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Thiazole-based chalcones as potent antimicrobial agents. Synthesis and biological evaluation

K. Liaras [a], A. Geronikaki [a,*], J. Glamočlija [b], A. Ćirić [b], M. Soković [b]

[a] Aristotle University, School of Pharmacy, Department of Pharmaceutical Chemistry of Aristotle University of Thessaloniki, Thessaloniki 54124, Greece
[b] Institute for biological research 'S.Stanković', Mycological laboratory, University of Belgrade, 11000, Serbia

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ABSTRACT

As part of ongoing studies in developing new antimicrobials, we report the synthesis of a new class of structurally novel derivatives, that incorporate two known bioactive structures a thiazole and chalcone, to yield a class of compounds with interesting antimicrobial properties.

Evaluation of antibacterial activity showed that almost all the compounds exhibited greater activity than reference drugs and thus could be promising novel drug candidates.

1. Introduction

Despite the rapid progress of science, the treatment of infectious diseases still remains a serious problem of concern to the scientific community, mainly because of the wide range of factors leading to the emergence of these diseases and also the increased number of pathogenic microorganisms with resistance to multiple drugs, including the Gram positive bacteria.[1–5]

To limit the emerging resistance of microorganisms requires the careful use of existing antimicrobial drugs. However, there is a need for the design of novel antimicrobial agents, particularly for the treatment of the infections of hospitalized patients and protection of immunosuppressed or HIV-infected patients.

A potential approach is the design of innovative drugs, with different mechanism of action, in effort to avoid cross resistance to existing therapeuticals.[6] Recently, linezolid,[7–9] a synthetic molecule containing oxazolidinone moiety has been approved for clinical use. It is effective against Gram positive bacteria while appearing to have a unique mechanism of action.

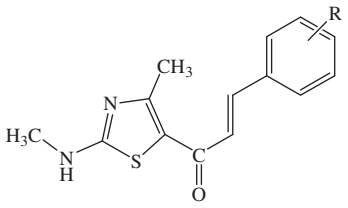
Our design supported from our previous findings[10–15] addressed the synthesis of a new class of structurally novel derivatives, that incorporate two known bioactive structures a thiazole[16–26] and a chalcone,[27–29] to yield a class of compounds with interesting antimicrobial properties

Ten new thiazole-based chalcones (Fig. 1) were synthesized and tested for their in vitro antimicrobial properties against Gram positive and Gram negative bacteria and also against a series of fungi.

2. Results and discussion

2.1. Chemistry

The synthesis of the target chalcones **1–10** was accomplished by a Claisen–Schmidt condensation of 1-(4-methyl-2-(methylamino) thiazol-5-yl)ethanone with various aromatic aldehydes, in methanol, and 10% aq NaOH,[24] as indicated to Scheme 1. The reactions



1.	H	6.	2-Cl
2.	4-NO_2	7.	4-OMe
3.	3-NO_2	8.	2-OMe
4.	4-Cl	9.	2,6-diCl
5.	3-Cl	10.	2,4-diCl

Figure 1. Structure of the synthesized compounds.

* Corresponding author. Tel.: +30 2310 997616; fax: +30 2310 998559.
E-mail address: geronik@pharm.auth.gr (A. Geronikaki).

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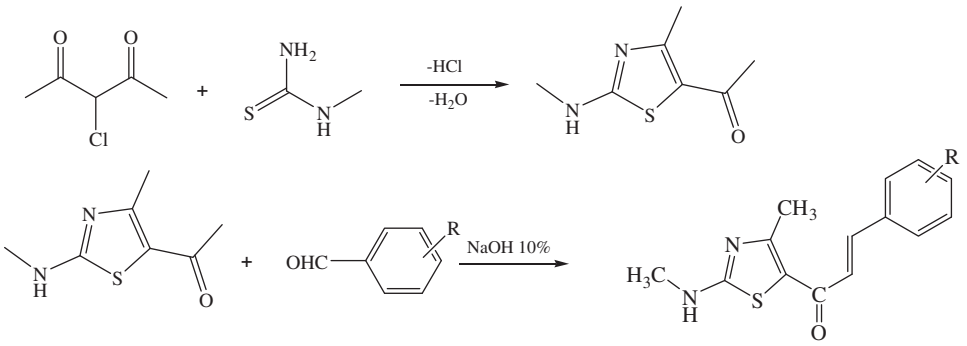
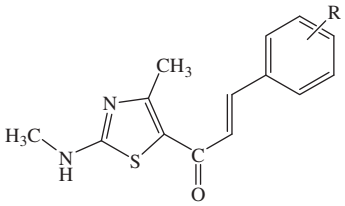


Scheme 1. Synthesis of title compounds.

