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Fungicidal evaluation of substituted 4-(1,3,4-thiadiazol-2-yl) benzene-1,3-diols

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Abstract: Fungicidal properties of 4-(1,3,4-thiadiazol-2-yl)benzene-1,3-diols set under *in vitro* conditions against five phytopathogenic fungi have been evaluated. The substitution panel includes amino-, alkyl-, alkoxyl-, aryl- and heteroarylderivatives. The most active compound with the benzyl substituent exhibits fungistatic effects amounting to 90-100% with the concentration of 20 μg mL^{-1} against *R. solani,* similar to the standard fungicides. The derivatives with amine moiety generally display lower activity than other analogues. *F. culmorum* seems to be the most refractory fungus compared to studied compounds. The influence of substitution of C-5 at the constant fragment at C-2 of 1,3,4-thiadiazole ring on the antifungal effect is discussed. To explain differences in the activity the quantum-chemical calculations were made.

Keywords: 4-(1,3,4-thiadiazol-2-yl)benzene-1,3-diols, fungicidal activity, *in vitro* studies, structure-activity

INTRODUCTION

Fungicides commonly used in agriculture against phytapathogenic filamentous fungi, include the compounds characterized by widely diverse chemical structures and functional groups [1, 2]. The compounds with the heterocyclic ring constitute an important group. As fungicides differently substituted five- or six-membered hetrocycles (also fused with the homoaromatic ring) with one or some heteroatoms are used. These include pyridine, pyrimidine, piperazine, piperidine, morpholine, azoles and others [3]. Particularly the derivatives of azoles and benzimidazole exhibit useful properties in plant protection. However, the intensive use of these

compounds leads to development of resistance in this field [4]. Therefore various structural modifications of heterocycles based on the structure-activity studies are undertaken to obtain compounds of higher efficacy [5-7].

Among azoles 1,3,4-thiadiazoles are an interesting group of fungistatic compounds [8, 9]. Vikani reported *p*,*p'*-bis(2-substituted-benzalamino/benzoylamino/sulphonamido-1,3,4-thiadiazol-5-yl-methylamino)diphenyl sulphones displaying good activity against *A. niger* [10]. Other sulphone derivatives containing a trimethoxyphenyl substituent were synthesized and evaluated for antifungal activities against *G. zeae* and *B. cinerea* [11]. 2-(3,4,5-Trimethoxyphenyl)-5-(substitutedsulfinyl)-1,3,4-thiadiazoles act against *G. zeae*, *F. oxysporun*, *C. mandshurica* [12], 2-(substituted thio)-5-(2,4-dinitrophenylthio)-1,3,4-thiadiazoles against *V. inaequalis, B. cinerea, F. bulbigenum* and *C. melonis* [13]. For 2-(4-chloro-3-ethyl-1-methyl-1H-pyrazol-5-yl)-5-(alkylthio)-1,3,4-thiadiazoles and 6-methyl-1-phenyl-3-(5-(phenylamino)-1,3,4-thiadiazol-2-yl)pyridazin-4(1H)-ones *in vivo* antifungal properties against *R. solani* and *P. recondita* were also confirmed [14, 15].

In our previous papers, many heterocyclic compounds containing 2,4-dihydroxyphenyl moiety were reported with good fungicidal activities [16, 17]. To extend our research in this field we have studied a series of benzenodiols with 1,3,4-thiadiazole functionality.

The paper presents *in vitro* fungicidal properties of some compounds from 5-substituted (1,3,4-thiadiazol-2-yl)benzene-1,3-diols against five phytopathogenic fungi. The effect of substitution of C-5 at the constant fragment at C-2 of the thiadiazole ring on antifungal activity is discussed. To explain differences in the activity receptor quantum-chemical calculations were made with the *ab initio* Hartree-Fock model.

MATERIALS AND METHODS

The compounds were obtained according to the previously described synthesis method from 4-substituted 3-thiosemicarbazides or hydrazides and sulfinylbis(2,4-dihydroxythiobenzoyl) [18-21].

