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# Synthesis and antifungal activity of new series of compounds against *Ascosphaera apis*

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## ABSTRACT

The fungicidal compounds synthesized by us and applied in the studies are: 3,1-benzothiazine, variously substituted thioamides, N-heterocyclic carbothionyl derivatives and 2,5-disubstituted 1,3,4-thiadiazole. Depending on their structure their action can be oriented towards zoo- and geophylic fungi destroying crops and raw material of vegetation origin as well as pathogenic for animals and man. The compounds are characterized by differentiated mechanism of molecular interactions in energetic cell processes and disubstituted 1,3,4-thiadiazole inhibiting squale epoxidase and squale accumulation seem to be an interesting group. Selective estimation of action of some compounds from individual groups against the strain *Ascosphaera apis* of the fungus isolated from insects infected with calciferous mycosis was made. 

## KEYWORDS

Calciferous mycosis;
Ascosphaera apis;
"In vitro" fungicidal activity.

## INTRODUCTION

Orthoalbino mycosis of bees ascosphaerosis has been one of the most dangerous diseases of bee colonies throughout the world recently. It is induced by the orthoalbino, *Ascosphaera apis*. Development of mycosis is due to common occurrence of this fungus in the honey-bee colonies, population of recluse bees and other wild insects as well as to the factors decreasing bee colonies immunity[1,2].

Susceptibility or its lack to mycosis in honey-bee (*Apis mellifera L.*) is strictly associated with behavioral immunity of bee colonies. This is a genetically conditioned feature. Behavioral immunity is determined in a simple way as the capacity of worker bees for searching out and tearing apart of dead bee maggot and its removal from the cells before the pathogenic agent acquires the invasion capacity[3-5].

Periodical growth of orthoalbino mycosis makes it necessary to apply chemical preparations to fight against it. In practice organic acids are applied locally or fungicidal compounds are administered in the food[6,7].

Action of "in vitro" compounds is estimated by determination of MIC (minimal inhibitory concentration) and MFC (minimal fungicidal concentration). Disk methods by Gliński[8] or series of compound concentrations in the substrates are mostly frequently used by for example Sanami[9].

At present di- and triazole preparations are most commonly applied in therapy of ascophaeriosis. They inhibit ergosterol biosynthesis limiting the action of demethylase changing properties of cytoplasm mem-

branes and capability of cells transformation in mycelium[10,11]. Literature on action of derivatives of this group of compounds devotes more and more space to diminished susceptibility of fungi suggesting also the increase in cytochromium P-450 activity. Particular attention is paid to increase of varied resistance and strict dependence between fungus virulence and chemoresistance extent Chorbiński[12]. Occurrence of primary and secondary resistance to fungicidal compounds, more frequent appearance of strains of diminished susceptibility and also differences between species and strains promote search for new fungicidal compounds.

## MATERIAL AND METHODS

### Synthesis of combinations

The compounds in question (TABLES 1 and 2) are obtained in the reactions of sulfinyl bis(2,4-dihydroxythiobenzoyl) = STB by us with nitrogen nucleophiles. The synthesis employs aromatic amines(heteroaromatic and cycloalkyl), hydrazines, hydrazones, hydrazides of carboxylic acids as well as 3-thiosemicarbazides and depending on the kind of substrate linear or heterocyclic systems are obtained.

