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Thiazole-based aminopyrimidines and N-phenylpyrazolines as potent antimicrobial agents. Synthesis and biological evaluation.

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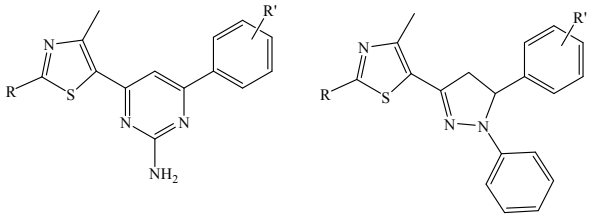

	R	R´
	Pyrimidines	
3a	$NHCH_3$	H
3b	Ph	H
	Pyrazolines	
4a	$NHCH_3$	H
4b	$NHCH_2CH_3$	H
4c	Ph	H
4d	$NHCH_3$	4-CH_3
4e	$NHCH_3$	4-OCH_3
4f	$NHCH_3$	4-NO_2
4g	$NHCH_3$	4-F
4h	$NHCH_3$	4-Cl

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A series of eight thiazole-based N-phenylpyrazolines and two aminopyrimidines having as precursors several chalcone derivatives have been synthesized and evaluated for their antimicrobial activity. All compounds showed antimicrobial activity. The best activity was achieved for compounds 3a and 3b, while compounds 4d and 4g showed the lowest antimicrobial potential.

1. Introduction

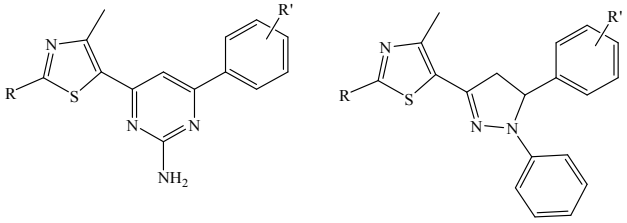
Despite the success to date in development of antimicrobial agents, the inexorable, ongoing emergence of resistance worldwide continues to spur the search for novel compounds to replace or supplement conventional antibiotics and antifungals. [1-6] In addition, the need for effective antimicrobial drugs became even greater considering the difficulties of dealing with the treatment of infections of hospitalized patients and protection of immunosuppressed and HIV-infected patients.[7-8]

Thus, the challenge of developing new categories of antimicrobial agents reignited the interest of the scientists during the last decades. A potential approach is the design of innovative drugs, with different mechanisms of action, in an attempt to avoid cross resistance to existing therapeuticals (e.g. linezolid).[9-11]

N-phenylpyrazolines and aminopyrimidines display a broad spectrum of potential pharmacological activities such as antimicrobial,[12-15] anti-inflammatory,[16-17] antileishmanial,[18-19] antidepressant[20] etc.

Taking also into account our promising findings regarding the antibacterial and antifungal activities of thiazole derivatives,[21-25] a series of ten structurally new compounds incorporating thiazole and the above mentioned aza-heterocyclic moieties were designed and synthesized.

Eight thiazole-based N-phenylpyrazolines and two aminopyrimidines (Figure 1) were synthesized having as precursors several chalcone derivatives. The title compounds were tested for their in vitro antimicrobial properties against Gram positive and Gram negative bacteria and also a series of fungi.



	R	R´
	Pyrimidines	
3a	$NHCH_3$	H
3b	Ph	H
	Pyrazolines	
4a	$NHCH_3$	H
4b	$NHCH_2CH_3$	H
4c	Ph	H
4d	$NHCH_3$	4-CH_3
4e	$NHCH_3$	4-OCH_3
4f	$NHCH_3$	4-NO_2
4g	$NHCH_3$	4-F
4h	$NHCH_3$	4-Cl

Fig. 1 Synthesized compounds

2. Results and discussion

2.1. Chemistry

The synthesis of the target N-phenylpyrazolines and aminopyrimidines was accomplished by heterocyclization of corresponding chalcones with phenylhydrazine hydrochloride and guanidine hydrochloride in the presence of NaOH respectively (Scheme 1). The reactions proceed smoothly and in good yields for the majority of the compounds.

Structures of synthesized compounds **3a-b** and **4a-h** were satisfactorily confirmed by IR, ^{1}H NMR and elemental analysis.

