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Novel (*E*)-1-(4-methyl-2-(alkylamino)thiazol-5-yl)-3-arylprop-2-en-1-ones as potent antimicrobial agents

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ABSTRACT

New (*E*)-1-(4-methyl-2-(alkylamino)thiazol-5-yl)-3-arylprop-2-en-1-ones, unsubstituted or carrying fluoro, bromo, methoxy, nitro, methyl and chloro groups on the benzene ring, were synthesized and assayed in vitro for their antimicrobial activity against Gram positive and Gram negative bacteria and fungi. The compounds were very potent towards all tested microorganisms and in most cases their activity was better than that of reference drugs.

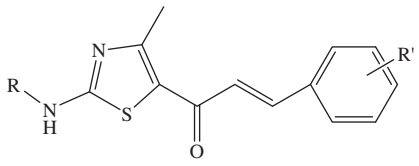
1. Introduction

The rapid progress of science in the past decades led to the significant improvement in the diagnosis and treatment of infectious diseases. Despite these efforts, the emerging resistance of microorganisms to known antibiotics kept the scientific interest in developing new classes of antimicrobial compounds.[1–5]

In addition, the need for effective antimicrobial drugs became even greater considering the difficulties of dealing with the treatment of infections of hospitalized patients and protection of immunosuppressed and HIV-infected patients.[6]

Therefore, designing innovating drugs with different mode of action could be the choice of preference in order to overcome the problem of cross resistance to existing therapeuticals. A recent example of this kind of approach is the introduction of Linezolid, a synthetic molecule, member of the oxazolidinone class of drugs. Linezolid is active against most Gram-positive bacteria, including streptococci, vancomycin-resistant enterococci (VRE), and methicillin-resistant *Staphylococcus aureus* (MRSA) and seems to have a unique mechanism of action compared to existing drugs.[7–9]

Taking into account the interesting properties of thiazole derivatives[10–19] and chalcones,[20–24] as well as our promising findings regarding, specifically, the antimicrobial activity of thiazole-based chalcones,[25] herein is presented the synthesis of a series of novel thiazole-based chalcones (Fig. 1) with significantly improved antibacterial and antifungal activities.



	R	R´
1.	Me	4-F
2.	Me	3-F
3.	Me	3-Br
4.	Me	3-Me
5.	Me	2,3-diCl
6.	Et	H
7.	Et	4-NO_2
8.	Et	3-NO_2
9.	Et	4-Cl
10.	Et	3-Cl
11.	Et	2-Cl
12.	Et	4-F
13.	Et	3-F
14.	Et	3-Br
15.	Et	4-OMe
16.	Et	2-OMe
17.	Prop	H

Figure 1.

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2. Results and discussion

2.1. Chemistry

The synthesis of the target chalcones **1–17** was accomplished by a Claisen–Schmidt condensation (Scheme 1) as described in our previous publication.[25] The reactions proceeded smoothly and in good yields for the majority of compounds. Structures of synthesized compounds **1–17** were satisfactorily confirmed by IR, ^{1}H NMR and elemental analysis. In IR spectra were observed absorptions at 1650–1710 cm^{-1} (C=O), and sharp bands at 3200 cm^{-1} (NH). In the ^{1}H NMR spectra, peaks of thiazole-based chalcones appeared in the region of δ 2.50–2.78 (thiazole 4-CH_3), 1.17–1.69 (methyl NCH_2CH_3, compounds 6–16), 2.86–2.94 (NCH_3, compounds 1–5), 3.26–3.41 (NCH_2, compounds 6–16), 7.14–7.46 (CO–CH) and 7.46–7.87 (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 chalcone exhibited CH=CH protons around 7.14–7.87 ppm, with J >15, and therefore would suggest that the compounds adopted (*E*) configuration.[26] Theoretical calculations of lipophilicity as *C*log*P* were performed (Table 1).

2.2. Biological evaluation

2.2.1. Antimicrobial activity

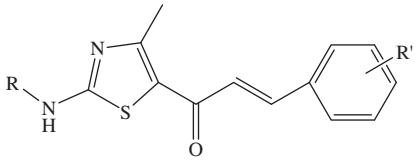
The synthesized thiazole-based chalcones were then assayed in vitro for their antibacterial and antifungal activity against Gram positive and Gram negative bacteria, and the minimal inhibitory concentrations that inhibited the growth of the tested microorganisms (MIC) and minimal bactericidal/fungicidal concentrations were determined. The results of antimicrobial testing are reported in Table 2, along with those of reference drugs Ampicillin and Streptomycin.[2] All compounds tested could be divided into two groups, namely methylamino and ethylamino/propylamino chalcones.

The structural modifications to previously synthesized thiazole-based chalcones[25] involve the nature of the substituent R as well as R′.

The results of antibacterial activity of the first group (compounds **1–5)** are presented in Table 2. All the compounds showed very strong antibacterial activity against all the species tested with MIC 1.48–11.03 μmol × 10^{-2}/ml, and MBC 1.09–11.00 μmol × 10^{-2}/ml. The antibacterial potential follows the order **3>1>5>2>4**. The best antibacterial effect exhibited compounds **3** and **1** with inhibitory activity at 1.48–9.93 and 3.26–9.06 μmol × 10^{-2}/ml respectively and bactericidal effect at 5.93–8.9 and 1.09–9.06 μmol × 10^{-2}/ml. respectively. The lowest antibacterial activity observed for compound **4** with MIC of 5.51–11.03 μmol × 10^{-2}/ml and MBC 7.35–11.03 μmol × 10^{-2}/ml. The most sensitive bacterial species on these compounds are *Micrococcus flavus*, followed by *Salmonella typhimurium* and *Bacillus cereus*, while *Listeria monocytogenes* is the most resistant species.

