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**Short communication**

# Synthesis and mycological activity of the compounds obtained in the reaction of $N^3$-substituted amidrazones with sulphinyl-bis-2,4-dihydroxybenzenethioyl

Bożena Modzelewska-Banachiewicz[a]*, Joanna Matysiak[b], Andrzej Niewiadomy[b]

[a]Department of Organic Chemistry, Faculty of Pharmacy, Medical University, Lublin, Poland
[b]Department of Chemistry, Faculty of Agriculture, Agriculture University, Lublin, Poland

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**Abstract** – 2-Phenyl-5-(2,4-dihydroxybenzene)-1,3,4-thiadiazole (**4**), 2-(2-pyridyl)-2,4-dihydroxybenzene-1,3,4-thiadiazole (**5**), $N^1$-2,4-dihydroxybenzenecarbothio-$N^3$-phenyl-benzamidrazone (**6**) and $N^1$-2,4-dihydroxybenzenecarbothio-$N^3$-phenyl-2-picoline-amidrazone (**7**) were prepared and tested for their antimycotic activity. The chemical structures were confirmed by IR, $^1$H-NMR, EI-MS and elemental analysis. The minimal inhibitory concentration (MIC) values against dermatophytes, yeasts and moulds were determined for the estimation of potential activity *in vitro*. The strongest fungistatic activity for compound **5** in relation to dermatophytes was found with MIC 0.48–0.99 μg mL$^{-1}$. © 2001 Éditions scientifiques et médicales Elsevier SAS

**2,5-diaryl-1,3,4-thiadiazoles / *N*′-aryl-carbothio-$N^3$-aryl-arylamidrazones / antifungal activity / *in vitro* study / MIC**

## 1. Introduction

The derivatives of 1,3,4-thiadiazole show according to the type of substituent different pharmacological activity, e.g. fungistatic, bacteriostatic and so on [1–4]. The compounds containing in their structure 2,4-dihydroxybenzenecarbothioacyl moiety, i.e. thiobenzanilides with the differently modified *N*-aromatic ring [5–9], *N*-heterocyclic derivatives of thiobenzamide [10] show also valuable biological properties. Most of them exhibit antimycotic activity against dermatophytes, moulds, yeasts [7–10] and phytopathogenic fungi [6], some bacteriostatic ones in relation to Gram-positive cells [11]. Especially, strong inhibitory activity against dermatophytes was found with minimal inhibitory concentration (MIC) 1.9 μg mL$^{-1}$ [8]. In the search for new leading structures with 2,4-dihydroxybenzenecarbothioacyl moiety, the synthesis of $N^3$-substituted amidrazones (**1**,**2**) with sulphinyl-bis-2,4-dihydroxybenzenethioyl (**3**) was elaborated. The studies were aimed at possible application of amidrazone as nucleophilic reagents to react with the thioacylating reagent prepared by us. In the case of $N^3$-substituted amidrazones, the $S_E$ proceeds in two directions, resulting in both linear and cyclic structures. The cyclization processes connected with splitting of phenylamine groups (–NHC$_6$H$_5$) facilitate local changes of free-electron density on the atoms *N* and reactions *S* caused by the tautomeric rearrangement [12]. The presence of relatively hydrophilic fragment of β-resorcil bonded in the 1,3,4-thiadiazole and $N^3$-carbothio-substituted amidrazones is expected to cause a non-typical increase in the antifungal activity of the connections. The introduction of balanced structures of appropriate lipophilicity leads to the hydrophilic–hydrophobic equilibrium and facilitates the penetration of the molecules through the cell membranes.

This paper presents preparation and biological activity of 2-phenyl-5-(2,4-dihydroxybenzene)-1,3,4-thiadiazole (**4**), 2-(2-pyridyl)-2,4-dihydroxybenzene-1,3,4-thiadiazole (**5**), $N^1$-2,4-dihydroxybenzenecarbothio-$N^3$-phenyl-benzamidrazone (**6**) and $N^1$-2,4-dihydroxybenzenecarbothio-$N^3$-phenyl-2-picolineamidrazone (**7**) obtained in the above reaction. For the

* Correspondence and reprints.
*E-mail address:* jmaty@agros.ar.lublin.pl (B. Modzelewska-Banachiewicz).

estimation of potential activity *in vitro*, the MIC values against four strains of moulds, five yeasts and six dermatophytes were determined.