The test *in vitro* estimating inhibition of mycelium in the agar culture medium caused by the compound under investigation was performed. The five strains of phytopathogenic fungi: *Alternaria alternata, Botrytis cinerea, Rhizoctonia solani, Fusarium culmorum,* and *Phytophthora cactorum* were used. The solutions (suspensions) were prepared with the concentration making it possible to obtain 200 and 20 μg mL^{-1} of the studied substance after dilution with the

agar culture medium (PDA). There were used Petri scale pans into which the agar culture medium and the studied substance were poured. When the culture medium sets, the infectious material of the tested fungus in the form of agar disc overgrown with mycelium is placed at three sites of its surface. After 3-5 days (temperature 22 (±1)°C) depending on the mycelium culture, the linear growth of the mycelium is measured. The compound action was determined from the percentage of mycelium growth inhibition compared with the control using the equation:

$$J = \frac{(C - T)}{C} 100\%,$$

where:
J – percentage of mycelium growth inhibition; C – zone of mycelium growth in the control combination (mm); T – zone of the mycelium growth in the combination with the compound (mm).

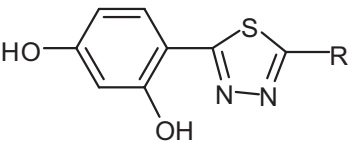
As standards the following fungicides tested under the same experimental conditions were used: carbendazime (Sarfun 500 SC, Organica, Chemical Comp. S.A., Nowa Sarzyna, Poland); prochloraz (Mirage 450 EC, Makhteshim Agan Industries Ltd Israel); procymidone (Sumilex 500 SC, Sumitomo Chemical Comp. Ltd., Japan). The results are given in the four-degree scale determining the percentage of mycelium growth inhibition compared with the control (Table 1). Biological studies were carried out in the Institute of Industrial Organic Chemistry in Warsaw with the SPR/BFF/01/b procedures (certificate GLP-OECD – 1997).

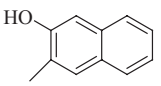
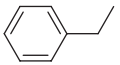
Quantum-chemical calculations were performed using the PC SPARATN Pro Ver 1.08 molecular modelling program [22]. The compounds were built with a standard bond length and angles. The energy was minimised by the molecular mechanics methods (SYBYL) and then by the Hartree-Fock method at 6-31G**. Charges of atoms from the Mullicane population and potential distribution were determined.

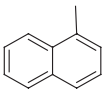
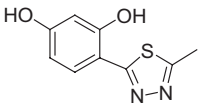
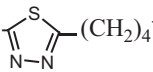
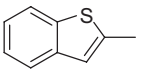
RESULTS AND DISCUSSION

The structures of compounds and the results of *in vitro* screening against five strains of the phytopathogenic fungi (*A. alternata*, *B. cinerea*, *R. solani*, *F. culmorum*, and *P. cactorum*) are given in Tables 1 and 2. Activity is expressed as relative inhibition of growth (%) on the four-degree scale at two different concentrations (200 and 20 μg mL^{-1}). Some fungicides studied under the same conditions were used as reference systems (Table 1).

Table 1. Growth-inhibitory activity of 5-substituted 4-(1,3,4-thiadiazol-2-yl)benzene-1,3-diols according to a 4-point scale[a] at two different compound concentrations (200 and 20 μg mL^{-1})



