* for

Table 4
Results of QSAR calculations

Eq.	Strain; incubation time	Results of QSAR calculations
9	*Trichosporon asahii* 1188; 24 h	Log MIC = −0.386 (±0.146) log *P* −0.560 (±0.218) *I* −0.174 (±0.360) *σ* +3.500 (±0.599) *R* = 0.80 *s* = 0.400 *F* = 8.69 *n* = 19
10	*Trichosporon asahii* 1188; 48 h	Log MIC = −0.335 (±0.118) log *P* −0.550 (±0.176) *I* −0.140 (±0.290) *σ* +3.427 (±0.484) *R* = 0.83 *s* = 0.323 *F* = 11.35 *n* = 19
11	*Aspergillus fumigatus* 231; 24 h	Log MIC = −0.394 (±0.114) log *P* −0.577 (±0.170) *I* −0.277 (±0.281) *σ* +3.749 (±0.468) *R* = 0.87 *s* = 0.313 *F* = 15.62 *n* = 19
12	*Aspergillus fumigatus* 231; 48 h	Log MIC = −0.403 (±0.126) log *P* −0.525 (±0.186) *I* −0.594 (±0.270) *σ* +3.925 (±0.488) *R* = 0.90 *s* = 0.299 *F* = 18.85 n= 18
13	*Absidia corymbifera* 272; 24 h	Log MIC = −0.582 (±0.133) log *P* −0.321 (±0.198) *I* +0.452 (±0.327) *σ* +3.798 (±0.545) *R* = 0.83 *s* = 0.364 *F* = 10.92 *n* = 19
14	*Absidia corymbifera* 272; 48 h	Log MIC = −0.480 (±0.132) log *P* −0.524 (±0.197) *I* +0.091 (±0.325) *σ* +3.699 (±0.542) *R* = 0.84 *s* = 0.362 *F* = 11.68 *n* = 19
15	*Trichophyton mentagrophytes* 445; 72 h	Log MIC = −0.375 (±0.136) log *P* −0.488 (±0.202) *I* −0.404 (±0.333) *σ* +2.945 (±0.555) *R* = 0.81 *s* = 0.371 *F* = 9.74 *n* = 19
16	*Trichophyton mentagrophytes* 445; 120 h	Log MIC = −0.453 (±0.141) log *P* −0.212 (±0.210) *I* −0.459 (±0.347) *σ* +3.213 (±0.577) *R* = 0.77 *s* = 0.386 *F* = 7.34 *n* = 19

Table 5
Comparison between the evaluated and calculated antifungal activity of *N*-benzyl-5-methoxysalicylthioamide

	Log MIC/log MIC calculated; 24 h[a]	Log MIC/log MIC calculated; 48 h[b]
Candida albicans ATCC 44859	2.10/2.21	2.40/2.48
Candida tropicalis 156	2.40/2.29	2.40/2.51
Candida krusei E28	2.40/2.09	2.40/2.30
Candida glabrata 20/I	2.40/2.22	2.40/2.50
Trichosporon asahii 1188	2.10/2.20	2.40/2.29
Aspergillus fumigatus 231	2.40/2.42	2.40/2.56
Absidia corymbifera 272	1.49/1.83	2.10/2.08
Trichophyton mentagrophytes 445	1.80/1.68	1.80/1.68

[a] 72 h.
[b] 120 h in the case of *Trichophyton mentagrophytes*.

halogens as substituents, and the substituent constants of polarity σ, expressed with regard to the carbonyl group of the acyl moiety.

The lipophilicity (log *P*) used for the QSAR calculations was confirmed by the correlation between the calculated log *P* and R_M values obtained using thin-layer chromatography on silica gel impregnated with trioctadecylsilane (See Supplementary data).

Equations **1–16** (Tables 3 and 4) show that the biological activity of the substances increases with lipophilicity, with the presence of halogens, and mostly with the increasing values of constants σ. The influence of halogens on the activity could be explained by their electron-withdrawing effect.

The antifungal activity of *N*-benzyl-5-methoxysalicylthioamide (log *P* 3.38, pK_a 7.8) was additionally evaluated to verify the QSAR calculations. The logarithms of measured and calculated antifungal activity against all examined strains (both 24 and 48 h incubation) were compared and no big differences were found between the evaluated and calculated log MIC (Table 5).

In conclusion, *N*-benzylsalicylthioamides form a new group with a promising antimycotic activity, and the possibility of how to obtain the efficient antifungal compounds is to ensure their optimal lipophilicity and pK_a.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.023.

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