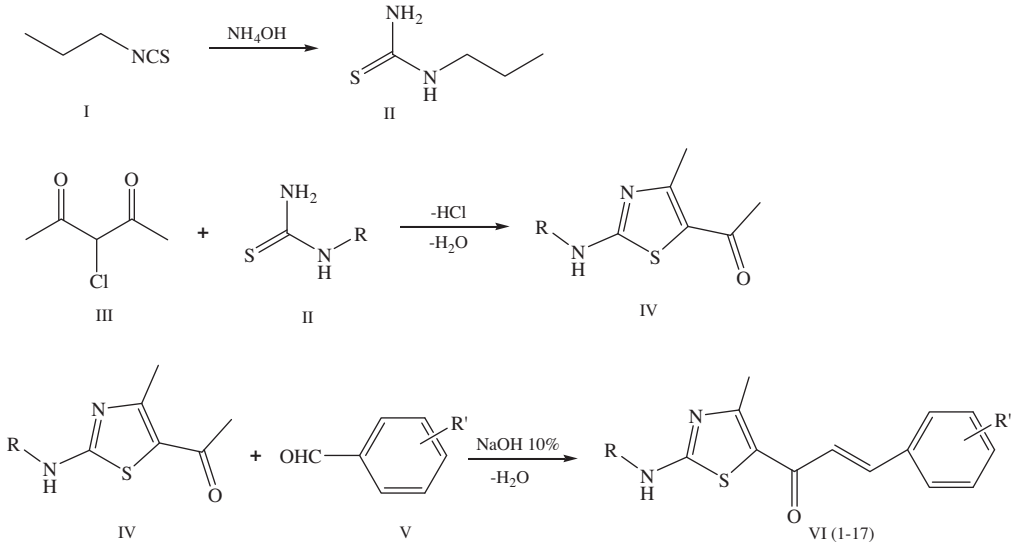
As far as the second group is concerned (compounds **6–17**), namely ethylamino and propylamino chalcones results of their antibacterial activity are presented in Table 2. The inhibitory activity for compounds **6–17** is obtained at 3.0–11.03 μmol × 10^{-2}/ml and

Table 1
Calculated lipophilicity of synthesized compounds



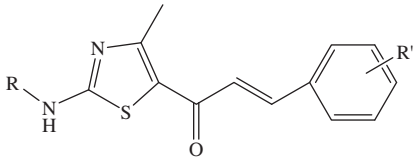
Compounds	*C*log*P*[a]	*A*Log*P*s[b]	*A*log*P*[b]	*C*log*P*[c]
1	3.11	3.46	3.29	2.99
2	3.11	3.46	3.29	2.99
3	3.74	3.96	3.83	3.71
4	3.48	3.71	3.57	3.35
5	4.18	4.05	4.41	4.15
6	3.33	3.89	3.43	3.38
7	3.27	3.56	3.33	3.12
8	3.27	3.61	3.33	3.12
9	3.93	4.39	4.09	4.09
10	3.93	4.39	4.09	4.09
11	3.93	4.40	4.09	4.09
12	3.47	3.78	3.64	3.52
13	3.47	3.83	3.64	3.52
14	4.10	4.48	4.18	4.24
15	3.17	3.86	3.41	3.30
16	3.17	3.79	3.41	3.30
17	3.85	4.30	3.95	3.91

[a] Calculated by online program of Chemaxon.
[b] Calculated by online program ALOGPS 2.1.
[c] Calculated by program *C*log*P* 4.0, BioByte Corp.



Scheme 1.

Table 2
Antibacterial activity of substituted/non substituted (*E*)-1-[4-methyl-2-(alkylamino)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	7.25	3.62	3.26	9.06	7.25	5.43	5.43	5.43
	MBC	7.25	5.43	7.25	9.06	11.09	7.25	7.25	7.25
2	MIC	7.25	7.25	1.81	9.06	5.43	7.25	5.43	5.43
	MBC	9.06	9.06	7.25	9.06	7.25	9.06	7.25	7.25
3	MIC	5.93	9.93	1.48	8.90	7.42	5.93	7.42	7.42
	MBC	7.42	7.42	5.93	8.90	7.42	7.42	7.42	7.42
4	MIC	7.35	7.35	7.35	11.03	7.35	7.35	5.51	5.51
	MBC	11.03	9.19	9.19	11.03	9.19	9.19	7.35	7.35
5	MIC	6.12	4.59	3.06	9.17	6.12	6.12	4.59	7.65
	MBC	9.17	6.12	9.17	9.17	9.17	7.65	6.12	7.65
6	MIC	7.35	7.35	9.19	11.03	7.35	3.68	7.35	5.51
	MBC	11.03	7.35	11.03	11.03	7.35	9.19	11.03	7.35
7	MIC	9.46	6.31	9.46	9.46	4.73	4.73	9.46	4.73
	MBC	9.46	9.46	9.46	9.46	6.31	6.31	9.46	6.31
8	MIC	9.46	4.73	6.31	9.46	7.89	4.73	6.31	4.73
	MBC	9.46	6.31	9.46	9.46	7.89	6.31	7.89	6.31
9	MIC	9.80	8.17	6.53	9.80	6.53	4.90	6.53	8.17
	MBC	9.80	8.17	8.17	9.80	9.80	4.90	9.80	8.17
10	MIC	9.80	4.91	3.27	9.80	6.53	4.91	4.91	4.91
	MBC	9.80	6.53	8.17	11.44	8.17	6.53	6.53	6.53
11	MIC	8.17	3.27	6.53	9.81	4.89	4.89	4.89	4.89
	MBC	9.81	6.53	9.81	11.43	6.11	6.11	6.11	6.11
12	MIC	6.89	8.62	5.17	8.62	6.89	5.17	5.17	5.17
	MBC	6.89	8.62	6.89	10.34	6.89	5.17	6.89	6.89
13	MIC	6.89	5.17	3.45	8.62	6.89	5.17	6.89	5.17
	MBC	10.34	6.89	8.62	8.62	6.89	5.17	8.62	6.89
14	MIC	5.71	2.86	5.71	8.57	5.71	5.71	5.71	5.71
	MBC	7.14	7.14	8.57	10.00	7.14	7.14	7.14	7.14
15	MIC	6.62	6.62	3.13	8.28	6.62	4.97	4.97	6.62
	MBC	9.93	8.28	8.25	9.93	8.28	4.97	6.62	9.93
16	MIC	6.62	6.62	3.13	8.28	8.28	3.31	8.28	8.28
	MBC	9.93	8.28	8.28	11.59	9.93	8.28	8.28	8.28
17	MIC	9.00	3.50	7.00	9.00	7.00	7.00	7.00	9.00
	MBC	10.50	7.00	10.50	10.50	9.00	9.00	8.80	9.00
Ampicilin	MIC	24.80	24.80	24.80	37.20	74.40	24.80	37.20	24.80
	MBC	37.20	37.20	37.20	74.40	124.00	49.20	49.20	37.20
Streptomycin	MIC	17.20	4.30	8.60	25.80	17.20	17.20	17.20	4.30
	MBC	34.40	8.60	17.20	51.60	34.40	34.40	34.40	8.60

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).

bactericidal at 5.17–11.59 μmol × 10^{-2}/ml. The antibacterial potential could be presented as following: **12>14>11>13>15>8>7>10>9>17>16>6**. It can be seen that compound **6** showed the worst antibacterial activity with MIC 3.68–11.03 μmol × 10^{-2}/ml and MBC 7.35–11.03 μmol × 10^{-2}/ml while compound **12** exhibited the strongest antibacterial potency at MIC 5.17–8.62 μmol × 10^{-2}/ml and MBC at 5.17–10.34 μmol × 10^{-2}/ml.