**TABLE 1 : Analytical data of compounds**

| No | Formula Mol.weight | Mp[°C] | %C | %H | %N | $M^+$ and fragmentation (m/z), B% | 1H NMR δ(ppm) | IR($cm^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| | | | calced/found | | | | | |
| 1a | $C_{14}H_{10}F_3NO_2S$ 313.30 | 185-186 | 53.62<br>53.74 | 3.19<br>3.11 | 4.47<br>4.66 | 313($M^+$; 50.58) 280(100), 153, 145, 136, 97, 81, 69 | 11.76 (2-COH), 11.03 NH, 10.09 (4-COH), | 1621 C=N 1468 N··C\|S\| 1130 C-F 1018 C\|=S\| |
| 1d | $C_{29}H_{26}N_2O_4S$ 498.4 | 193-195 | 69.82<br>69.70 | 5.22<br>5.29 | 5.61<br>5.48 | 498 ($M^+$; 1.25) 428, 397, 344, 278 (100) 249, 182, 137, 106, 66,39 | 11.51 (2-COH), 11.23 NH, 10.06 (4-COH), 3.91 $CH_2$, 3.47 CH(2H), 3.31 $CH_2$, -endo (2H), | 2926, 2855 CH 1621 C=N 1498NHC\|=S\| 1415 N··C(S) 1268 C=N 1025 C\|=S\| |
| 4a | $C_{13}H_{11}NO_4S_2$ 309.37 | 195-197 | 50.43<br>50.37 | 3.56<br>3.68 | 4.52<br>4.50 | 309 ($M^+$; 90.34) 276, 250 (100), 234, 208, 157, 153, 137, 125, 97, 69, 53 | 12.79C\|=O\|··HN- 11.56 (2-COH), 10.25 (4-COH), | 2952, 2830 CH, 1677 C\|=O\| 1469 N=C\|S\| 1267C=N 672 C-S-C |
| 4b | $C_{18}H_{21}NO_4S_2$ 379.34 | 130-131 | 56.94<br>57.11 | 5.53<br>5.46 | 3.69<br>3.78 | 379 ($M^+$; 7.00) 377,331, 304, 298, 273, 242, 225 (100), 196, 179, 153, 137, 97, 69 | 14.08 C\|=O\|··HN- 11.51 (2-COH), 10.23 (4-COH), | 2930,2850 CH, 1670 C(=O), 1120N=C\|SH\| 1095 C(=S).. |
| 5a | $C_{14}H_{16}N_2O_3S$ 292.3 | 201-201 | 57.47<br>57.38 | 5.46<br>5.59 | 9/58<br>9.76 | 291 ($M^+$; 100) (in MeOH) 268, 240, 211, 153, 134, 120, 105 77, 63 | 11.37 (2-COH), 10.09 (4-COH), 7.64 C\|=O\|··HN- 2.50 $CH_2$, | 2937 CH, 1646 C\|=O\| 1587, 1490, 1329, 1006, C\|=S\| |
| 5b | $C_{19}H_{12}F_3NO_3S$ 391.26 | 106-108 | 58.27<br>58.32 | 3.07<br>2.96 | 3.58<br>3.71 | 392 ($M^+$; 8.40) 351, 269, 239, 184(100), 137, 124, 117, 89, 69 | 11.79 (2-COH), 10.04 (4-COH), 3.84 (HC=CH), | 2945,2830CH 1720 C(=O) 1025 C(=S) |