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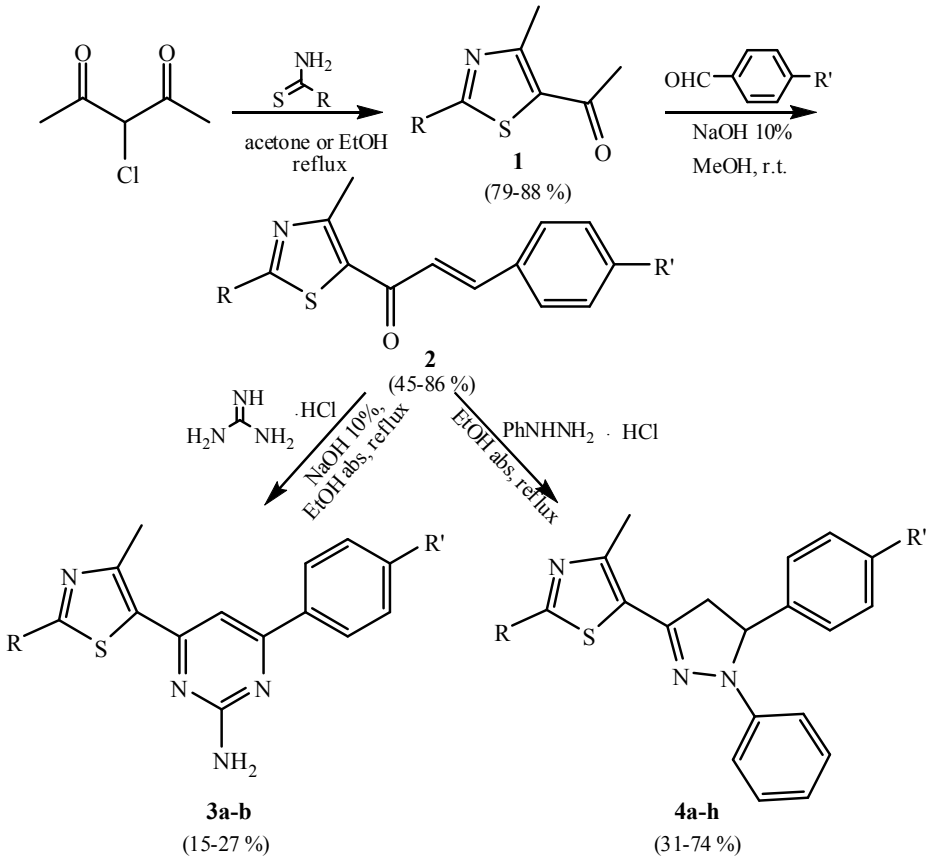

Scheme 1 Synthesis of title compounds

In IR spectra were observed absorptions at 1592-1596 cm^{-1} (C=C arom.), 1638-1644 cm^{-1} (C=N) and sharp bands at 3156-3164 (NH). As far as the aminopyrimidine derivatives are concerned, the characteristic band of primary amine (NH_2) was observed at 3311-3384 cm^{-1}. In the ^1H NMR spectra, chemical shifts of the final compounds appeared in the region of δ 2.35-2.88 (thiazole 4-CH_3), 3.04-3.26 (NCH_3), 3.09-3.73 ($C\underline{H}_A\underline{H}_BC{=}N$, compounds 4a-h), 3.82-4.06 ($CH_A\underline{H}_BC{=}N$, compounds 4a-h).

2.2. Biological evaluation

2.2.1. Antimicrobial activity

The synthesized thiazole-based aminopyrimidines and N-phenylpyrazolines were assayed *in vitro* for their antibacterial and antifungal activity against Gram positive, Gram negative bacteria and molds obtained from ATCC-American Type of Culture Collection. These bacterial and fungal species are chosen because of their properties; among them are plant, animal and human pathogenic species, also food spoilage and contaminators, mycotoxin producers could be found. More than 90 percent of the cases of food poisoning each year are caused by *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Bacillus cereus*, and entero-pathogenic *Escherichia coli*.[26] *Pseudomonas aeruginosa,* as an example, is an opportunistic human pathogen that infects immunocompromised individuals and people with cystic fibrosis. This bacterium has low sensitivity to antibiotic treatment and has become multidrug resistant, which causes an increasing public health threat. No new broad spectrum antibiotics have been developed recently for most Gram negative bacteria.[27]Moulds have various health effects. Excessive mould growth in the human environment needs to be taken care of, regardless of the species, as it may lead to increased number of allergy cases, toxicity, and house/building structural problems. Some *Aspergillus, Penicillium, Trichoderma, Fusarium, Alternaria* species are able to produce mycotoxins that are potent hepatocarcinogens in animals and humans. Therefore, the presence of toxigenic fungi in foods, grains, environment presents a potential hazard to human and animal health.[28] Minimal inhibitory concentrations that inhibited the growth of the tested microorganisms (MIC) as well as 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 1, along with those of reference drugs Ampicillin and Streptomycin. [22]