Streptomycin showed MIC in range of 4.3–25.8 μmol × 10^{-2}/ml and MBC of 8.6–51.6 μmol × 10^{-2}/ml while Ampicillin showed inhibitory effect at 24.8–74.4 μmol × 10^{-2}/ml and bactericidal at 37.2–124.0 μmol × 10^{-2}/ml.

Compounds **1–5** showed better antibacterial activity than streptomycin with exception compounds **1, 2, 3** and **4** against *B. cereus*. Compounds**8, 10, 11, 17** showed almost the same or slightly higher inhibitory activity than Streptomycin against *B. cereus*, while compound **13** showed slightly lower bactericidal activity against the same bacterial species. All other compounds are less potent antibacterial agents than Streptomycin. On the other side, chalcones from both series (**1–17)** possessed better antibacterial activity than Ampicillin even ten-times lower than compounds in some cases (against *Pseudomonas aeruginosa*).

As regards the relationships between the structure and the detected antibacterial activity compounds **1–5** showed a significant antibacterial activity, greater (5–10 fold) than compounds from the same series previously synthesized.[25] Among the methylamino derivatives the inhibitory effect appears to be dependent on the substitution at the benzene ring. Thus the introduction of halogen substituents led to increase of activity 1.2–5-fold, compared to the unsubstituted derivative. The best results were observed for fluoro- and bromo-derivatives, compared to chloro—ones.[25] It should be mentioned that antibacterial activity is dependent not only on the nature of substituent but on the position of it in the benzene ring as well. It seems that position 3 in the benzene ring favored the antibacterial activity.

As far as regards the second group of compounds with R = Et it was observed that like in the series of methylamino chalcones the introduction of halogen substituents as well as methoxy group at the benzene ring led to increased activity. The introduction of

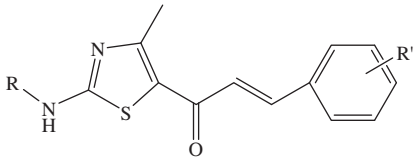
F- and Br-substituents, independently of their position, increase the antibacterial activity. Position of chloro- and nitro groups in the benzene ring seems to exert a certain effect. Thus 3-Cl and 2-Cl derivatives are mostly endowed with higher activity with respect to para substitution on the contrary with methylamino chalcones where the best activity was observed for fluoro-substitution. 4-Cl Derivative as well as 4-NO_2 exhibited activity equal to unsubstituted chalcone, while 3-Cl and 3-NO_2 derivatives are slightly more active than unsubstituted one. Furthemore, it seems that not only the nature of substituent (R′) in benzene ring but also substituent R influences much the activity. Thus the introduction in position 2 of thiazole ring of ethylamino group led to compounds with increased antibacterial activity (2.8–6 fold).

Comparison of novel ethylamino thiazole-based chalcones (Table 2) with methylamino ones previously tested[25] shows that insertion of ethyl group led to the production of more potent compounds. Only in case of 4- and 3-fluoro substitution the antibacterial activity of ethylamino chalcones was lower than in case of 4-and 3-fluoro methylamino chalcones.

The results of antifungal activity of methylamino (**1–5**) and ethylamino/propylamino (**6**–**17**) derivatives against eight fungi are presented in Table 3, in comparison with those of the reference drugs bifonazole and ketoconazole, respectively. All compounds showed remarkable antifungal effect with MIC 2.97–7.35 μmol × 10–^{2}/ml and MFC 5.93–9.19 μmol × 10^{-2}/ml. The antifungal potential could be presented as: **3>5>4>2>1**. Compound **3** showed the best antifungal activity among other tested with MIC 2.97–4.45 μmol × 10^{-2}/ml and MFC with 5.93–7.42 μmol × 10^{-2}/ml, while compound **1** possessed the lowest antifungal potential with inhibitory activity at 3.5–7.0 μμmol × 10^{-2}/ml and fungicidal activity at 7.0–9.0 μmol × 10^{-2}/ml. The majority of the compounds showed the best activity against *Penicillium funiculosum*, while *Candida albicans* was the most resistant species.

The ethylamino chalcones as well as propylamino (**17**) derivative also showed great antifungal potential with MIC at 3.15–7.35 μmol × 10^{-2}/ml and MFC at 4.0–9.0 μmol × 10^{-2}/ml. The antifungal potential is ranged in following order: **14>11>17>13>12>7>8>10>16>9>15>6**. The best antifungal activity was observed for compound **14** with MIC at 2.89–4.29 μmol × 10^{-2}/ml and MFC at 4.29–5.72 μmol × 10^{-2}/ml while the worst was obtained for compound **6** with MIC at 3.68–7.35 μmol × 10^{-2}/ml and MFC at 7.35–9.19 μmol × 10^{-2}/ml. The most sensitive fungus to tested