No.	Substituent R-	Estimation of mycelium growth inhibition					
		Concentr. [μg mL^{-1}]	*A. alternata*	*B. cinerea*	*F. culmorum*	*P. cactorum*	*R. solani*
1a.	CH_3-	200	2	3	1	2	3
		20	1	1	1	1	2
2a.	$CH_3(CH_2)_{14}$-	200	1	1	0	1	1
3a.	CH_3O-	200	1	1	1	1	2
4a.	$HO(CH_2)_3$-	200	0	0	0	0	0
5a.	$CH_3CH_2OC(=O)CH_2$-	200	2	1	1	2	2
6a.	4-CH_3-C_6H_4-	200	1	0	0	1	1
7a.	4-$(CH_3)_3C$-C_6H_4-	200	0	0	0	1	0
8a.	2-Br-C_6H_4-	200	1	1	0	1	1
9a.	4-C_2H_5O-C_6H_4-	200	3	3	3	3	3
		20	1	2	0	2	1
10a.	3-HO-C_6H_4-	200	3	3	3	3	3
		20	1	1	0	1	1
11a.	2,4-di-HO-C_6H_3-	200	0	0	0	0	0
12a.	3-CH_3-2-HO-C_6H_3-	200	1	1	0	1	1
13a.		200	0	0	0	0	1
14a.		200	- *b*	3	- *b*	- *b*	3
		20	- *b*	2	- *b*	- *b*	3
15a.	4-F-C_6H_4-OCH_2-	200	2	2	1	2	2
16a.	4-Cl-C_6H_4-OCH_2-	200	0	1	0	1	1
17a.	2,4-di-Cl-C_6H_3-OCH_2-	200	0	0	0	1	1
18a.	4-Cl-2-CH_3-C_6H_3-OCH_2-	200	0	1	0	1	0
19a.	4-NO_2-C_6H_4-CH_2O-	200	1	0	0	1	1

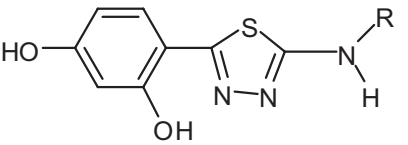
20a.	C_6H_5-CH(OH)-	200	0	1	0	1	1
21a.		200	2	1	0	0	2
22a.		200	2	2	1	2	2
23a.	2,4-di-HO-C_6H_3  $(CH_2)_4$-	200	0	0	0	0	1
24a.		200	3	2	0	0	2
25a.		200	2	2	1	2	2
26a.		200	- [b]	- [b]	3	3	- [b]
		20	- [b]	- [b]	1	1	- [b]
27.	prochloraz	200	3	3	3	- [b]	- [b]
		20	3	3	3	- [b]	- [b]
28.	carbendazym	200	- [b]	- [b]	- [b]	3	3
		20	- [b]	- [b]	- [b]	3	3
29.	procymidon	200	2	3	- [b]	- [b]	- [b]
		20	2	3	- [b]	- [b]	- [b]

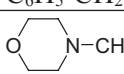
a The results are given in the four-degree scale determining the percentage of mycelium growth inhibition compared with the control: 0: < 20% lack of action, 1: 20-50% poor action, 2: 50-90% medium action, 3: > 90% good action.

- [b] Test was not performed.

The results in Tables 1 and 2 show that antifungal properties of the compounds under consideration are varied. In the laboratory studies at the concentration of 200 μg mL^{-1}, compounds 9a and 10a revealed significant fungistatic action (amounting to 91-100%) against all bioindicators. Compounds 1a, 14a and 26a show strong antifungal effects against two fungi only (Table 1). Compounds 15a, 22a and 25a inhibit development of four pathogens but on a slightly lower level (50-90%). However, the fungistatic action of substance 24a is selective, quite differentiated and the highest against *A. alternata.* Relatively high activity of compounds 22a and 24a-26a can be associated with their biheterocyclic structure. An additional heterocyclic ring may promote antifungal effect.

Table 2. Structure and inhibitory activity of N-substituted 4-(5-amino-1,3,4-thiadiazol-2-yl)benzene-1,3-diols according to a 4-point scale[a] at the compound concentrations of 200 μg mL^{-1}