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| No | Formula Mol.weight | Mp[°C] | %C %H %N calced/found | $M^+$ and fragmentation (m/z), B% | 1H NMR δ(ppm) | IR($cm^{-1}$) |
|---|---|---|---|---|---|---|
| 5d | $C_{27}H_{30}N_3O_3S$ 476.31 | 91-92 | 68.02 6.30 8.82<br>68.26 6.47 8.96 | no peak $M^+$ 460, 361, 324, 307, 289, 252, 196 154(100), 137, 108, 91, 82 | 11.56 (2-COH), 10.34 (4-COH), 7.09 (1-COH), 3.35, 1.74 $CH_2$, 1.36 $CH_3$, (6H), 0.74 $CH_3$ (9H), | 2951,CH-$CH_3$ 2601 C\|=N\| 1070 C\|=S\| |
| 6a | $C_{14}H_{18}N_2O_4S$ 310.36 | 204-205 | 54.13 5.80 9.02<br>54.02 5.89 8.83 | 310 ($M^+$; 100) 272, 264, 194, 157, 153, 141, 114, 97, 69, 56, 42, | 9.74 (2-COH), 9.56 (4-COH), 4.27 ($OCH_2$), 3.61 $CH_2$, 1.19 $CH_3$, | 1669 C\|=O\| 1640C\|=O\|O 1558NC\|=O\|O 1031 C\|=S\| |
| 6b | $C_{20}H_{22}N_2O_2S$ 354.28 | 108-110 | 67.74 6.21 7.90<br>67.97 6.30 7.92 | 354 ($M^+$; 9.56) 321, 196, 185, 172, 153, 117(100), 91, 69, 56, 44 | 9.80 (2-COH), 9.61 (4-COH), 4.29 $CH_2$, 4.13 (HC=CH), | 2921, 2818CH 1520 –NC\|=S\| 1028 C\|=S\| |
| 7 | $C_{16}H_{12}N_2O_4S$ 328.33 | 193-195 | 58.47 3.65 8.53<br>58.36 3.51 8.42 | 328 ($M^+$; 48.20) 296, 162, 149, 132, 104 76, 70, 51, 40 | 11.14 (2-COH), 10.08 (4-COH), 3.71 ($OCH_3$), | 2950, 2770CH, 1670 C\|=O\| 1435C=N |
| 8 | $C_{14}H_{13}N_2O_3S$ 289.27 | 206-207 | 58.06 4.49 9.68<br>57.82 4.53 9.65 | 288 ($M^+$; 1.15) 273, 167, 153 | 11.34 (2-COH), 11.22 NH, 9.96 (4-COH), 9.00 (1'-COH), | 1618C=N 1472 -N\|NH\|C=S 1035C\|=S\| |

**TABLE 2 : Structure of preparing compounds**

**R = $C_6H_3(OH)_2$**

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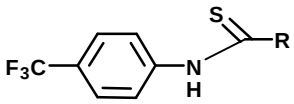
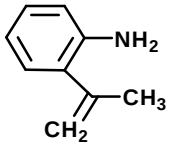
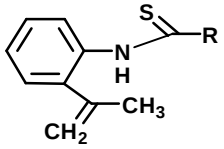
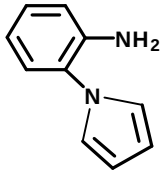
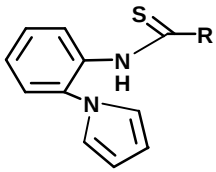
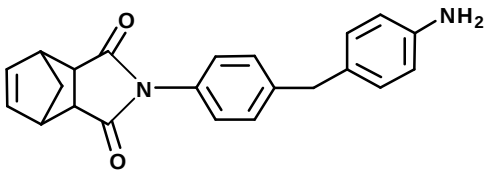
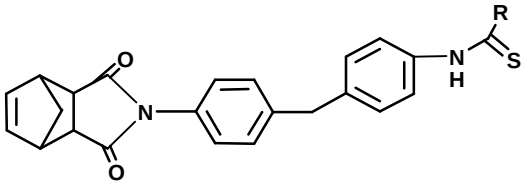
| 1a |  |  |
| 1b |  |  |
| 1c |  |  |
| 1d |  |  |