## 2. Chemistry

The synthetic pathway for the compounds described is illustrated in *figure 1*. Sulphinyl-bis-2,4-dihydroxybenzenethioyl and $N^3$-substituted amidrazones, as the starting materials, were prepared by patent pending and according to Modzelewska and Pyra (1995–1996) [13] and Spassow et al. (1995) [14], respectively. The obtained precipitate in this reaction was filtered and crystallized from methanol, giving compounds **4** and **5**. The fraction of the precipitate insoluble in methanol was crystallized from isopropanol and, in this way, compounds **6** and **7** were obtained. Physico-chemical data were in agreement with the proposed structures.

The chemical structures of compounds **4–7** were confirmed by IR, $^1$H-NMR, EI-MS spectral data and elemental analysis. In $^1$H-NMR spectra of compounds **4** and **5**, apart from the signals of aromatic hydrogen atoms (6.4–8.7 ppm), the single absorption band localized in the region of 11.2 ppm and corresponding to OH groups was observed. In $^1$H-NMR spectra of compounds **6** and **7**, apart from the signals corresponding to aromatic protons (6.4–8.7 ppm), there appear also the characteristic absorption bands of NH groups (10.0–10.4) and a single absorption band of 11.1–11.2 ppm corresponding to OH groups. More chemical and physical data about compounds **4–7** are given in *table I*.