Almost all tested compounds were potent against all the used bacteria with minimal inhibitory concentrations (MIC) at range of 1.45-44.16 x 10^{-2} µmol/ml and minimal bactericidal concentrations (MBC) at 5.80-95.5 x 10^{-2} µmol/ml. All tested compounds showed inhibitory activity in concentration at 1.45-27.6 x 10^{-2} µmol/ml towards Gram (+) bacteria, and 2.73-44.16 x 10^{-2} µmol/ml towards Gram (-) bacteria. Bactericidal effect was achieved at 5.80-95.5 x 10^{-2} µmol/ml against Gram (+) bacteria and 6.74-58.6 x 10^{-2} µmol/ml on Gram (-) bacteria but the majority of tested compounds did not influenced *E. facealis* and *E. cloacae*, which are also Gram (-) bacteria. So, it can be seen that tested compounds were more potent against Gram (+) than Gram (-) bacterial species. It should be mentioned that their activity was comparable to that of Ampicillin with MIC at 24.8–74.4 x 10^{-2} µmol/ml and bactericidal at 37.2–124.0 x 10^{-2} µmol/ml.

Table 1 Antibacterial activity of title compounds towards Gram (+) bacteria (MIC and MBC in µmol/ml x 10^{-2})

Compounds		*S.a.*	*B.c.*	*M.f*	*L. m.*
3a	**MIC**	8.41±0.02^{b}	3.37±0.02^{a}	3.37±0.02^{a}	10.10±0.10^{c}
	MBC	10.10±0.03^{b}	6.74±0.10^{a}	8.41±0.10^{a}	11.81±0.10^{a}
3b	**MIC**	1.45±0.00^{a}	5.80±0.10^{a}	5.80±0.10^{a}	1.45±0.00^{a}
	MBC	5.80±0.07^{a}	40.60±0.10^{d}	29.00±0.00^{d}	36.25±0.00^{b}
4a	**MIC**	11.50±0.07^{b}	23.00±0.70^{c}	11.50±0.00^{b}	5.70±0.10^{b}
	MBC	20.10±0.00^{c}	28.70±0.00^{bc}	23.00±0.30^{c}	11.50±0.20^{a}
4b	**MIC**	2.76±0.01^{a}	5.52±0.07^{a}	11.04±0.01^{b}	5.52±0.07^{b}
	MBC	27.60±0.10^{d}	27.60±0.10^{b}	44.16±0.05^{f}	11.04±0.01^{a}
4c	**MIC**	12.6±0.20^{c}	15.20±0.07^{b}	12.60±0.02^{b}	7.60±0.10^{b}
	MBC	20.20±0.07^{d}	30.4 ±0.10^{c}	20.20±0.07^{c}	10.10±0.00^{a}
4d	**MIC**	11.04±0.00^{b}	5.52±0.07^{a}	27.60±0.10^{c}	9.60±0.10^{bc}
	MBC	27.60±0.10^{d}	27.60±0.10^{b}	58.60±0.20^{g}	11.04±0.00^{a}
4e	**MIC**	10.60±0.20^{b}	15.80±0.10^{b}	10.60±0.20^{b}	5.30±0.00^{a}
	MBC	15.80±0.07^{bc}	26.40±0.10^{b}	15.80±0.10^{b}	10.60±0.20^{a}
4f	**MIC**	10.20±0.07^{b}	20.30±0.10^{c}	12.70±0.10^{b}	7.60±0.10^{b}
	MBC	20.30±0.03^{c}	25.40±0.10^{b}	20.30±0.00^{c}	10.20±0.07^{a}
4g	**MIC**	2.73±0.01^{a}	5.46±0.02^{a}	5.46±0.00^{a}	5.46±0.20^{b}
	MBC	27.30±0.00^{d}	27.30±0.10^{b}	47.80±0.30^{f}	95.50±0.20^{e}
4h	**MIC**	10.50±0.20^{bc}	15.70±0.07^{b}	10.50±0.02^{b}	5.20±0.07^{a}
	MBC	21.00±0.50^{c}	31.40±0.10^{c}	21.00±0.10^{cd}	10.50±0.20^{a}
Ampi-cilin	**MIC**	24.80±0.07^{d}	24.80±0.10^{c}	24.80±0.02^{c}	37.20±0.07^{e}
	MBC	37.20±0.00^{e}	37.20±0.07^{d}	37.20±0.07^{e}	74.40±0.10^{d}
Strepto-mycin	**MIC**	17.20±0.07^{cd}	4.30±0.10^{a}	8.60±0.10^{ab}	25.80±0.10^{d}
	MBC	34.40±0.10^{e}	8.60±0.00^{a}	17.20±0.00^{c}	51.60±0.20^{c}