| Compound no. | Substrate | Product |
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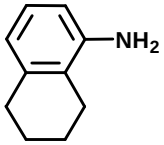
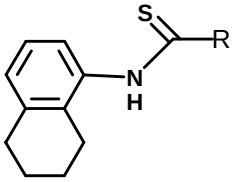
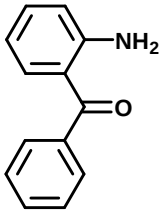
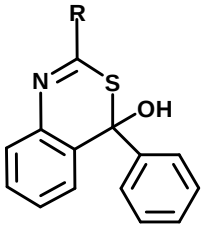
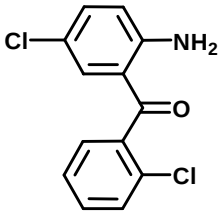
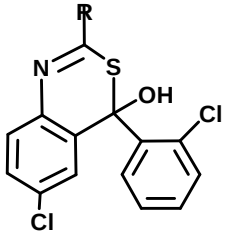
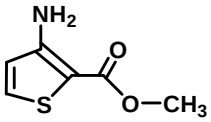
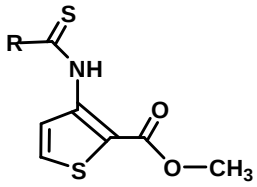
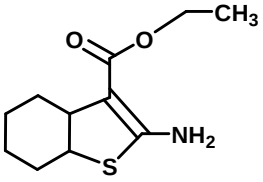
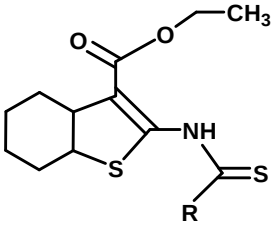
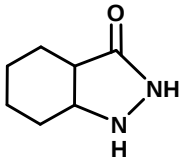
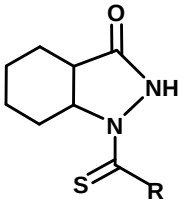
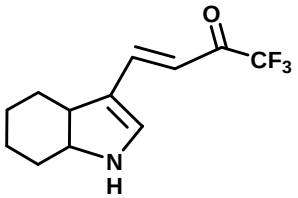
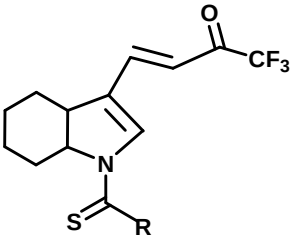
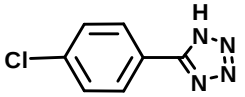
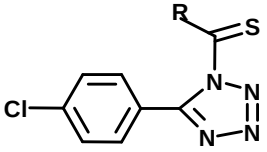
| 2 |  |  |
| 3a |  |  |
| 3b |  |  |
| 4a |  |  |
| 4b |  |  |
| 5a |  |  |
| 5b |  |  |
| 5c |  |  |