No.	Substituent R-	Estimation of mycelium growth inhibition				
		A. alternata	*B. cinerea*	*F. culmorum*	*P. cactorum*	*R. solani*
1b.	H-	1	1	0	1	1
2b.	$(CH_3)_3C$-	0	1	0	1	0
3b.	CH_2=$CHCH_2$-	2	2	1	2	2
4b.	cyclohexyl-	2	2	2	2	2
5b.	2,6-di-CH_3-C_6H_3-	2	2	1	2	2
6b.	4-C_2H_5-C_6H_4-	1	1	0	1	0
7b.	2-F-C_6H_4-	2	2	1	2	1
8b.	3-F-C_6H_4-	1	0	0	0	0
9b.	2-Cl-C_6H_4-	2	2	1	2	2
10b.	3-Cl-C_6H_4-	1	1	1	0	0
11b.	2,4-di-Cl-C_6H_3-	2	2	1	2	2
12b.	2,5-di-Cl-C_6H_3-	1	0	0	1	1
13b.	2,6-di-Cl-C_6H_3-	2	2	0	2	2
14b.	2-CH_3-5-Cl-C_6H_3-	1	1	0	1	1
15b.	4-CH_3O-C_6H_4-	1	0	0	1	1
16b.	4-$C_2H_5OC(=O)$-C_6H_4-	0	0	0	0	1
17b.	4-C_6H_5-O-C_6H_4-	2	1	1	2	2
18b.	C_6H_5-CH_2-	1	1	0	1	2
19b.		2	2	1	2	2

[a] – see Table 1 ([a])

With the compounds at 20 μg mL^{-1} concentration only compound 14a with the benzyl substituent inhibits growth of *R. solani* on the highest level and *B. cinerea*

on the level 50-60%. Interesting activity is also exhibited by compound 9a (4-ethoxyphenyl derivative) at this concentration. For the other derivatives at the concentration of 20 μg mL^{-1} under the *in vitro* conditions, poor or no fungistatic activity was observed (Table 1). The most active compound 14a is characterized by the activity similar to carbendazym applied as the reference substance and a bit lower then that of prochloraz and procymidon (Table 1). *F. culmorum* seems to be the most refractory fungus compared to the evaluated 1,3,4-thiadiazole derivatives.

In the case of the compounds of structure b with the amine moiety the growth-inhibitory effect is slightly weaker. Only one derivative with the cyclohexyl substituent (2b) shows antifungal activity against all pathogens amounting to 50-90% at the concentration of 200 μg mL^{-1}. Compounds 5b, 9b, 11b, 13b and 19b inhibit growth of four fungi on the same level.

Comparing general activity of amine derivatives (compounds of structure b) with their corresponding structural analogs without the amine group (compounds of structure a) considerably larger efficacy of combinations without -NH- group was found. Particularly it is clear in the case of benzyl derivatives 14a and 18b. This can be associated with lower lipophilicity of compounds with the hydrophilic -NH- group and the electron properties which influence on character of molecule.

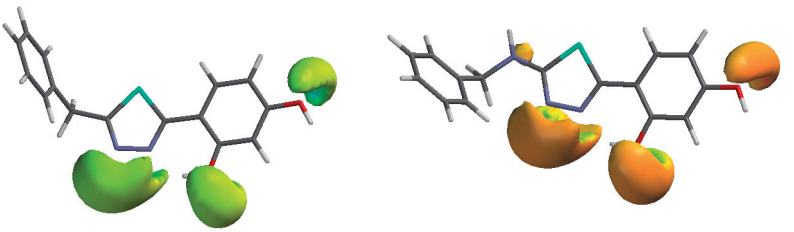
To compare the structure of the most active compound 14a with its analogue without the amine group 18b quantum-chemical calculations were performed with the *ab initio* Hartree-Fock model at the 6-31G** basis set. The determined values of HF energy (E) of isolate molecules, their surface area, volume, solvatation energy (E_S) and electronic properties are presented in Table 3. As follows from Table 3 differences in the activity can be caused by various partial atomic charges (q) on carbon atom of 1,3,4-thiadiazole ring calculated by the potential distribution (q_p) as well as from the Mulliken population analysis (Mulliken charges, q_M). A large electron gap on C-5 compared to C-2 of 1,3,4-thiadiazole ring for the amine derivative appears (Table 3).