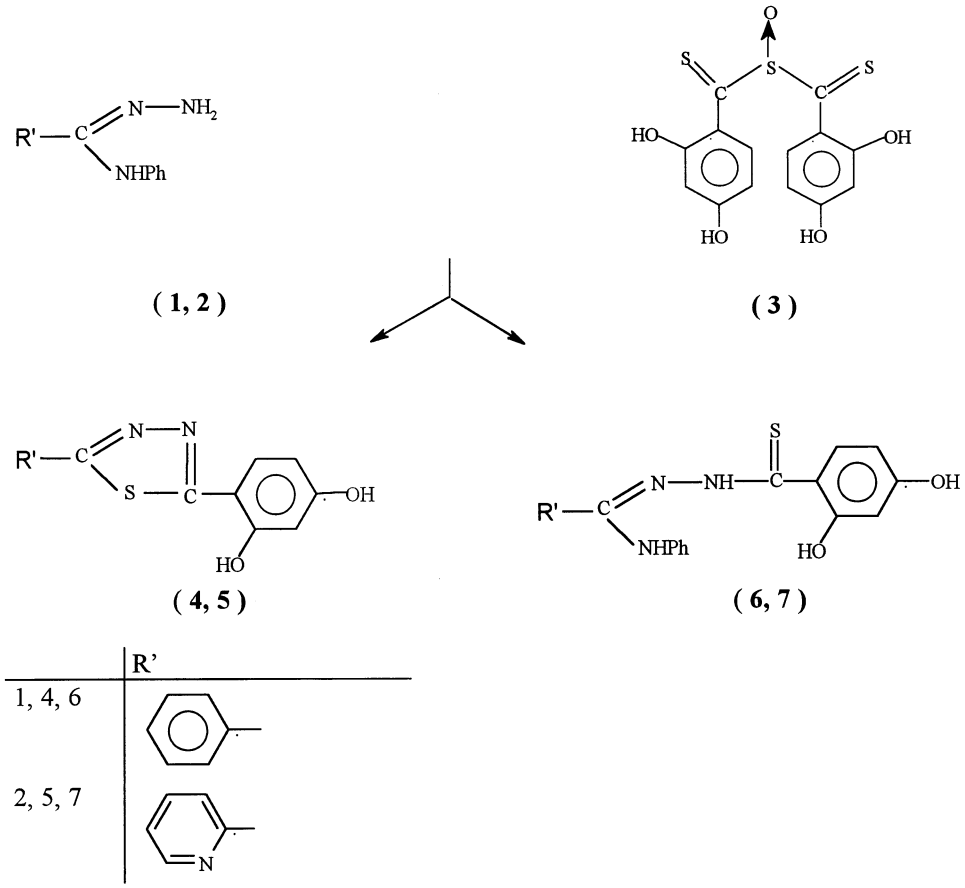


**Figure 1.** Synthetic pathway.

**Table I.** Physicochemical and spectral properties of compounds **4–7**.

| Compound | $R^1$ | Formula m.w. | m p. (°C) | Yield (%) | IR ($cm^{-1}$) | $^1$H-NMR (ppm, TMS), MS *m/e* (%) |
|---|---|---|---|---|---|---|
| **4** | $C_6H_5$ | $C_{14}H_{10}N_2O_2S$ 270.1 | 240–2 | 40 | 3060 OH; 1629 arom.; 1600 C=N; 1180 OH | 6.0–8.1 (m.8H arom.) 11.2 (s. 2H, 2OH) 270 (100.0 $M^+$, 194 (5.3) 193 (4.8), 180 (7.8), 167 (46.0), 135 (39.8), 121 (13.7), 91 (10.4), 77 (33.5) |
| **5** | 2-$C_5H_4N$ | $C_{13}H_9N_3O_2S$ 271.1 | 234–6 | 40 | 3020 OH; 1631 arom.; 1605 C=N; 1170 OH | 6.4–8.7 (m.7H arom.) 11.2 (s. 2H, 2OH) 271 (2.6 $M^+$), 260 (5.7) 258 (35.3), 256 (100.0), 238 (32.0), 192 (32.6), 162 (10.3), 128 (56.6), 105 (24.4), 78 (16.1), 77 (9.8) |
| **6** | $C_6H_5$ | $C_{20}H_{17}N_3O_2S$ 363.2 | 280–2 | 30 | 3338 NH; 3064 OH; 1627 arom.; 1591 C=N; 1274 C=S; 1174 OH | 6.0–7.4 (m.13H arom.) 10.0 (s. 1H, NH), 10.4 (s. 1H, NH), 11.1 (s. 2H, 2OH 361 (4.7 $M^+$, 330 (22.6) 329 (100.0), 328 (84.6), 297 (48.6), 296 (32.9), 194 (12.2), 180 (16.6), 91 (15.3), 77 (35.8) |
| **7** | 2-$C_5H_4N$ | $C_{19}H_{16}N_4O_2S$ 364.2 | 240–2 | 30 | 3320 NH; 3067 OH; 1627 arom.; 1580 C=N; 1243 C=S; 1170 OH | 6.4–8.7 (m.12H arom.) 10.1 (s. 2H, 2NH), 11.2 (s. 2H, 2OH) |

**Table II.** Comparison of activities of compounds 4–7 expressed by MIC and standards — batrafen and ketoconazole.

| | Strain | MIC μg $mL^{-1}$ | | | | 4 | | 5 | | 6 | | 7 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **4** | **5** | **6** | **7** | $X^{KETa}$ | $X^{BATb}$ | $X^{KET}$ | $X^{BAT}$ | $X^{KET}$ | $X^{BAT}$ | $X^{KET}$ | $X^{BAT}$ |
| **1** | *Penicillium* sp. | 31.25 | 7.8 | >62.5 | 15.6 | 2 | 1 | 0.5 | 0.25 | >8 | >4 | 1 | 0.5 |
| 2 | *Aspergillus fumigatus* | 15.6 | 7.8 | 62.5 | 15.6 | 0.25 | 0.5 | 0.125 | 0.25 | 1 | 2 | 0.25 | 0.5 |
| 3 | *Aspergillus niger* | 15.6 | 15.6 | 62.5 | 15.6 | 0.25 | 0.5 | 0.25 | 0.5 | 1 | 2 | 0.25 | 0.5 |
| 4 | *Scopulariopsis brevicaulis* | 31.25 | 15.6 | 62.5 | 15.6 | 1 | 1 | 0.5 | 0.5 | 2 | 2 | 0.5 | 0.5 |
| 5 | *Candida albicans* | 15.6 | 15.6 | 31.25 | 31.25 | 0.25 | 1 | 0.25 | 1 | 0.5 | 2 | 0.5 | 2 |
| 6 | *Candida albicans* ATCC 10231 | 31.25 | 15.6 | 31.25 | 31.25 | 0.5 | 2 | 0.25 | 1 | 0.5 | 2 | 0.5 | 2 |
| 7 | *Cryptococcus neoformans* | 15.6 | 7.8 | 31.25 | 15.6 | 4 | 1 | 2 | 0.5 | 8 | 2 | 4 | 1 |
| 8 | *Geotricum candidum* | 31.25 | 15.6 | 31.25 | 15.6 | 8 | 1 | 4 | 0.5 | 8 | 1 | 4 | 0.5 |
| 9 | *Trichosporon* sp. | 31.25 | 7.8 | 31.25 | 15.6 | 8 | 2 | 2 | 0.5 | 8 | 2 | 4 | 1 |
| 10 | *Epidermophyton floccosum* | 1.98 | 0.99 | 3.9 | 1.98 | 660 | 0.127 | 330 | 0.063 | 1300 | 0.25 | 660 | 0.127 |
| 11 | *Microsporum gypseum* | 3.9 | 1.98 | 3.9 | 1.98 | 32.5 | 0.123 | 16.5 | 0.063 | 32.5 | 0.125 | 16.5 | 0.063 |
| 12 | *Trichophyton interdigitale* | 1.98 | 0.99 | 3.9 | 1.98 | 8.25 | 0.063 | 4.125 | 0.032 | 16.25 | 0.125 | 8.25 | 0.063 |
| 13 | *Trichophyton galline* | 1.98 | 0.99 | 3.9 | 1.98 | 4.125 | 0.125 | 2 | 0.063 | 8.125 | 0.25 | 4.125 | 0.125 |
| 14 | *Trichophyton mentagrophytes* | 3.9 | 0.99 | 3.9 | 1.98 | 32.5 | 0.125 | 8.25 | 0.032 | 32.5 | 0.125 | 16.5 | 0.063 |
| 15 | *Trichophyton rubrum* | 3.9 | 0.48 | 3.9 | 1.98 | 8.125 | 0.125 | 1 | 0.015 | 8.125 | 0.125 | 4.125 | 0.063 |