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| Compound no. | Substrate | Product |
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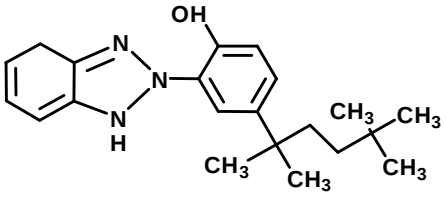
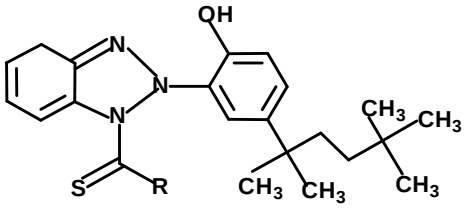
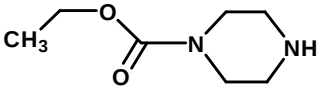
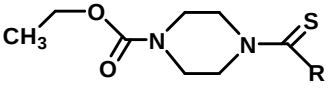
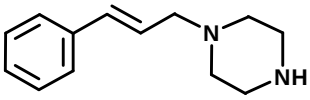
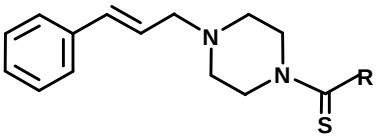
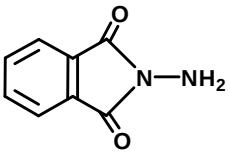
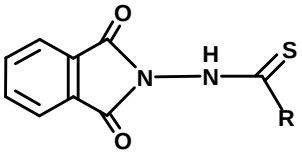
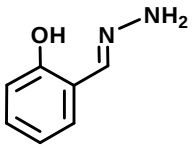
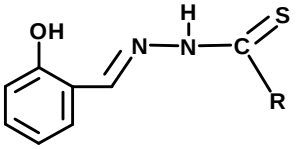
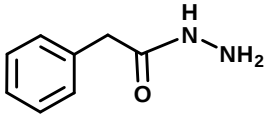
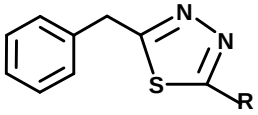
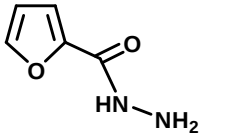
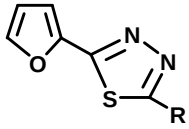
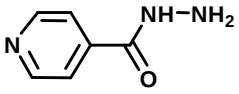
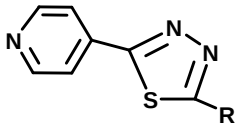
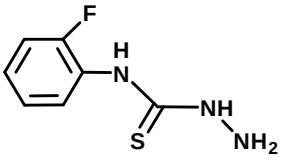
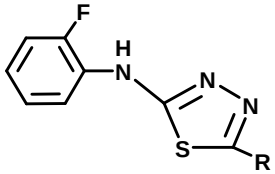
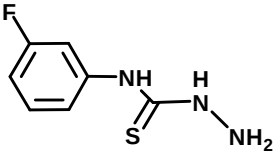
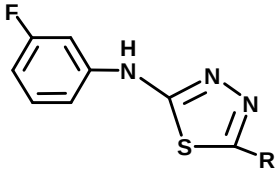
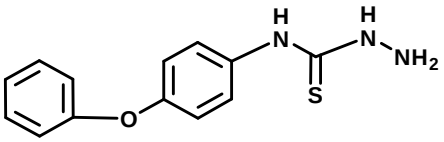
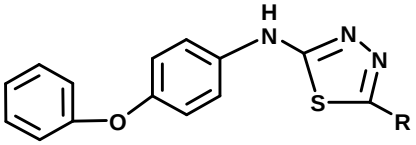
| 5d |  |  |
| 6a |  |  |
| 6b |  |  |
| 7 |  |  |
| 8 |  |  |
| 9 |  |  |
| 10a |  |  |
| 10b |  |  |
| 11a |  |  |
| 11b |  |  |
| 11c |  |  |

**Synthesis of compounds**

**N-[4-(trifluoromethyl)phenyl]-2,4-dihydroxythioamide (1a)**

0.01 mole 4-aminobenztrifluoromethyl and 0.0075 mole STB were moved to methanol and heated to boiling (2h). The reaction mixture was filtered hot and the filtrate was concentrated to small volume. The removed compound was recrystallized from the aqueous-mathanol (1:1) solution (50ml).

**N-(2-isopropenylphenyl)-2,4-dihydroxythioamide (1b)**

Synthesis of the compound was described earlier[13]

**N-2-(pyrol-1-yl-phenyl)-2,4-dihydroxythiobenzamide (1c)**

Synthesis of the compound was described earlier[13]

**N-4-[4-(benzyl) 4-phenyl-3-norbornene-2,3-dicarboximide] – 2,4-dihydroxythiobenzamide (1d)**

0.01 mole N-[4-(4-aminobenzyl)phenyl]-3-norbornene -2,3-dicarboximide and 0.0075 mole STB were moved to methanol (75ml) and heated to boiling (3h). The reaction mixture was filtered hot. The filtrate was evaporated almost dry (10ml). The obtained product was dissolved in the aqueous-methanol (1:1) solution (40ml) and after removal and drying it was dissolved again in methanol (30ml) and water (30ml) was added. After removal the compound was filtered, washed with methanol and dried.