Table 1
Calculated lipophilicity of synthesized compounds expressed as *C* log *P*, *A* Log *Ps* και *A* Log *P*



A/A	*C* log *P*[a]	*C* log *P*[b]	*A* Log *Ps*[c]	*A* Log *P*[d]
1	3.43	2.97	3.41	3.08
2	3.17	2.91	3.02	2.98
3	3.17	2.91	3.15	2.98
4	4.14	3.57	3.87	3.75
5	4.14	3.57	3.80	3.75
6	4.14	3.57	3.80	3.75
7	3.57	3.11	3.46	3.29
8	3.57	3.11	3.46	3.29
9	4.29	3.74	3.96	3.83
10	3.93	3.48	3.71	3.57
11	3.35	2.81	3.49	3.07
12	3.35	2.81	3.43	3.07
13	4.73	4.18	4.05	4.41
14	4.85	4.18	4.04	4.41
15	4.85	4.18	3.93	4.41

[a] Calculated by ChemDraw Ultra 10.0.
[b] Calculated by online program of ChemAxon.
[c,d] Calculated by online program ALOGPS 2.1.

proceeded smoothly and in good yields (32–84%). All the compounds formed were recrystallized from dioxane or ethanol.

All new structures of compounds **1–10** were satisfactorily confirmed by IR, ^{1}H NMR and elemental analysis. IR spectra showed absorptions at 1650–1720 cm^{-1} (C=O), and sharp bands at 3200 cm^{-1} (NH). In the ^{1}H NMR spectra chalcones showed peaks in the region of δ 2.54–2.78 (CH_3), 2.88–2.98 (N–CH_3), 7.14–7.26 (CO–CH) and 7.53–7.58 (Ar-CH).

The C_α–C_β double bond in the enone moiety of chalcones can potentially adopt either a *Z* or an E configuration. The ^{1}H NMR spectrum of each compounds exhibited CH=CH protons around 7.14–7.63 ppm, with J >15, would suggest that the compounds were produced with an (*E*) configuration.[30]

Theoretical calculations of lipophilicity using the method of additivity to generate *C* log *P* values, was performed (Table 1).[31]

2.2. Biological evaluation

2.2.1. Antimicrobial activity

The synthesized thiazole-based chalcones were then assayed in vitro for their antibacterial and antifungal activity against Gram positive and Gram negative bacteria, and molds and the minimal inhibitory concentrations that inhibited the growth of the tested microorganisms (MIC) and minimal bactericidal/fungicidal concentration were determined. The results of antimicrobial testing against a panel of selected Gram positive and Gram negative bacteria are reported in Table 2, along with those of reference drugs Ampicillin and Streptomycin.[2]

All the compounds tested have activity against all the bacteria tested roughly comparable to that of Ampicillin, and rather less than of Streptomycin and minimal inhibitory concentration (MIC) is at range of 7.64–45.87 μmol/ml × 10^{-2} and minimal bactericidal concentration (MBC) is 30.58–68.37 μmol/ml × 10^{-2}. Exceptions to this generalization are the compound **6** possesses threefold better MIC against *Enterococcus Faecalis* than does Ampicillin, and the compounds **4** and **7** possess threefold better MIC against *Micrococcus flavus* than does Ampicillin. Probably these two bacteria are more sensitive to title compounds than to Ampicillin.

The lowest activity was observed for compound **4** against *Staphylococcus aureus* with MIC 51.28 × 10^{-2} and MBC 68.37 μmol/ml × 10^{-2}.

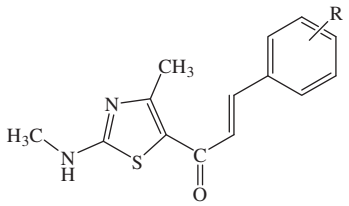
It was found that the most sensitive bacteria to compounds tested was Gram negative bacteria *E. faecalis*, while, Gram-positive *Listeria monocytogenes*, was the most resistant one.

It is noteworthy, that all tested compounds exhibited activity equal or better than commercial antibiotic Ampicillin but they were less active compared to Streptomycin. DMSO in used concentration did not show any effect on bacteria.