Table 3. Structural, electronic and thermodynamic properties of 14a and 18b

No.	E [kJ/mol]	volume [Å^3]	area [Å^2]	E_{Lumo} [eV]	E_{Homo} [eV]	E_S [kJ/mol]	$q_{P(C-5)}$ [e]	$q_{P(C-2)}$ [e]	$q_{M(C-5)}$ [e]	$q_{M(C-2)}$ [e]	μ [D]
14a	-3231787.3	299.87	310.62	2.342	-7.921	-17.23	0.47	0.32	0.07	0.16	2.71
18b	-3376230.9	314.49	323.44	2.661	-8.186	-19.63	0.70	0.33	0.40	0.15	3.04

Comparing the molecular potential density distribution of both compounds (Figure 1), it results that the most negative potential is located on nitrogen atoms of the thiadiazole ring and on oxygen atom of hydroxyl groups. In the case of

the amine derivative it also involves amine nitrogen atom. There are significant differences in the Lumo energy (energy of the lowest unoccupied molecular orbital) (Table 3) and its orbital distribution (Figure 2). Lumo involves the amine group of compound 18b or carbon atom of N-aryl ring of 14a and indicates the region reacting with potential biological nucleophiles. Some differences in the Homo orbital (highest occupied molecular orbital) distribution are also observed (Figure 2). It is located additionally on nitrogen atom of amine moiety in the case of 18b and represents the space of the greatest electron density [23].



14a **18b**

Figure 1. The electrostatic potential profile at –20 kcal/mol of compounds 14a and 18b. The structures are shown with a tube rendering.

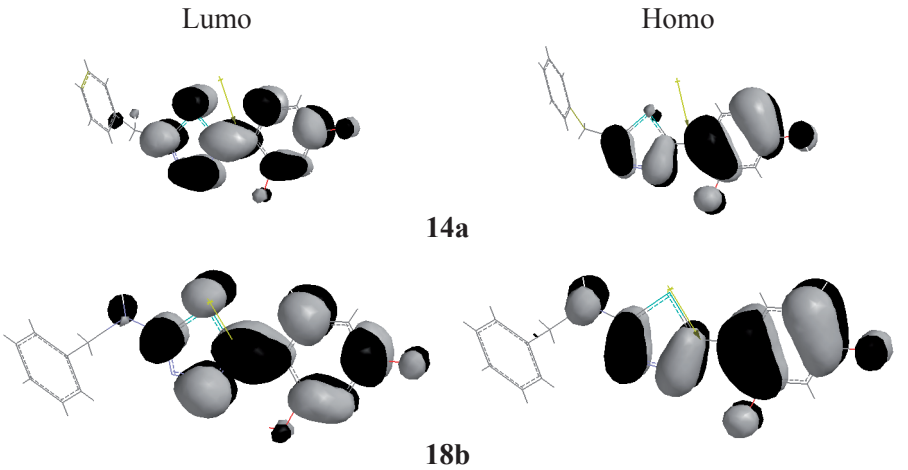


Figure 2. Lumo and Homo isosurfaces for the 14a and 18b. Different surface colours represent opposite signs of the wavefunction.

CONCLUSION

The compounds under discussion exhibit interesting extent of fungicidal activity against phytopathogenic fungi. Some of them are more efficient than these reported in the literature [11, 12]. The structure-activity studies show that amine derivatives are less active than their analogues without -NH- moiety. The presence of -NH- group in the molecule causes decrease of their lipophilicity and gives the possibility to create hydrogen bonds. The amine group also changes distribution of charge in 1,3,4-thiadiazole ring and dipole moment of molecule. Probably the changes caused by -NH- moiety reduce antifungal effect of compounds against pathogens. The most active compound with the benzyl substituent shows fungicidal effect similar to that of commonly applied fungicides. The compounds of different group studied previously with 2,4-dihydroxythiobenzoyl moiety also showed strong antifungal effect. So, the activity of these compounds may be promoted by the 2,4-dihydroxypheny substituent. It encourages us to carry out further research in this series of analogues, including the *in vivo* studies and toxicological tests.

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