**N-1-(5,6,7,8-tetrahydronaphtyl)-2,4-dihydroxythiobenzamide (2)**

Synthesis of the compound was described earlier[13]

**2-(2,4-dihydroxyphenyl)-4hydroxy -4-phenyl-4*H*-3,1-benzothiazine (3a)**

Synthesis of the compound was described earlier[13]

**6-chloro-4-(2-chlorophenyl)-4-hydroxy-2-(2,4-dihydroxyphenyl)-4*H*-3,1-benzothiazine (3b)**

Synthesis of the compound was described earlier[13]

**N-3-(2,4-dihydroxyphenyl)-2-(methylcarboxylate) thiophenocarbothioamide (4a)**

0.01 mole methyl-3-amino-2-thiophenocarboxylate and 0.0075 mole STB were moved to methanol (50ml) and heated to boiling (3h). The reaction mixture was filtered hot and the filtrate was evaporated dry. The product was washed with water and recrystallized from the aqueous-methanol (3:1) solution (60ml).

**N-2-(4,5,6,7-tetrahydrobenz[b]thiophene-3-yl-carboxylate)-2,4-dihydroxythiobenzamide (4b)**

0.005 mole 2-amino-4,5,6,7-tetrahydrobenz[b]-thiopheno-3-ethyl-carboxylate and 0.0075 mole STB were moved to methanol (50ml) and heated to boiling (3h). After reaction mixture was filtered hot. The filtrate was concentrated almost to dry and the remaining part was recrystllized from methanol (30ml).

**N-3-(2,4-dihydroxythiobenzoyl)-indazolinon (5a)**

0.01 mole 3-indazolinone and 0.0075 mole STB were moved to methanol (50ml) and heated to boiling (3h). After reaction mixture was filtered hot. The filtrate was concentrated almost dry and the remaining part was recrystallized from the aqueous-methanol (2:1) solution (75ml).

**N-1-(2,4-dihydroxythiobenzoyl)-*trans*-1,1,1-trifluoro-4-(3-indolyl)-butene-2-on (5b)**

0.01 mole *trans*-1,1,1-trifluoro-4-(3-indolylo)-3-butene-2-onu and 0.0075 mole STB were moved to methanol (70ml) and heated to boiling (3h). The reaction mixture was filtered hot. The filtrate was concentrated dry and the removed product was crystallized from benzene (60ml).

**[5-(4-chlorophenyl)]-1-(2,4-dihydroxythiobenzoyl)-1-*H*-tetrazole (5c)**

Synthesis of the compound was described earlier[14]

**N-1-(2,4-dihydroxythionenzoyl)-2-(2*H*-benztriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)-phenol (5d)**

0.01 mole 2-(2*H*-benzotriazolo-2-ylo)-4-(1,1,3,3-tetramethylbutyl)-phenol and 0.0075 mole STB were moved to methanol (50ml) and heated to boiling (3h). The reaction mixture was filtered hot and left at room temperature (24h). The removed compound was recrystallized from aqueous-methanol (2:1) solution (75ml).

**N-4-(2,4-dihydroxythiobenzoyl)-1-ethoxycarbonylopiperazine (6a)**

0.02 mole 1-ethoxycarbonylpiperazine and 0.015

mole STB were moved to methanol (80ml) and heated to boiling (3h). The reaction mixture was filtered hot. 50 ml of water were added to the filtrate. The removed compound was recrystallized from methanol (20ml).

**N-4-(2,4-dihydroxythiobenzoyl)-*trans*-1-cinnamyl-piperazine (6b)**

0.01 mole *trans*-1-cinnamyl-piperazine and 0.0075 mole STB were moved to methanol (40ml) and heated to boiling (3h). The reaction mixture was filtered hot and concentrated to dry. The removed compound was recrystallized from the aqueous-methanol (2:1) solution.

**N-(2,4-dihydroxythiobenzoyl)-phthalimidamide (7)**

0.02 mole N-aminophthalimide and 0.015 mole STB were moved to methanol (50ml) and heated to boiling (3h). The reaction mixture was filtered hot and 50 ml of water were added to the filtrate. The removed compound was filtered and crystallized from methanol (40ml).