The results of antifungal activity are presented in Table 3. All the compounds tested possess modest activity against all the fungal species tested; but that either ketoconazole or bifonazole, or both, are more active than any of the chalcones tested. The antifungal properties of the chalcones are rather broad spectrum: all the activities determined were within a threefold concentration range. Noteworthy results are that: (i) Compounds **2**, **4**, **5**, **6**, **8**, **9**, and **10** are all only 1.5- to 2-fold less active against *Fusarium sporotrichoides* compared to bifonazole, the standard possessing the better activity. (ii) All 10 novel compounds are only twofold less active against *Trichoderma viride* than compared to ketoconazole, the standard possessing the better activity. (iii) Additionally compound **4** is only 3.5-fold less active against *Aspergillus niger* than compared to bifonazole; compound **8** is only fourfold less active against *Aspergillus ochraceus* than compared to bifonazole.

Among compounds tested the best antifungal activity was expressed by chalcone **6** (MIC 15.29 μmol/ml × 10^{-2}) against *Aspergillus versicolor*, T. *viride* and *F. sporotrichoides*, as well as compound **9** against T. *viride*, *Penicillium ochrochloron* and *F. sporotrichoides*., even though these compounds are less potent than reference drugs (ketoconazole and bifonazole). Quite good antifungal activity was observed for compounds **7** and **8** while

Table 2
Antibacterial activity of substituted/non substituted (*E*)-1-[4-methyl-2-(methylamino)thiazol-5-yl)-3-phenylprop-2-en-1-ones (MIC and MBC, μmol/ml × 10^{-2})



Compounds		*S.a.*	*B.c.*	*M.f.*	*L.m.*	*Ps. aer.*	*S. typhi*	*E.coli*	*En.f.*
1	MIC	38.75	38.75	19.37	38.75	29.06	32.94	32.94	19.37
	MBC	58.13	58.13	38.75	58.13	38.75	38.75	38.75	38.75
2	MIC	33.00	33.00	33.00	33.00	33.00	24.75	24.75	16.50
	MBC	49.50	49.50	49.50	49.50	49.50	33.00	33.00	33.00
3	MIC	33.00	33.00	33.00	33.00	28.05	28.05	24.75	33.00
	MBC	49.50	33.00	49.50	49.50	33.00	33.00	33.00	33.00
4	MIC	51.28	34.18	8.54	34.18	25.64	29.05	29.05	17.10
	MBC	68.37	51.28	34.18	51.28	34.18	34.18	34.18	34.18
5	MIC	34.18	34.18	25.64	34.18	34.18	29.05	29.05	8.54
	MBC	51.28	51.28	34.18	51.28	51.28	34.18	34.18	34.18
6	MIC	30.58	22.93	22.93	30.58	25.99	30.58	22.93	7.64
	MBC	45.87	30.58	25.99	45.87	30.58	45.87	30.58	15.29
7	MIC	29.05	29.05	8.54	34.18	34.18	34.18	34.18	17.09
	MBC	34.18	34.18	17.09	51.28	51.28	51.28	51.28	17.09
8	MIC	34.72	34.72	26.04	34.72	29.51	34.72	26.04	26.04
	MBC	34.72	34.72	29.51	52.08	34.72	52.08	34.72	34.72
9	MIC	30.58	25.99	22.93	30.58	22.93	30.58	22.93	15.29
	MBC	45.87	30.58	30.58	45.87	30.58	30.58	30.58	30.58
10	MIC	30.58	22.93	30.58	30.58	30.58	22.93	30.58	30.58
	MBC	45.87	30.58	45.87	45.87	30.58	30.58	45.87	45.87
Ampicillin	MIC	24.79	24.79	24.79	24.79	24.79	24.79	24.79	24.79
	MBC	37.19	37.19	37.19	37.19	37.19	37.19	37.19	37.19
Streptomycin	MIC	1.71	0.20	0.20	1.71	0.20	0.20	0.86	0.43
	MBC	4.29	0.43	0.43	4.30	0.43	0.43	1.71	0.86

S.a.—Staphylococcus aureus (ATCC 6538); *B.c.—Bacillus cereus* (clinical isolate); *M.f.—Micrococcus flavus* (ATCC 10240); *L.m.—Listeria monocytogenes* (NCTC 7973); *Ps. aer.—Pseudomonas aeruginosa* (ATCC 27853); *S. typhi—Salmonella typhimurium* (ATCC 13311); *E.coli—Escherichia coli* (ATCC 35210); *En.f.—Enterococcus faecalis* (human isolate).