[a] $X^{BAT} = MIC_{compound}/MIC_{batrafen}$
[b] $X^{KET} = MIC_{compound}/MIC_{ketoconazole}$

## 3. Pharmacology

*In vitro* activities of compounds obtained against potentially pathogenic fungi were compared. The series of four strains of moulds, five yeasts and six dermatophytes were tested. All strains under study were clinical isolates, identified with conventional morphological and biochemical methods. The broth dilution method for estimation of MIC values (MIC caused full inhibition of growth) was applied to evaluate the antimycotic activity. The antifungal potency of the compounds under conditions was compared with the activities of two common topical antifungal drugs, batrafen and ketoconazole.

## 4. Results and discussion

The results of *in vitro* screening compounds obtained are summarized in *table II*. The MIC values against dermatophytes are ranging from 3.9 to 0.48 μg $mL^{-1}$, against yeasts 31.25–7.8 μg $mL^{-1}$ and moulds 62.5–7.8 μg $mL^{-1}$. Assuming 15–25 μg $mL^{-1}$ as the conventionally determined breakpoint for susceptibility to topical antifungal agents [15, 16], compound **5** inhibits growth of all strains below these concentrations. However, all compounds exhibit much stronger fungistatic activity against dermatophytes. This indicates significantly greater sensitivity of dermatophytes to the tested compounds compared with other studied fungi. Similar tendencies were observed while studying fungistatic activity of 2,4-dihydroxythiobenzanilides [8, 9]. From the analysis of the structure of compounds studied, it can be stated that the cyclic systems, i.e. compounds **4** and **5**, are characterized by higher activity compared with linear compounds **6** and **7**. The presence of 2-pirydyl moiety compared with phenyl has also a positive effect on fungistatic activity. Therefore, compound **5** exhibits the strongest inhibitory activity, i.e. 0.48 μg $mL^{-1} \leq$ MIC $\leq$ 15.6 μg $mL^{-1}$.

As biological tests are relative measures, batrafen and ketoconazole were used as reference system. MIC values of these antimycotic drugs are compared with MIC values of the compounds studied, i.e. $X = \mathrm{MIC}_{\mathrm{compound}}/\mathrm{MIC}_{\mathrm{Batrafen\ or\ Ketoconazole}}$ (*table II*). It can be generally stated that the activity of compounds against moulds and yeasts can be compared with the standards, but significant differences in activity are observed against dermatophytes. Compounds **5** and **7** with 2-pyridyl moiety show two or four times higher activity than the standards with regard to moulds. However, substances **4** and **6** exhibit weaker inhibitory activity. The compounds studied exhibit higher activity against *Candida* compared with ketoconazole, but lower compared with batrafenem. The standards are insignificantly more effective against other strains of yeasts except compound **5** compared with batrafen. The obtained compounds are characterized by stronger inhibitory activity against dermatophytes than batrafen, but much weaker than ketoconazole.