**N-1-(2,4-dihydroxyphenylcarbothion)-salicylaldehydehydrazone (8)**

0.01 mole salicylaldehydehydrazone and 0.0075 mole STB were moved to methanol (50mL) and heated to boiling (3h). The reaction mixture was filtered hot and the filtrate was left at room temperature (24h). The removed compound was recrystallized from the aqueous-methanol (2:1) solution (45ml).

**2-(benzyl)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (9)**

Synthesis of the compound was described earlier[15]

**2-(2-furyl)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (10a)**

Synthesis of the compound was described earlier[15]

**2-(4-pyridil)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (10b)**

Synthesis of the compound was described earlier[15]

**2-(2-fluorophenylamine)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (11a)**

Synthesis of the compound was described earlier[16]

**2-(3-fluorophenylamine)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (11b)**

Synthesis of the compound was described earlier[13]

**2-(4-phenoxyphenylamine)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (11c)**

Synthesis of the compound was described earlier[13]

**Analytical studies**

The melting point (mp) was determined on a BUCHI B-540 (Switzerland) melting point apparatus. The elemental analysis was performed in order to determine C, H and N contents (Perkin-Elmer 2400). The analyses (C,H,N) were within ± 0.4% of the theoretical values. The vibrational spectra were recorded with a Perkin-Elmer FF-IR 1725 X spectrophotometer (in KBr). The spectra were made in the range of 600-4000 $cm^{-1}$.

NMR spectra were recorded in DMSO-$d_6$ on a Varian Mercury 400 instrument. Chemical shifts ($\delta$, ppm) were given in with TMS. The spectra MS (EI, 70eV) were recorded using the apparatus AMD-604. (TABLE 1)

**Biological investigations**

Action of the compounds against *Ascospaera apis* was examined in the Department of Pesticides Formulation and Application (Laboratory of Microbiocides Application) in the Organic Industry Institute in Warsaw according to the regulations Good Laboratory Practice (OECD 1997, Statement of GLP Compliance No. GO13).

Susceptibility of *Ascosphaera apis* to the studied compounds was determined by means of cylinder method of dilutions in agar. Preliminary studies of compounds were made for two the concentrations 125 and 62.5 mg/l. the values of minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) were determined for the compounds acting at the concentration 125 mg/l.

**Preliminary studies**

The compounds in question were dissolved in acetone (DMSO) and then water was added to obtain 10% concentration of the solvent. The culture medium was added to get 1% solvent contribution. The solution of the studied compound was added to the liquefied agar culture medium (Sabouraud) in the 1:9 ratio and after mixing it was spread over the Petri plates obtaining the concentration 125mg/l. After the substrate solidification, 3 holes of a 5 mm diameter were made with

a cork-drill in each of them. Agar cylinders cut out of homogeneous culture of fungus grown on the Petri plate (inoculum) were incorporated into these holes. The plates containing only culture medium or culture medium with the addition of solvent were the control.

The plates were incubated at 25°C for 5 days. The compounds which at the concentration 125 mg/l inhibited the growth of colony up to 7 mm were suitable for the second stage of investigations and determination of MIC value. In the case of compounds with weaker action against *Ascospahaera apis* (fungus colonies over 7 mm) the value MIC was assumed as equal or greater than 125 mg/l.

### Determination of MIC

For the compounds which at the concentration 125 mg/l inhibited colony growth up to 7 mm dilution with agar culture medium was made twice. After inoculation the plates were incubated at 25°C for five days and then the size of grown colonies was determined. The compound concentration inhibiting the growth of fungus colony to 7 mm i.e. 2 mm beyond the diameter of the incorporated inoculum was assumed as the MIC value.

### Criteria of compound activity estimation

The diameter of fungus colony (mm) at a given compound concentration was the basis of 4-degree scale estimation.

| | Colony diameter | Kind of activity |
|---|---|---|
| 1 | 0-7 mm | strong activity |
| 2 | 8-20 mm | medium activity |
| 3 | 21-50 mm | weak activity |
| 4 | over 50 mm | no activity |

Azole preparations of the second and third generations as well as amphotericine were applied as the reference (TABLE 3).