Table 3
Antifungal activity of substituted/non substituted (*E*)-1-[4-methyl-2-(methylamino)thiazol-5-yl)-3-phenylprop-2-en-1-ones (MIC and MFC in μmol/ml × 10^{-2})

Compounds		*A.o.*	*A.v.*	*A.n.*	*A.f.*	*T.v.*	*P.o.*	*P.f.*	*F.s.*
1	MIC	38.80	19.38	38.80	38.80	19.38	19.38	38.80	38.80
	MFC	58.10	58.10	77.52	58.10	38.80	77.52	58.10	38.80
2	MIC	25.00	33.00	33.00	33.00	16.50	33.00	33.00	16.50
	MFC	33.00	49.60	82.50	49.60	33.00	49.60	49.60	66.00
3	MIC	33.00	33.00	33.00	33.00	16.50	33.00	33.00	33.00
	MFC	49.60	49.60	66.00	49.60	33.00	49.60	49.60	66.00
4	MIC	34.00	17.00	17.00	17.00	17.00	34.00	17.00	17.00
	MFC	51.30	34.00	34.00	34.00	34.00	51.30	34.00	17.00
5	MIC	34.00	34.00	34.00	34.00	17.00	34.00	34.00	17.00
	MFC	51.30	51.30	85.40	51.30	34.00	51.30	51.30	68.30
6	MIC	30.58	15.29	30.58	30.58	15.29	30.58	30.58	15.29
	MFC	45.87	45.87	61.16	61.16	30.58	45.87	61.16	45.87
7	MIC	34.00	17.00	34.00	17.00	17.00	34.00	34.00	34.00
	MFC	51.30	51.30	68.30	51.30	51.30	51.30	51.30	51.30
8	MIC	17.00	34.00	34.00	34.00	17.00	34.00	34.00	17.00
	MFC	34.00	52.00	87.00	52.00	34.00	52.00	69.00	34.00
9	MIC	45.87	30.58	30.58	30.58	15.29	15.29	30.58	15.29
	MFC	61.16	45.87	61.16	30.58	30.58	45.87	45.87	45.87
10	MIC	30.58	15.29	45.87	15.29	15.29	30.58	15.29	15.29
	MFC	61.16	61.16	61.16	61.16	30.58	61.16	30.58	30.58
Ketoconazole	MIC	4.70	1.88	28.25	4.70	9.42	1.88	0.94	9.42
	MFC	9.42	4.70	47.00	9.42	18.80	4.70	1.88	18.80
Bifonazole	MIC	40.30	2.51	5.03	0.32	80.60	40.30	161.16	8.06
	MFC	80.60	161.16	20.14	6.12	161.16	161.16	241.74	80.60

A.o.—Aspergillus ochraceus (ATCC 12066); *A.v.—Aspergillus versicolor* (ATCC 11730); *A.n.—Aspergillus niger* (ATCC 6275); *A.f.—Aspergillus fumigatus* (plant isolate); *T.v.—Trichoderma viride* (IAM 5061); *P.o.—Penicillium ochrochloron* (ATCC 9112); *P.f.—Penicillium funiculosum* (ATCC 36839); *F.s.—Fusarium sporotrichoides* (IMT 496).

the lowest activity was observed for chalcone **1**, with MIC 19.38–38.80 μmol/ml × 10^{-2}. DMSO in used concentration did not show any effect on fungi.

As regards the relationships between the structure of the heterocyclic scaffold and the detected antibacterial properties, clearly, the introduction of the bulky electron-withdrawing nitro and

chloro groups enhances the antibacterial properties. It was observed that the introduction of methoxy group is favored too for antibacterial activity (compounds **7** and **8**) with respect to unsubstituted derivative **1.** In generally, compounds **4**, **6**, **7**, and **8** stand out as the compounds possessing the more noteworthy properties, which are either mono-chloro or mono-methoxy substituted.

It seems also interesting to point out that, among the isomeric chloro substituted compounds **4–6**, as well as among the nitro substituted **2–3**, the position of the substituent exerts, in general, a certain effect, even if not univocal, on the activity against all the microorganisms. Conversely, among the nitro substituted **2**, **3**, the *meta*—derivative (compound **3)** are endowed with the higher activity, whereas, for the chloro substituted compounds **4–6** it is evident the greater efficacy of the *ortho* derivative **6** with respect to *m*- and *p*-derivatives.

The majority of compounds showed the worst activity against *A. ochraceus* followed by *P. ochrochloron*, while *T. viride*, followed by *A. versicolor* and *F. sporotrichoides*, are the most sensitive species. The last two species are in general more sensitive than others used in experiment.[11,14]

No correlation of antimicrobial activity, and lipophilicity, calculated as *C* log *P*, was observed.