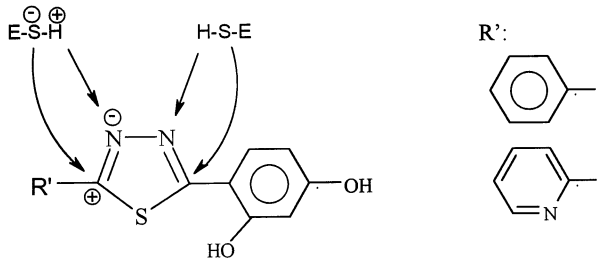


**Figure 2.** Mechanism of enzymatic thiols reaction with 1,3,4-thiadiazoles.

Higher activity of cyclic connections can confirm greater probability of their penetration than of any boundary ionic forms of the linear structure, though specific interactions of another pharmacophoric system in the 1,3,4-thiadiazole form should be taken into account. In linear structures **6** and **7**, the influence of thiocarbonyl groups seems to be more significant than of the amidrazone groups, which exhibit poor antimicrobial activity due to their nature [17, 18]. However, the reactions of elimination and the structural arrangement change in compounds **4** and **5** lead to formation of 2,5-diaryl-substituted derivatives of 1,3,4-thiadiazoles and pharmacophore of another type. The cyclization mechanism in the synthesis of connections leads to the formation of the π-deficit ring with the –C=N– bond system. Their coupling with the sulfur atom and mesomeric stabilization strengthen the electron gaps on carbon atoms and the tendency to the addition reaction. Multidirectional antimicrobial and nematocidal activities resulting in enzymatic thiol blocking are observed for the substituted thiadiazoles [19]. The presented mechanism seems to be justified and, in this case, it can correspond to the addition reaction depending kinetically on the size of local charge on nitrogen atoms and spherical effects (*figure 2*).

With a relatively large difference of standard hydrophobicity parameters of aryl fragments [20], a positive effect of 2-pyridyl substituent should be considered, based on an inductive increase of charges on atoms of the corresponding pharmacophores, i.e. on the interaction forces with enzymatic thiols. Its advantageous effects can be observed for both linear and cyclic combinations. Though pyridine is a benzene azaanalogue, the presence of both types of electrons (δ and π) in the heteroatom ring of the acceptor character causes a relative increase of activity of compounds **5** and **7**.

Taking into account the strong inhibitory activity, particularly against dermatophytes, and a wide spectrum of biological activity of the obtained compounds, the research in this field will be continued. This refers to both modification of the presented structures and synthesis of the analogous systems.

## 5. Experimental protocols

### 5.1. Chemistry

Melting-point measures on a Boetius apparatus are given uncorrected. $^1$H-NMR spectra were recorded on Tesla BS 567A (100 MHz) apparatus in $D_6$-DMSO with TMS as an external standard. IR spectra were recorded as KBr disks with a Specord IR-74 spectrophotometer. MS fragmentation was recorded as EI MS (15 eV) on AMD apparatus. Chemicals were purchased from Merck Co. or Fluca Ltd. and used without further purification. The results of elemental analysis for C, H, and N by the microanalysis method, obtained in the Department of Organic Chemistry, Medical University in Lublin, were acceptable in accordance with the calculated values (0.7% for C, 1.0% for N, and 1.2% for H).

#### 5.1.1. 2-Phenyl-5-(2,4-dihydroxybenzene)-1,3,4-thiadiazole (**4**, **5**)

Amidrazone (0.1 mol) (**1**, **2**) was dissolved in 50 cm$^3$ of methanol and then 0.005 mol of sulphinyl-bis-2,4-dihydroxybenzenethioyl (**3**) was added. The mixture was heated in the flask under reflux on water bath (2 h). Then, the mixture was hot-filtered and the separated solution was kept for 24 h. The precipitate obtained was filtered off and purified by crystallization from methanol.

#### 5.1.2. $N^1$-2,4-dihydroxybenzenecarbothio-$N^3$-phenylacylamidrazone (**6**, **7**)

The insoluble precipitate in methanol (obtained according to the above procedure) was purified by crystallization from isopropanol. The yields and physical data of compounds **4–7** are given in *table I*.

### 5.2. Microbiology

Using the dilution method, the MIC of individual compounds against four strains of moulds, five of yeasts and six of dermatophytes has been determined. These were either reference strains of known sensitivity to antifungal drugs or the strains isolated directly from the clinical material. Microorganisms 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 agar medium accurately measured, dissolved and cooled to 45°C, and then mixed and emptied onto Petri plates. The medium of more and more decreasing concentration, ranging from 1000 to 0.003 μg mL$^{-1}$, was obtained. The medium containing 0.5 mL of the substance had also 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 activity of ketoconazole and batrafen against all fungi was also estimated. The presented results were obtained from three independent measurements. The investigations were carried out in the Department of Pharmaceutical Microbiology, Medical University, Lublin.

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