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



Compounds		*A. o.*	*A. fl.*	*A. n.*	*A. fum.*	*T. v.*	*P. o.*	*P. f.*	*C. a.*
1	MIC	3.62	3.62	3.62	3.62	7.25	3.62	3.62	7.25
	MFC	7.25	9.06	7.25	7.25	9.06	7.25	7.25	9.06
2	**MIC**	3.62	3.62	5.43	3.62	3.62	3.62	3.62	5.13
	MFC	7.25	9.06	7.25	7.25	7.25	7.25	7.25	7.25
3	**MIC**	2.97	2.97	4.45	2.97	2.97	2.97	2.97	4.45
	MFC	5.93	7.42	5.93	5.93	5.93	5.93	5.93	5.93
4	**MIC**	3.68	3.68	3.78	3.68	3.68	3.68	3.68	5.51
	MFC	7.35	9.19	7.35	7.35	7.35	7.35	7.35	7.35
5	**MIC**	3.06	3.06	3.06	3.06	3.06	3.06	3.06	4.59
	MFC	6.12	7.65	6.12	6.12	6.12	6.12	6.12	6.12
6	**MIC**	7.35	7.35	5.51	3.68	3.68	3.68	3.68	3.68
	MFC	9.19	9.19	7.35	7.35	7.35	7.35	7.35	7.35
7	**MIC**	6.31	6.31	4.73	4.73	4.73	6.31	3.15	3.15
	MFC	7.88	7.88	6.31	6.31	6.31	7.88	6.31	6.31
8	**MIC**	6.31	6.31	6.31	6.31	4.73	6.31	3.15	3.15
	MFC	7.88	7.88	7.88	7.88	6.31	7.88	6.31	6.31
9	**MIC**	6.53	6.53	6.53	4.90	3.27	6.53	3.27	3.27
	MFC	8.17	8.17	8.17	6.53	6.53	8.17	6.53	6.53
10	**MIC**	6.53	6.53	6.53	3.27	3.27	6.53	3.27	3.27
	MFC	8.17	8.17	8.17	6.53	6.53	8.17	6.53	6.53
11	**MIC**	3.27	4.90	4.90	4.90	3.27	3.27	3.27	3.27
	MFC	4.90	6.53	6.53	6.53	6.53	6.53	6.53	6.53
12	**MIC**	6.89	6.89	3.45	5.17	3.45	6.89	3.45	3.45
	MFC	8.62	8.62	5.17	6.89	6.89	8.62	6.89	6.89
13	**MIC**	6.89	6.89	3.45	3.45	3.45	3.45	3.45	3.45
	MFC	8.62	8.62	6.89	6.89	6.89	6.89	6.89	6.89
14	**MIC**	2.89	4.29	4.29	4.29	2.89	2.89	2.89	2.89
	MFC	4.29	5.71	5.71	5.71	5.72	5.82	4.29	5.72
15	**MIC**	6.62	6.62	3.31	6.62	3.31	6.62	3.31	6.62
	MFC	8.28	8.28	6.62	8.28	6.62	8.28	6.62	8.28
16	**MIC**	6.62	6.62	4.97	4.97	3.31	6.62	3.31	3.31
	MFC	8.28	8.28	6.62	6.62	6.62	8.28	6.62	6.62
17	**MIC**	3.50	5.24	5.24	5.24	2.62	3.50	5.24	3.50
	MFC	5.24	7.00	7.00	7.00	3.50	7.00	7.00	7.00
Ketoconazole	**MIC**	38.00	285.00	38.00	38.00	475.00	380.00	38.00	38.00
	MFC	95.00	380.00	95.00	95.00	570.00	380.00	95.00	95.00
Bifonazole	**MIC**	48.00	48.00	48.00	48.00	64.00	48.00	64.00	32.20
	MFC	80.00	64.00	64.00	64.00	80.00	64.00	80.00	48.00

Aspergillus ochraceus (ATCC 12066), *Aspergillus fumigatus* (human isolate), *Aspergillus niger* (ATCC 6275), *Aspergillus flavus* (ATCC 9643), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112), *Trichoderma viride* (IAM 5061) and *Candida albicans* (human isolate).

compounds was found to be *Trichoderma viride*, while *Aspergillus flavus* is the most resistant one.

The commercial antifungal agent, Bifonazole, showed MIC at 38.0–64.0 μmol × 10^{-2}/ml and MFC at 48.0–80.0 μmol × 10^{-2}/ml while Ketoconazole showed fungistatic activity at 38.0–475.0 μmol × 10^{-2}/ml and fungicidal effect at 95.0–570.0 μmol × 10^{-2}/ml. All compounds tested exhibited much higher antifungal potential than bifonazole and ketoconazole, ten-fifty fold higher.

As regards the relationships between the structure and the detected antifungal activity these five methylamino chalcone derivatives exhibited significant antifungal efficacy (5–6 fold), greater than those of previous published.[25] In this case antifungal activity among methylamino chalcones seems to be dependent on the substitution at the benzene ring, as it was observed for antibacterial activity. The introduction of fluoro- and bromo- substituents as well as methyl group is endowed with better antifungal activity in respect to unsubstituted chalcone.

Regarding the structure–activity relationship of ethylamino chalcones it was observed that the introduction of different substituents at the benzene ring has as a result the increase of antifungal potency. The efficacy depends more on the kind of substituents than their position at the ring. Thus, chloro- substitution seems in general to endow antifungal activity which ranged in following order 2-Cl>3-Cl>4-Cl. The same order (2-OMe >4-OMe) was observed for methoxy derivatives. In case of fluoro- derivatives, *m*-position is more favorable compared to *p*-, while for nitro ones it is the opposite.

Comparing the results of antifungal effect of methylamino derivatives (**1–5**) and ethylamino chalcones (**6–16**) it can be seen that the last ones are more potent. It should be mentioned that ethylamino derivatives possess also higher activity compared to methylamino chalcones with the same substitution in the phenyl ring.[25] Thus, the antifungal potency of such kind of compounds seems to be dependent not only on the substituent of the phenyl ring, but also on the elongation of the alkylamino group. It is interesting to point out that in this case the elongation of alkylamino group leads to higher lipophilicity (Table 1).

It was observed that the activity of unsubstituted derivatives followed the order: methylamino<ethylamino<propylamino (MIC: 19.38–38.8 μmol × 10^{-2}/ml, 3.68–7.35 μmol × 10^{-2}/ml, 2.62–5.24 μμmol × 10^{-2}/ml and MFC: 38.8–77.52 μmol × 10^{-2}/ml, 7.35–9.19 μmol × 10^{-2}/ml, 3.50–7.0 μmol × 10^{-2}/ml respectively) which are in agreement with the above findings.

It can be noticed that compound **14** exhibited the best antifungal and strong antibacterial activity while compound **1** possessed the best antibacterial activity but the lowest antifungal potency. From ethylamino/propylamino derivatives compound **12** showed the best antibacterial but modest antifungal activity.

3. Conclusion

The newly synthesized thiazole-based chalcones **1–17** exhibit a remarkable inhibition of the growth of a wide spectrum of Gram positive, Gram negative bacteria and fungi. Almost all our compounds exhibited better antibacterial activity than reference drugs, with most potent methylamino chalcone **3** and ethylamino **12**, followed by compound **14**. The most sensitive bacterial species on these compounds is *M. flavus*, followed by *B. cereus*, while *L. monocytogenes* is the most resistant species.

As far as the fungi are concerned, the tested compounds possess excellent activity against all the fungal species tested being more active than ketoconazole and bifonazole. The most promising are compounds **3** (methylamino group), **14** and **11** (ethylamino). The most sensitive fungi is *P. funiculosum*, while *C. albicans* was the most resistant species.