3. Conclusion

The newly synthesized thiazole-based chalcones **1–10** exhibit a remarkable inhibition of the growth of a wide spectrum of Gram positive bacteria, Gram negative bacteria and fungi. The most sensitive bacterial species on compounds tested is Gram negative bacteria *E. faecalis*, while, Gram-positive *L. monocytogenes*, is the most resistant one. As far as the fungi are concerned, the tested compounds posses modest activity against all the fungal species tested being less active than ketoconazole and bifonazole. The majority of compounds showed the worst activity against *Aspergillus ochraceus* followed by *Penicillium ochrochloron,* while *T. viride*, followed by *A. versicolor* and *F. sporotrichoides*, are the most sensitive species. The last two species are in general more sensitive than others used in experiment.[11,14]

It should be noticed that some compounds tested exhibited better activity than commercial antimicrobial agents used as reference drugs while there were less active than ketoconazole and bifonazole.

The outstanding properties of this new class of antibacterial substances deserve further investigation in order to clarify the mode of action at molecular level, responsible for the activity observed.

4. Experimental part

Melting points (°C) were determined with a MELTEMP II capillary apparatus (LAB Devices, Holliston, MA, USA) without correction. Elemental analyses were performed on a Perkin–Elmer 2400 CHN elemental analyzer and all compounds synthesized were within a 0.4% of theoretical values. IR spectra were recorded, in Nujol, on a Perkin Elmer Spectrum BX and a DR-8001 Shimadzu. Wave numbers in the IR spectra are given in cm^{-1}. ^1H- NMR spectra of the newly synthesized compounds, in DMSO-d_6 solutions, were recorded on a Bruker AC 300 instrument (Bruker, Karlsruhe, Germany) at 298 K. Chemical shifts are reported as δ (ppm) relative to TMS as internal standard. Coupling constants *J* are expressed in Hertz (Center of Instrumental Analysis of the University of Thessaloniki).

The reactions were monitored by TLC on F_{254} silica-gel precoated sheets (Merck, Darmstadt, Germany) and the purified compounds each showed a single spot.

Solvents, unless otherwise specified were of analytical reagent grade or of the highest quality commercially available. Synthetic starting materials, reagents and solvents were purchased from Aldrich Chemie (Steinheimm, Germany).

4.1. Synthesis of 1-(4-methyl-2-(methylamino)thiazol-5-yl)ethanone

1-Methylthiourea (1.8 g, 0.02 mol) was dissolved in 50 ml of acetone. 3-Chloroacetylacetone (2.26 ml, 0.02 mol), diluted in acetone (5 ml) was added dropwise and mixture was refluxed for 1.5 h. The solid product was filtered and recrystallized from ethanol.[32]

4.2. General procedure for the synthesis of (*E*)-1-(4-methyl-2-(methylamino)thiazol-5-yl)-3-phenylprop-2-en-1-ones

1-(4-Methyl-2-(methylamino)thiazol-5-yl)ethanone (1 mol) in methanol (4.0–4.1 l), was added dropwise to a cooled solution of corresponding aromatic aldehydes (1 mol) in 10% NaOH (600–650 ml). The solution was maintained at 0 °C for 1.5 h and then was allowed to stir at room temperature. After some time (5–12 h) solid started separating out. The solid was filtered under vacuum and recrystallized from dioxane or ethanol to give the title chalcones. The yield range after recrystallization of chalcones was 32–84%.

4.2.1. (*E*)-1-(4-Methyl-2-(methylamino)thiazol-5-yl)-3-phenyl prop-2-en-1-one (1)

Yield: 32%, mp: 186–187 °C. IR (Nujol) 3250, 1625 (C=C), 1720 (C=O), 1600 (C–H arom) cm^{-1}; ^1H NMR (δ ppm, DMSO-d_6, 300 MHz): 2.54 (s, 3H, thiazole 4′-CH_3), 2.88 (s, 3H, N–CH_3), 7.14 (d, *J* = 15.6 Hz, 1H, CO–CH), 7.34–7.59 (m, 6H, phenyl 2′ and 6′, phenyl 3′ and 5′, phenyl 4′, Ph-CH), 8.31 (s, 1H, thiazole 2′-NH). Anal. Calcd for $C_{14}H_{14}N_2OS$ (MW 258): C, 65.09; H, 5.46; N, 10.84. Found: C, 65.29; H, 5.36; N, 10.90.