S. a.-Staphylococcus aureus (ATCC 6538); *B. c.-Bacillus cereus* (clinical isolate); *M. f.-Micrococcus flavus* (ATCC 10240); *L. m.-Listeria monocytogenes* (NCTC 7973);

Streptomycin showed MIC in range of 4.3-25.8 x 10^{-2} µmol/ml and MBC of 8.6-51.6 x 10^{-2} µmol/ml. However, it should be noticed that some compounds showed activity against some bacterial species better than streptomycin.

The best inhibitory effect exhibited compound **3b** with inhibitory activity at 1.45-5.8 x 10^{-2} µmol/ml against Gram (+) bacteria. Compound **4g** exhibited also very high inhibitory activity on all bacterial species, but bactericidal effect was higher than that for other compounds esspecialy against *L. monocytogenes* where MBC was 27.3-95.5 x 10^{-2} µmol/ml. In general compound **3a** showed the best antibacterial activity on both Gram (+) and Gram (-) bacterial species. The lowest antibacterial activity was observed for compound **4d** with MIC at 5.50-44.1 x 10^{-2} µmol/ml and MBC, 11.04-58.6 x 10^{-2} µmol/ml, and this compound was inactive against bacteria *En. cloacae*.

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Table 2 Antibacterial activity of title compounds towards Gram (-) bacteria (MIC and MBC in µmol/ml x 10^{-2})