All the compounds showed remarkable antimicrobial activity indicating that the diverse substitutions were well tolerated on the benzylidene moiety as well as on amino group.

The outstanding properties of this series of novel chalcones and especially of compound **3** as antimicrobial agents deserve further investigation in order to clarify the mode of action at molecular level, responsible for the activity observed.

4. Experimental

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 and were within a 0.4% of theoretical values. IR spectra were recorded, as Nujol mulls, 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). ^{13}C NMR spectra of compounds in DMSO-d_6 were recorded on a Bruker AC 300 instrument or an INOVA 600 (Varian, USA). Mass spectra were recorded on ESI-MS (Micromass ZMD Waters) at cone voltage 30 V.

The reactions were monitored by TLC on F_{254} silica-gel precoated sheets (Merck, Darmstadt, Germany) and each of the purified compounds 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-propylthiourea[27,28]

1-Propyl isothiocyanate (25.29 g, 0.25 mol), was added dropwise in NH_4OH (29 ml) and the mixture was refluxed until the excess of NH_3 was evaporated (30–45 min). Then the reaction mixture was treated with activated charcoal (0.5 g) and filtered. After cooling white crystals of 1-propylthiourea were precipitated. The product was filtered under vacuum and recrystallized from petroleum ether/ethanol.

4.2. General synthesis of 1-(4-methyl-2-(alkylamino)thiazol-5-yl)ethanones[25]

1-Alkylthiourea (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.

4.3. General procedure for the synthesis of unsubstituted/substituted (*E*)-1-(4-methyl-2-(alkylamino)thiazol-5-yl)-3-phenylprop-2-en-1-ones[25,29]

1-(4-Methyl-2-(alkylamino)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 reaction mixture was kept under stirring at 0 °C for 30 min and afterwards at room temperature for several hours (5–12 h) until solid started separating out. The solid was filtered under vacuum and recrystallized from dioxane to give the title chalcones. The yield range after recrystallization of chalcones was 18–73%.

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

Yield: 29%, mp: 189–192 °C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 2.78 (s, 3H, thiazole 4-CH_3), 2.94 (s, 3H, N–CH_3), 7.26 (d, J = 15.6 Hz, 1H, CO–CH), 7.23–7.28 (m, 2H, arom. 3 and 5), 7.53 (d, J = 15.6 Hz, 1H, Ar–CH), 7.56–7.84 (m, 2H, arom. 2 and 6). ^{13}C NMR (δ ppm, DMSO-d_6, 150 MHz): 19.02, 31.10, 115.92, 120.93, 130.66, 131.37, 139.74, 159.34, 161.90, 164.27, 171.33, 179.78. MS, (ES^+) (m/z) 299 (100% M^++Na,). (ES^-) (m/z) 276 (M^+, 16%), 275 (100%), 233 (7%). Anal. Calcd for $C_{14}H_{13}FN_2OS$ (MW 276): C, 60.85; H, 4.74; N, 10.14. Found: C, 60.90; H, 4.71; N, 10.10.

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

Yield: 67%, mp: 191–193 °C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 2.55 (s, 3H, thiazole 4-CH_3), 2.87 (s, 3H, N–CH_3), 7.25–7.65 (m, 6H, arom., alkene). ^{13}C NMR (δ ppm, DMSO-d_6, 75 MHz): 19.10, 32.23, 114.09, 114.38, 117.18, 121.63, 124.42, 130.45, 137.24, 140.96, 160.05, 164.68, 172.80, 180.60. MS (ES^+). (m/z) 299 (100%, M^++Na). (ES^-) (m/z) 276 (20%, M^+), 275 (100%). Anal. Calcd for $C_{14}H_{13}FN_2OS$ (MW 276): C, 60.85; H, 4.74; N, 10.14. Found: C, 60.81; H, 4.68; N, 10.09.

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

Yield: 73%, mp: 215–217 °C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 2.54 (s, 3H, thiazole 4-CH_3), 2.86 (s, 3H, N–CH_3), 7.38–7.58 (m, 3H, arom. 5, alkene), 7.62 (d, J = 8.4 Hz, 1H, arom. 4), 7.74 (d, J = 7.8 Hz, 1H, arom. 6), 7.99 (s, 1H, arom. 2). ^{13}C NMR (δ ppm, DMSO-d_6, 150 MHz): 19.05, 31.08, 120.93, 122.35, 126.50, 127.36, 130.72, 132.63, 137.28, 139.25, 159.73, 169.54, 171.55, 179.71. MS (ES^+). (m/z) 361 (100%, M^+ +Na), 339 (8%), 301 (8%), 193 (8%) MS (ES^-) (m/z) 339 (7%), 337 (M^+, 93%), 335 (100%). Anal. Calcd for $C_{14}H_{13}BrN_2OS$ (MW 337): C, 49.86; H, 3.89; N, 8.31. Found: C, 49.80; H, 3.83; N, 8.29.

4.3.4. (*E*)-1-(4-Methyl-2-(methylamino)thiazol-5-yl)-3-m-tolylprop-2-en-1-one (4)

Yield: 63%, mp: 186–188 °C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 2.35 (s, 3H, Ar–CH_3), 2.57 (s, 3H, thiazole 4-CH_3), 2.89 (s, 3H, N–CH_3), 7.26–7.33 (m, 3H, CO–CH, arom. 2 and 4), 7.49–7.56 (m, 3H, arom. 5 and 6, Ar–CH). ^{13}C NMR (δ ppm, DMSO-d_6, 75 MHz): 19.44, 21.78, 32.60, 122.18, 124.86, 125.87, 129.19, 129.31, 131.48, 135.31, 138.97, 143.08, 159.92, 173.08, 181.52. MS (ES^+) (m/z) 295(100%, M^++Na). MS (ES^-) (m/z) 272 (23%, M^+), 271 (100%). Anal. Calcd for $C_{15}H_{16}N_2OS$ (MW 272): C, 66.15; H, 5.92; N, 10.29. Found: C, 66.10; H, 5.97; N, 10.32.