4.2.2. (*E*)-1-(4-Methyl-2-(methylamino)thiazol-5-yl)-3-(4-nitrophenyl)prop-2-en-1-one (2)

Yield: 58%, mp: 248–249 °C. IR (Nujol) 3390, 3200 (NH), 1722 (C=O), 1653 (CH=CH), 1602 (C–H arom), 1525 (assym. N=O), 1340 (NO_2) cm^{-1}; ^1H NMR (δ ppm, DMSO-d_6, 300 MHz): 2.58 (s, 3H, thiazole 4′-CH_3), 2.89 (s, 3H, N–CH_3), 7.51 (d, *J* = 15.6 Hz, 1H, CO–CH), 7.63 (d, *J* = 15.6 Hz, 1H, Ar-CH), 8.04 (d, *J* = 9 Hz, 2H, Ar 3′ and 5′), 8.26 (d, *J* = 8.7 Hz, 2H. Ar 2′ and 6′), 8.65 (s, 1H, thiazole 2′-NH). Anal. Calcd for $C_{14}H_{13}N_3O_3S$ (MW 303): C, 55.43; H, 4.32; N, 13.85. Found: C, 55.35; H, 4.20; N, 13.65.

4.2.3. (*E*)-1-(4-Methyl-2-(methylamino)thiazol-5-yl)-3-(3-nitrophenyl)prop-2-en-1-one (3)

Yield: 47%, mp: 246–247 °C. IR (Nujol) 3390, 3250 (NH), 1680 (C=O),1652 (CH=CH), 1602, 1525 (assym. N=O), 1350 (NO_2) cm^{-1}; ^1H NMR (δ ppm, DMSO-d_6, 300 MHz): 2.49 (s, 3H, thiazole 4′-CH_3), 2.79 (s, 3H, N–CH_3), 7.42–7.63 (m, 3H, alkene, Ar 5′), 8.15 (dd, 2H, Ar 6′, Ar 4′), 8.48 (s, 1H, Ar 2′). MS: (*m*/*z*): 303 (M^+, 5%), 289 (4%), 273 (2%), 181 (6%), 171 (7%), 170 (58%), 155 (100%), 151 (25%), 150 (23%), 127 (11%), 114 (10%), 105 (12%), 95 (9%), 86 (30%), 81 (24%), 77 (19%), 69 (42%), 57 (10%), 55 (11%), 51 (14%), 45 (11%), 43 (19%), 42 (10%), 41 (15%). Anal. Calcd for $C_{14}H_{13}N_3O_3S$ (MW 303): C, 55.43; H, 4.32; N, 13.85. Found: C, 55.38; H, 4.22; N, 13.75.

4.2.4. (*E*)-3-(4-Chlorophenyl)-1-(4-methyl-2-(methylamino) thiazol-5-yl)prop-2-en-1-one (4)

Yield: 60%, mp: 191–193 °C. IR (Nujol) 3200 (NH), 1680 (C=O), 1650 (CH=CH), 1602, 1100 (Cl) cm^{-1}; ^1H NMR (δ ppm, DMSO-d_6,

300 MHz): 2.50 (s, 3H, thiazole 4′-CH_3), 2.82 (s, 3H, N–CH_3), 7.27 (d, J = 15.6 Hz, 1H, CO–CH), 7.42–7.50 (m, 3H, Ar 3′ and 5′, Ar-CH), 7.74 (d, J = 8.4 Hz, 2H, Ar 2′ and 6′). Anal. Calcd for $C_{14}H_{13}ClN_2OS$ (MW 292.5): C, 57.43; H, 4.48; Cl, 12.11; N, 9.57. Found: C, 57.40; H, 4.30; N, 9.68.

4.2.5. (*E*)-3-(3-Chlorophenyl)-1-(4-methyl-2-(methylamino) thiazol-5-yl)prop-2-en-1-one (5)

Yield: 61%, mp: 209–212 °C. IR (Nujol) 3200 (NH), 1685 (C=O), 1651 (CH=CH), 1602, 1075 (Cl) cm^{-1}; ^1H NMR (δ ppm, DMSO-d_6, 300 MHz): 2.48 (s, 3H, thiazole 4′-CH_3), 2.80 (s, 3H, N–CH_3), 7.32–7.46 (m, 4H, CO–CH, Ar 2′, Ar 5′, Ar 4′), 7.72 (dd, 2H, Ar 6′, Ar-CH), 8.52 (s, 1H, NH). Anal. Calcd for $C_{14}H_{13}ClN_2OS$ (MW 292.5): C, 57.43; H, 4.48; Cl, 12.11; N, 9.57. Found: C, 57.44; H, 4.40; N, 9.60.