**TABLE 3 : Action of compounds against the strain *Ascosphaera apis***

| Compound/ preparation | Diameter of colony [mm] | MIC [mg/l] | Action of compound |
|---|---|---|---|
| 1a | 5.0 | 62 | 1 |
| 1b | 5.0 | 125 | 1 |
| 1c | 5.0 | 125 | 1 |
| 1d | 60.0 | >125 | 4 |
| 2 | 5.0 | 62 | 1 |
| 3a | 7.0 | 125 | 1 |
| 3b | 13.5 | >125 | 2 |
| 4a | 6.1 | 125 | 1 |
| 4b | 31.5 | >125 | 3 |
| 5a | 60.0 | >125 | 4 |
| 5b | 33.1 | >125 | 3 |
| 5c | 5.0 | 62 | 1 |
| 5d | >60.0 | >125 | 4 |
| 6a | >60.0 | >125 | 4 |
| 6b | 22.5 | >125 | 3 |
| 7 | 13.2 | >125 | 2 |
| 8 | 24.6 | >125 | 3 |
| 9 | 13.1 | >125 | 2 |
| 10a | 11.9 | >125 | 2 |
| 10b | 19.0 | >125 | 2 |
| 11a | 13.3 | >125 | 2 |
| 11b | 9.6 | >125 | 2 |
| 11c | 12.0 | >125 | 2 |
| chlorimidazol | | 125 | |
| amphotericin B | | 250 | |
| miconazol | | 500 | |
| thioconazol | | 1000 | |
| ketoconazol | | >1000 | |
| klotrimazol | | >1000 | |
| fluconazol | | >1000 | |

*** values 60 mm show the growth of fungus on the whole surface of the plate**

## DISCUSSION

The studied compounds from various systematic groups **(1-11)** exhibited differentiated activity against *Ascosphaera apis*. The strongest activity was found for compounds **(1a)**, **(1b)**, **(2)**, **(4a)**, **(5c)** and **(3a)**. The MIC value for compounds **(1a)**, **(2)** and **(5c)** was 62 mg/l but in the other combinations it was 125 mg/l.

Only compound **(5c)** exhibit fungicidal activity at this concentration (other compounds had larger MFC values). The results of studies indicate large activity of N-aryl thioamides **(1a)**, **(1b)** or variable order heteroaryl thioamides **(4a)**, **(5c)**. In these groups there is evident effect of N-ring substituents determining electron effects and lipophilicity (they seem to be optimized in the parametric intervals of the calculated values – log P, or log k'- determined experimentally).

While planning new structures, the compounds prepared from endocyclization of 2-substituted-

arylthioamides like 1,3-benzoxazoles and 3,1-benzotiazines **(3a)**, **(3b)** as well as 2,5-disubstituted 1,3,4-thiadiazoles **(10a)**, **(10b)**, **(11a)**, **(11b)** and **(11c)** seem to be interesting. Similar to 5-sulfonyl analogues Foroumadi[17], the studied group of thiadiazoles exhibit very good and selective fungicidal activity depending on structure.

Therefore further studies aiming at ecotoxicological estimation of this group of compounds taking into account the quantities specified "per os" of $LD_{50}$ doses and high tolerance of regular cells, among others, in the test to hepato- and neurotoxic activities are advisable[18].

Promising predictions concerning activity are confirmed by the results concerning that of the reference preparations from the azole group and amphotericine B (TABLE 1). Growth of mycelium of *Ascosphaera apis* was most strongly inhibited by chloromidazol (125mg/l), amphotericine B (250mg/l) and miconazol (500mg/l). In the case of thioconazole it was the dose 1000mg/l in the substrate and with the other azoles the MIC values indispensable for complete inhibition of mycelium growth were larger than 1000mg/l.

The results of other studies also point to similar sizes of inhibitory doses, as well as significant and increasing resistance of the strains *Ascosphaera apis* against the applied chemiotherapeutic agents[19].

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