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

Yield: 42%, mp: 186–188 °C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 2.57 (s, 3H, thiazole 4-CH_3), 2.89 (s, 3H, N–CH_3), 7.39 (d, J = 15.4 Hz, 1H, CO–CH), 7.46 (d, J = 7.9 Hz, 1H, arom. 6), 7.72 (d, J = 7.9 Hz, 1H, arom. 5), 7.82 (d, J = 15.4 Hz, 1H, Ar–CH), 7.98 (d, J = 7.7 Hz, 1H, arom. 4), 8.65 (s, 1H, NH). ^{13}C NMR (δ ppm, DMSO-d_6), 75 MHz): 19.07, 32.23, 121.90, 128.03, 128.2, 128.85, 129.62, 132.53, 132.59, 135.22, 135.55, 160.11, 173.23, 180.37. MS (m/z) 330 (17%), 329 (13%), 328 (64), 327 (M^+ 36%), 326 (92%), 298 (7%), 293 (26%), 291 (67%), 263 (13%), 228 (9%), 209 (10%), 181 (100%), 155 (26%), 136 (22%), 100 (13%). Anal. Calcd for $C_{14}H_{12}Cl_2N_2OS$ (MW 327): C, 51.39; H, 3.70; N, 8.56. Found: C, 51.35; H, 3.68; N, 8.50.

4.3.6. (*E*)-1-(2-(Ethylamino)-4-methylthiazol-5-yl)-3-phenylprop-2-en-1-one (6)

Yield: 32%, mp: 150–152 °C. ^{1}H NMR (δ δ ppm, DMSO-d_6, 300 MHz): 1.18 (t, J = 7.2 Hz, 3H, methyl NCH_2CH_3), 2.50 (s, 3H, thiazole 4-CH_3), 3.26 (q, J = 7.2 Hz, 2H, N–CH_2), 7.29 (d, J = 15.3 Hz, 1H, CO–CH), 7.43–7.44 (m, 3H, arom. 3, 4 and 5), 7.54 (d, J = 15.3 Hz, 1H, Ar–CH), 7.72–7.74 (m, 2H, arom. 2 and 6), 8.55 (s, 1H, NH). ^{13}C NMR (δ ppm, DMSO-d_6, 150 MHz): 14.03, 18.99, 39.40, 120.77, 124.87, 128.31, 128.91, 130.13, 134.66, 140.89, 159.15, 170.24, 179.87. MS (m/z) 295 (M^+ + Na, 100%) 273 (12%). Anal. Calcd for $C_{15}H_{16}N_2OS$ (MW 272): C, 66.15; H, 5.92; N, 10.29. Found: C, 66.12; H, 5.89; N, 10.31.

4.3.7. (*E*)-1-(2-(Ethylamino)-4-methylthiazol-5-yl)-3-(4-nitrophenyl)prop-2-en-1-one (7)

Yield: 58%, mp: 248–249 °C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 1.17 (t, J = 6.9 Hz, 3H, methyl NCH_2CH_3), 2.55 (s, 3H, thiazole 4-CH_3), 3.27 (q, J = 7.2 Hz, 2H, N–CH_2), 7.46 (d, J = 15.3 Hz, 1H, CO–CH), 7.59 (d, J = 16.6 Hz, 1H, Ar–CH), 8.0 (d, J = 8.7 Hz, 2H, arom. 2 and 6), 8.23 (d, J = 8.4 Hz, 2H, arom. 3 and 5), 8.70 (s, 1H, NH). ^{13}C NMR (δ ppm, DMSO-d_6, 150 MHz): 13.89, 18.97, 39.42 120.75, 123.98, 128.73, 129.30, 138.22, 141.13, 147.76, 160.28, 170.85, 179.52. MS (m/z) 340 (M^+ + Na, 100%) 329 (6%), 318 (M^+, 5%), 302 (4%), 301 (15%), 239(5%), 229 (8%), 217 (5%), 213 (5%), 207 (11%), 199 (5%), 187 (5%), 185 (9%), 177 (29%), 169 (6%), 167 (14%), 165 (13%), 159 (6%), 155 (9%), 153 (17%), 151 (24%), 149 (15%), 147 (14%), 141 (11%), 137 (12%), 135 (21%), 133 (42%). Anal. Calcd for $C_{15}H_{15}N_3O_3S$ (MW 317): C, 56.77; H, 4.76; N, 13.24. Found: C, 56.73; H, 4.80; N, 13.19.

4.3.8. (*E*)-1-(2-(Ethylamino)-4-methylthiazol-5-yl)-3-(3-nitrophenyl)prop-2-en-1-one (8)

Yield: 47%, mp: 246–247 C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 1.17 (t, J = 7.2 Hz, 3H, methyl NCH_2CH_3), 2.55 (s, 3H, thiazole 4-CH_3), 3.27 (q, J = 7.2 Hz, 2H, N–CH_2), 7.46 (d, J = 15.6 Hz, 1H, CO–CH), 7.63 (d, J = 15.3 Hz, 1H, Ar–CH), 7.70–7.73 (m, 1H, arom. 5), 8.20–8.23 (m, 2H, arom. 4 and 6), 8.55 (s, 1H, arom. 2), 8.66 (s, 1H, NH). ^{13}C NMR (δ ppm, DMSO-d_6, 150 MHz): 14.05, 19.08, 39.72, 120.64, 122.81, 124.27, 127.74, 130.38, 134.23, 136.64, 138.40, 148.37, 159.93, 170.59, 179.54. MS (m/z) 340 (M^+ + Na, 100%) 328 (4%), 326 (3%), 318 (M^+, 7%), 311 (3%), 301 (12%), 263 (3%), 257 (3%), 245 (4%), 230 (4%), 229 (8%), 217 (6%), 207 (22%), 203 (4%), 191 (6%), 185 (9%), 177 (15%), 167 (7%), 165 (13%), 159 (6%), 153 (19%). Anal. Calcd for $C_{15}H_{15}N_3O_3S$ (MW 317): C, 56.77; H, 4.76; N, 13.24. Found: C, 56.72; H, 4.79; N, 13.20.

4.3.9. (*E*)-3-(4-Chlorophenyl)-1-(2-(ethylamino)-4-methylthiazol-5-yl)prop-2-en-1-one (9)

Yield: 67%, mp: 191–293 C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 1.68 (t, J = 6.9 Hz, 3H, methyl NCH_2CH_3), 2.54 (s, 3H, thiazole 4-CH_3), 3.26 (q, J = 7.2 Hz, 2H, N–CH_2), 7.31 (d, J = 15.3 Hz, 1H, CO–CH), 7.47–7.53 (m, 3H, Ar–CH, arom. 3 and 5), 7.77 (d, J = 7.8 Hz, 2H, arom. 2 and 6). ^{13}C NMR (δ ppm, DMSO-d_6, 150 MHz): 14.01, 19.05, 39.44, 120.72, 125.59, 128.92, 130.02, 133.63, 134.57, 139.43, 159.38, 170.34, 179.67. MS (m/z) 329 (M^++Na, 100%) 307 (M^+, 7%). Anal. Calcd for $C_{15}H_{15}ClN_2OS$ (MW 306.5): C, 58.72; H, 4.93; N, 9.13. Found: C, 58.68; H, 4.90; N, 9.11.