4.2.6. (*E*)-3-(2-Chlorophenyl)-1-(4-methyl-2-(methylamino) thiazol-5-yl)prop-2-en-1-one (6)

Yield: 32%, mp: 185–187 °C. IR (Nujol) 3200 (NH), 1680 (C=O), 1650 (CH=CH), 1602, 1025 (Cl) cm^{-1}; ^1H NMR (δ ppm, DMSO-d_6, 300 MHz): 2.50 (s, 3H, thiazole 4′-CH_3), 2.81 (s, 3H, N–CH_3), 7.3 (d, J = 15.3 Hz, 1H, CO–CH), 7.36 (dd, 2H, Ar 4′, Ar 5′), 7.49 (d, J = 9.3 Hz, 1H, Ar 6′), 7.76 (d, J = 15.6 Hz, 1H, Ar-CH), 7.93 (d, J = 9.3 Hz, 1H, Ar 3′). MS: (m/z): 292 (M^+, 100%), 291 (28%), 257 (43%), 236 (9%), 201 (9%), 182 (8%), 181 (73%), 167 (13%), 165 (9%), 155 (33%), 137 (13%), 128 (9%), 102 (14%), 101 (22%), 86 (15%), 81 (14%), 75 (9%), 69 (25%), 57 (9%). Anal. Calcd for $C_{14}H_{13}ClN_2OS$ (MW 292.5): C, 57.43; H, 4.48; Cl, 12.11; N, 9.57. Found: C, 57.41; H, 4.45; N, 9.55.

4.2.7. (*E*)-3-(4-Methoxyphenyl)-1-(4-methyl-2-(methylamino) thiazol-5-yl)prop-2-en-1-one (7)

Yield: 29%, mp: 219–223 °C. IR (Nujol) 3200 (NH), 1684 (C=O), 1654 (CH=CH), 1604, 1584, 1250 C-O–C) cm^{-1}; ^1H NMR (δ ppm, DMSO-d_6, 300 MHz): 2.61 (s, 3H, thiazole 4′-CH_3), 2.93 (s, 3H, N–CH_3), 3.87 (s, 3H, OCH_3), 7.05 (d, J = 8.7 Hz, 2H, Ar 3′ and 5′), 7.22 (d, J = 15.3 Hz, 1H, CO–CH), 7.58 (d, J = 15.6 Hz, 1H, Ar-CH), 7.77 (d, J = 8.7 Hz, 2H, Ar 2′ and 6′). Anal. Calcd for $C_{15}H_{16}N_2O_2S$ (MW 288): C, 62.48; H,5.50; N, 9.71. Found: C,62.45; H, 5.52; N, 9.71.

4.2.8. (*E*)-3-(2-Methoxyphenyl)-1-(4-methyl-2-(methylamino)thiazol-5-yl)prop-2-en-1-one (8)

Yield: 36%, mp: 213–215 °C. IR (Nujol) 3200 (NH), 1670 (C=O), 1643 (CH=CH), 1607, 1247 (C-O–C) cm^{-1}; ^1H NMR (δ ppm, DMSO-d_6, 300 MHz): 2.54 (s, 3H, thiazole 4′-CH_3), 2.86 (s, 3H, N–CH_3), 3.88 (s, 3H, OCH_3), 7.05 (m, 2H, CO–CH, Ar 3′), 7.41 (dd, 2H, Ar 5′, Ar 4′), 7.75 (dd, 2H, Ar 6′, Ar-CH). Anal. Calcd for $C_{15}H_{16}N_2O_2S$ (MW 288): C, 62.48; H,5.50; N, 9.71. Found: C,62.47; H, 5.53; N, 9.70.

4.2.9. (*E*)-3-(2,6-Dichlorophenyl)-1-(4-methyl-2-(methylamino) thiazol-5-yl)prop-2-en-1-one (9)

Yield: 84%, mp: 195–197 °C. IR (Nujol) 3200 (NH), 1690 (C=O), 1650 (CH=CH), 1607 cm^{-1}; ^1H NMR (δ ppm, DMSO-d_6, 80 MHz): 2.50 (s, 3H, thiazole 4′-CH_3), 2.91 (s, 3H, N–CH_3), 7.15–7.8 (m, 5H, Ar, alkene). Anal. Calcd for $C_{14}H_{12}Cl_2N_2OS$ (MW 327): C, 51.39; H, 3.70; Cl, 21.67; N, 8.56; O, 4.89; S, 9.80. Found: C, 51.40; H, 3.73; N, 8.58.

4.2.10. (*E*)-3-(2,4-Dichlorophenyl)-1-(4-methyl-2-(methylamino)thiazol-5-yl)prop-2-en-1-one (10)

Yield: 76%, mp: 239–240 °C. IR (Nujol) 3200 (NH), 1680 (C=O), 1650 (CH=CH), 1602, 1100 (Cl) cm^{-1}; ^1H NMR (δ ppm, DMSO-d_6, 300 MHz): 2.56 (s, 3H, thiazole 4′-CH_3), 2.88 (s, 3H, N–CH_3), 7.39 (d, J = 15.3 Hz, 1H, CO–CH), 7.51 (d, J = 8.4 Hz, 1H, Ar 5′), 7.74–7.78 (m, 2H, Ar 3′, Ar-CH), 8.05 (d, J = 8.3 Hz, 1H, Ar 6′), 8.64 (s, 1H, NH). Anal. Calcd for $C_{14}H_{12}Cl_2N_2OS$ (MW 327): C, 51.39; H, 3.70; Cl, 21.67; N, 8.56. Found: C, 51.43; H, 3.69; N, 8.55.

4.3. Biological evaluation

4.3.1. Antifungal activity

For the antifungal bioassays, eight fungi were used: Aspergillus ochraceus (ATCC 12066), Aspergillus fumigatus (plant isolate), Aspergillus niger (ATCC 6275), *A.* versicolor (ATCC 11730), Penicillium funiculosum (ATCC 36839), Penicillium ochrochloron (ATCC 9112), Trichoderma viride (IAM 5061) and *F.* sporotrichoides (IMT 496). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research 'Siniša Stanković', Belgrade, Serbia.

The micromycetes were maintained on malt agar and the cultures stored at 4 °C and sub-cultured once a month.[33] In order to investigate the antifungal activity of the compounds, a modified microdilution technique was used.[34–36] The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 μl per well. The inocula were stored at 4 °C for further use. Dilutions of the inocula were cultured on solid malt agar to verify the absence of contamination and to check the validity of the inoculum.

Minimum inhibitory concentration (MIC) determinations were performed by a serial dilution technique using 96-well microtiter plates. The compounds investigated were dissolved in 5% DMSO solution containing 0.1% Tween 80 (v/v) (1 mg/ml) and added in broth Malt medium with inoculum. The microplates were incubated at Rotary shaker (160 rpm) for 72 h at 28 °C. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs.

The fungicidal concentrations (MFCs) were determined by serial subcultivation of a 2 μl of tested compounds dissolved in medium and inoculated for 72 h, into microtiter plates containing 100 μl of broth per well and further incubation 72 h at 28 °C. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. DMSO was used as a negative control, commercial fungicides, bifonazole (Srbolek, Belgrade, Serbia) and ketoconazole (Zorkapharma, Šabac, Serbia), were used as positive controls (1–3000 μg/ml).

All experiments were performed in duplicate and repeated three times.

4.3.2. Antibacterial activity

The following Gram-negative bacteria were used: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *E. faecalis* (human isolate) and the following Gram-positive bacteria: *Bacillus cereus* (clinical isolate), *M. flavus* (ATCC 10240), *L. monocytogenes* (NCTC 7973), and *Staphylococcus aureus* (ATCC 6538). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research 'Siniša Stanković', Belgrade, Serbia.

The antibacterial assay was carried out by a microdilution method[34,35] in order to determine the antibacterial activity of compounds tested against the human pathogenic bacteria.

The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^5 CFU/ml. The inocula were prepared daily and stored at +4 °C until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum.

All experiments were performed in duplicate and repeated three times.

4.3.3. Microdilution test

The minimum inhibitory and bactericidal concentrations (MICs and MBCs) were determined using 96-well microtiter plates. The

bacterial suspension was adjusted with sterile saline to a concentration of 1.0×10^5 CFU/ml. Compounds to be investigated were dissolved in 5% DMSO solution containing 0.1% Tween 80 (v/v) (1 mg/ml) and added in broth LB medium (100 μl) with bacterial inoculum (1.0×10^4 CFU per well) to achieve the wanted concentrations. The microplates were incubated at Rotary shaker (160 rpm) for 24 h at 48 °C. The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The MBCs were determined by serial sub-cultivation of 2 μl into microtiter plates containing 100 μl of broth per well and further incubation for 24 h. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by Microplate manager 4.0 (Bio-Rad Laboratories) and compared with a blank and the positive control. Streptomycin (Sigma P 7794) and Ampicillin (Panfarma, Belgrade, Serbia) were used as a positive control (1 mg/ml DMSO). DMSO was used as a negative control.

All experiments were performed in duplicate and repeated three times.

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