4.3.10. (*E*)-3-(3-Chlorophenyl)-1-(2-(ethylamino)-4-methylthiazol-5-yl)prop-2-en-1-one (10)

Yield: 68%, mp: 209–212 C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 1.69 (t, J = 7.2 Hz, 3H, methyl NCH_2CH_3), 2.54 (s, 3H, thiazole 4-CH_3), 3.27 (q, J = 7.2 Hz, 2H, N–CH_2), 7.35 (d, 1H, J = 15.6 Hz, CO–CH), 7.43–7.46 (m, 3H, Ar–CH, arom. 4 and 5), 7.68–7.73 (m, 1H, arom. 6), 7.86 (s, 1H, arom. 2). ^{13}C NMR (δ ppm, DMSO-d_6, 150 MHz): 14.02, 19.03, 39.89, 120.66, 126.48, 126.94, 127.78, 129.65, 130.65, 133.72, 136.98, 139.17, 159.62, 170.46, 179.62. MS (m/z) 329 (M^+ + Na, 38%), 309 (38%), 307 (M^++H, 100%), 303 (13%), 185 (12%), 165 (3%), 153 (3%), 143 (6%). Anal. Calcd for $C_{15}H_{15}ClN_2OS$ (MW 306.5): C, 58.72; H, 4.93; N, 9.13. Found: C, 58.70; H, 4.90; N, 9.09.

4.3.11. (*E*)-3-(2-Chlorophenyl)-1-(2-(ethylamino)-4-methylthiazol-5-yl)prop-2-en-1-one (11)

Yield: 32%, mp: 185–187 C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 1.21 (t, J = 7.2 Hz, 3H, methyl NCH_2CH_3), 2.63 (s, 3H, thiazole 4-CH_3), 3.41 (q, J = 7.2 Hz, 2H, N–CH_2), 7.37 (d, J = 15.6 Hz, 1H, CO–CH), 7.43–7.57 (m, 3H, arom. 4, 5 and 6), 7.87 (d, J = 15.3 Hz, 1H, Ar–CH), 7.99–8.02 (m, 1H, arom. 3). ^{13}C NMR (δ ppm, DMSO-d_6, 150 MHz): 14.06, 19.07, 39.52, 120.70, 126.52, 126.99, 127.81, 129.70, 130.77, 133.76, 137.02, 139.21, 159.65, 170.49, 179.67. MS (m/z) 329 (M^+ + Na, 100%), 327(16%), 326 (12%), 309 (10%), 307 (21%), 276 (16%), 229 (4%), 216 (5%), 201 (4%), 174 (8%), 173 (14%), 153 (9%), 145 (10%), 135 (12%), 131 (24%), 129 (29%), 125 (12%), 123 (20%). Anal. Calcd for $C_{15}H_{15}ClN_2OS$ (MW 306.5): C, 58.72; H, 4.93; N, 9.13. Found: C, 58.76; H, 4.95; N, 9.10.

4.3.12. (*E*)-1-(2-(Ethylamino)-4-methylthiazol-5-yl)-3-(4-fluorophenyl)prop-2-en-1-one (12)

Yield: 32%, mp: 167–169 C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 1.17 (t, J = 7.2 Hz, 3H, methyl NCH_2CH_3), 2.55 (s, 3H, thiazole 4-CH_3), 3.28 (q, J = 6.9 Hz, 2H, N–CH_2), 7.24–7.30 (m, 3H, CO–CH, arom. 3 and 5), 7.54 (d, J = 15.6 Hz, 1H, Ar–CH), 7.81–7.86 (m, 2H, arom. 2 and 6), 8.62 (s, 1H, NH). ^{13}C NMR (δ ppm, DMSO-d_6, 150 MHz): 14.05, 19.01, 39.91, 115.88, 120.65, 130.62, 131.35, 139.64, 159.20, 162.27, 163.91, 170.22, 179.72. MS (m/z) 313 (M^++Na, 100%) 291 (M^+, 6.5%), 231 (4%), 229 (9%), 209 (29%), 201 (5%), 199 (10%), 197 (16%), 195 (12%), 187 (5%), 183 (9%), 177 (8%), 167 (5%), 165 (15%), 159 (6%), 155 (9%), 153 (23%), 151 (65%), 149 (8%), 141 (8%), 128 (9%), 127 (31%), 121 (11%). (Anal. Calcd for $C_{15}H_{15}FN_2OS$ (MW 290): C, 62.05; H, 5.21; N, 9.65. Found: C, 62.07; H, 5.19; N, 9.61.

4.3.13. (*E*)-1-(2-(Ethylamino)-4-methylthiazol-5-yl)-3-(3-fluorophenyl)prop-2-en-1-one (13)

Yield: 37%, mp: 177–179 C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 1.18 (t, J = 7.2 Hz, 3H, methyl NCH_2CH_3), 2.55 (s, 3H, thiazole 4-CH_3), 3.28 (q, J = 7.2 Hz, 2H, N–CH_2), 7.22–7.27 (m, 1H, arom. 2), 7.36 (d, J = 15.3 Hz, 1H, CO–CH), 7.43–7.51 (m, 2H, Ar–CH, arom. 4), 7.55–7.59 (m, 1H, arom. 5), 7.67 (d, J = 10.2 Hz, 1H, arom. 6). ^{13}C NMR (δ ppm, DMSO-d_6, 150 MHz): 14.05, 19.06, 39.48, 114.45, 116.77, 120.78, 124.77, 126.39, 130.85, 137.31, 139.48, 159.57, 161.29, 163.71, 179.75. MS (m/z) 313 (M^+ + Na, 100%) 291 (M^+, 2%), 207 (4%), 121 (2%), 117 (4%). Anal. Calcd for $C_{15}H_{15}FN_2OS$ (MW 290): C, 62.05; H, 5.21; N, 9.65. Found: C, 62.09; H, 5.19; N, 9.60.

4.3.14. (*E*)-3-(3-Bromophenyl)-1-(2-(ethylamino)-4-methylthiazol-5-yl)prop-2-en-1-one (14)

Yield: 47%, mp: 171–172 C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 1.19 (t, J = 6.9 Hz, 3H, methyl NCH_2CH_3), 2.55 (s, 3H, thiazole 4-CH_3), 3.30 (q, J = 7.2 Hz, 2H, N–CH_2), 7.33 (d, J = 15.6 Hz, 1H, CO–CH), 7.36–7.41 (m, 1H, arom. 5), 7.49 (d, J = 15.3 Hz, 1H, Ar–CH), 7.60 (d, J = 8.1 Hz, 2H, arom. 4), 7.73 (d, J = 7.8 Hz, 2H, arom. 6), 7.96 (s, 1H, arom. 2), 8.51 (s, 1H, NH). ^{13}C NMR (δ ppm, DMSO-d_6, 150 MHz): 14.05, 19.05, 39.46, 120.72, 122.35, 126.49, 127.32, 130.71, 130.96, 132.61, 137.27, 139.19, 159.63, 170.51, 179.71. MS (ES^+) (m/z) 376 (18%), 375 (100%), 374 (18% M^+ + Na), 373 (97%), 353 (8%). Anal. Calcd for $C_{15}H_{15}BrN_2OS$ (MW 351): C, 51.29; H, 4.30; N, 7.98. Found: C, 51.25; H, 4.28; N, 7.95.

4.3.15. (*E*)-1-(2-(ethylamino)-4-methylthiazol-5-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (15)

Yield: 18%, mp: 175–176 C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 1.18 (t, J = 7.2 Hz, 3H, methyl NCH_2CH_3), 2.54 (s, 3H, thiazole 4-CH_3), 3.27 (q, J = 7.2 Hz, 2H, N–CH_2), 3.81 (s, 3H, OCH_3), 6.99 (d, J = 8.4 Hz, 2H, arom. 3 and 5), 7.14 (d, J = 15.3 Hz, 1H, CO–CH), 7.51 (d, J = 15.3 Hz, 1H, Ar–CH), 7.69 (d, J = 8.7 Hz, 2H, arom. 2 and 6). ^{13}C NMR (δ ppm, DMSO-d_6, 150 MHz): 14.10, 18.98, 39.43, 55.36, 114.47, 120.85, 122.43, 127.28, 130.20, 141.01, 158.72, 161.06, 170.10, 180.05. MS (m/z) 325 (M^++Na, 100%), 305(5%), 303(M^+, 10%), 161 (5%), 153 (6%), 143 (5%), 131 (5%), 119 (5%), 117 (8%). Anal. Calcd for $C_{16}H_{18}N_2O_2S$ (MW 302): C, 63.55; H, 6.00; N, 9.26. Found: C, 63.50; H, 6.06; N, 9.26.

4.3.16. (*E*)-1-(2-(ethylamino)-4-methylthiazol-5-yl)-3-(2-methoxyphenyl)prop-2-en-1-one (16)

Yield: 19%, mp: 161–163 C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 1.17 (t, J = 7.2 Hz, 3H, methyl NCH_2CH_3), 2.55 (s, 3H, thiazole 4-CH_3), 3.28 (q, J = 6.9 Hz, 2H, N–CH_2), 3.89 (s, 3H, OCH_3), 6.98–7.12 (m, 2H, arom. 3 and 5), 7.34–7.44 (m, 2H, CO–CH, arom. 4), 7.71–7.80 (m, 2H, Ar–CH, arom. 6), 8.60 (s, 1H, NH). MS (m/z) 303 (M^+,100%), 179 (4%), 157 (4%), 155 (6%), 151 (6%). Anal. Calcd for $C_{16}H_{18}N_2O_2S$ (MW 302): C, 63.55; H, 6.00; N, 9.26. Found: C, 63.58; H, 6.03; N, 9.23.

4.3.17. (*E*)-1-(4-methyl-2-(propylamino)thiazol-5-yl)-3-phenylprop-2-en-1-one (17)

Yield: 41%, mp: 150–152 C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 0.93 (t, J = 7.2 Hz, 3H, methyl $NCH_2CH_2CH_3$), 1.60 (sextet, J = 7.2 Hz, 2H, β-methylene $NCH_2CH_2CH_3$), 2.55 (s, 3H, thiazole 4-CH_3), 3.24 (t, 2H, α-methylene $NCH_2CH_2CH_3$), 7.28 (d, J = 15.3 Hz, 1H, CO–CH), 7.43–7.44 (m, 3H, arom. 3, 4 and 5), 7.54 (d, J = 15.6 Hz, 1H, Ar-–CH), 7.71–7.72 (m, 2H, arom. 2 and 6), 8.51 (s, 1H, NH). ^{13}C NMR (δ ppm, DMSO-d_6, 150 MHz): 11.35, 19.03, 21.73, 46.49, 120.73, 124.91, 128.39, 128.96, 130.17, 134.69, 140.90, 159.23, 170.57, 179.89. MS (ES^+) (m/z) 309 (100%, M^++Na), 287 (5%), MS (ES^-) (m/z) 286 (M^+, 20%), 285 (100%). Anal. Calcd for $C_{16}H_{18}N_2OS$ (MW 286): C, 67.10; H, 6.33; N, 9.78. Found: C, 67.14; H, 6.30; N, 9.81.

4.4. Biological evaluation

4.4.1. Antibacterial activity

The following Gram-negative bacteria were used: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Enterococcus faecalis* (human isolate) and the following Gram-positive bacteria: *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), *Listeria 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.

4.4.2. Microdilution test

The minimum inhibitory and bactericidal concentrations (MICs and MBCs) were determined using 96-well microtitre plates. The bacterial suspension was adjusted with sterile saline to a concentration of 1.0×10^5 CFU/ml. Compounds to be investigated were disolved 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 microtitre 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 in sterile physiological saline). DMSO was used as a negative control.

All experiments were performed in duplicate and repeated three times.

4.4.3. Antifungal activity

For the antifungal bioassays, eight fungi were used: *Aspergillus ochraceus* (ATCC 12066), *Aspergillus fumigatus* (human isolate), *Aspergillus niger* (ATCC 6275), *Aspergillus flavus* (ATCC 9643), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112), *Trichoderma viride* (IAM 5061) and *Candida albicans* (human isolate). 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.[30] In order to investigate the antifungal activity of the compounds, a modified microdilution technique was used.[31–33] 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 disolved 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.

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