Compounds		*Ps. aer.*	*S. typh.*	*E.coli*	*En.f.*	*En. cl.*
3a	**MIC**	3.37±0.02^{a}	3.37±0.02^{a}	3.37±0.00^{a}	nt	6.74±0.08^{a}
	MBC	6.74±0.08^{a}	6.74±0.02^{a}	6.74±0.00^{a}	nt	8.41±0.10^{a}
3b	**MIC**	29.00±0.30^{d}	29.00±0.00^{d}	34.80±0.10^{d}	29.00±0.07^{c}	nt
	MBC	40.60±0.00^{d}	34.80±0.30^{d}	40.60±0.20^{d}	34.80±0.00^{c}	nt
4a	**MIC**	14.40±0.10^{b}	17.20±0.07^{bc}	17.20±0.00^{c}	nt	14.40±0.10^{bc}
	MBC	45.90±0.10^{d}	28.70±0.02^{d}	20.10±0.03^{b}	nt	20.10±0.00^{bc}
4b	**MIC**	27.60±0.00^{d}	27.60±0.10^{d}	27.60±0.10^{cd}	11.04±0.10^{b}	nt
	MBC	44.16±0.05^{d}	30.90±0.20^{d}	44.16±0.05^{d}	27.6±0.10^{b}	nt
4c	**MIC**	15.20±0.07^{b}	20.20±0.07^{c}	10.10±0.07^{b}	nt	12.60±0.20^{b}
	MBC	25.30±0.10^{b}	25.30±0.10^{c}	17.70±0.20^{b}	nt	17.70±0.10^{b}
4d	**MIC**	27.60±0.20^{d}	11.04±0.10^{b}	27.60±0.10^{cd}	44.16±0.05^{d}	nt
	MBC	33.12±0.04^{c}	27.60±0.20^{cd}	58.60±0.20^{e}	58.64±0.20^{d}	nt
4e	**MIC**	15.80±0.10^{b}	18.50±0.20^{bc}	18.50±0.20^{c}	nt	13.20±0.07^{b}
	MBC	26.40±0.10^{b}	26.40±0.00^{c}	21.10±0.00^{b}	nt	18.50±0.20^{b}
4f	**MIC**	20.30±0.10^{cd}	15.20±0.10^{b}	17.80±0.00^{c}	nt	12.70±0.10^{b}
	MBC	25.40±0.10^{b}	25.40±0.10^{c}	20.30±0.10^{b}	nt	20.30±0.10^{bc}
4g	**MIC**	2.73±0.01^{a}	5.46±0.02^{a}	5.46±0.02^{a}	5.46±0.20^{a}	nt
	MBC	27.30±0.10^{b}	27.30±0.10^{cd}	47.80±0.00^{d}	27.30±0.10^{b}	nt
4h	**MIC**	15.20±0.07^{b}	15.20±0.07^{b}	18.30±0.10^{c}	nt	13.10±0.10^{b}
	MBC	31.40±0.10^{c}	18.30±0.10^{b}	21.00±0.30^{b}	nt	18.30±0.10^{b}
Ampi-cilin	**MIC**	74.40±0.10^{e}	24.80±0.00^{c}	37.20±0.07^{d}	24.80±0.30^{c}	24.80±0.20^{c}
	MBC	124.00±0.70^{e}	49.20±0.07^{e}	49.20±0.07^{d}	37.20±0.07^{c}	37.20±0.07^{c}
Strepto-mycin	**MIC**	17.20±0.00^{c}	17.20±0.07^{bc}	17.20±0.00^{c}	4.30±0.10^{a}	4.30±0.01^{a}
	MBC	34.40±0.00^{cd}	34.40±0.10^{d}	34.40±0.10^{c}	8.60±0.10^{a}	8.60±0.10^{a}

Ps. aer.-Pseudomonas aeruginosa (ATCC 27853); *S. typh.-Salmonella typhimurium* (ATCC 13311); *E. coli-Escherichia coli* (ATCC 35210); *En. f.-Enterococcus faecalis* (human isolate); *En. cl-Enterobacter cloacae* (human isolate).

The most sensitive bacterial species on these compounds are *Listeria monocytogenes* (with exception for compound **4g**) *En. faecalis* and *En. cloacae* are the more resistant species.

Thus, all the compounds showed stronger antibacterial effect than streptomycin against *S. aureus* and *L. monocytogenes* (except **4g**), as well as against *P. aeruginosa, S. typhimurium* and *E. coli.* Almost all compounds showed higher antibacterial effect than Ampicillin, with some exception (Table 1, 2).

The SAR studies revealed that substitution both in thiazole as well as in benzene ring played important role on the antibacterial activity of the tested compounds. Thus, it was observed that replacement of methylamino group in compound **4a** with ethylamino group (**4b**) led to decrease of antibacterial potential, while introduction of phenyl ring had the opposite effect. Thus, the activity observed among these three compounds followed the order **4c>4a>4b**. In case of methylamino derivatives, the introduction of 4-OMe, 4-Cl and 4-NO_2 (**4e, 4f and 4h**) slightly increased the activity compared to compound **4a**, while introduction of 4-Me or 4-F- substituent (**4d, 4g**) had the opposite effect.

The results of antifungal activity of compounds **3a-b, 4a-h** tested by microdilution method are presented in Tables 3, 4. Minimal inhibitory concentration (MIC) is at range of 1.45-47.80 x 10^{-2} µmol/ml and minimal fungicidal concentration (MFC) is in range of 3.37-95.5 x 10^{-2} µmol/ml.

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Table 3 Antifungal activity of title compounds (MIC and MFC in μmol/ml x 10^{-2})

Compounds		*A.o.*	*A.ver.*	*A.fl.*	*A.n.*	*A.fum.*
3a	**MIC**	3.37±0.10^{a}	nt	5.05±0.02^{a}	5.05±0.02^{a}	5.05±0.02^{a}
	MFC	6.74±0.10^{a}	nt	6.74±0.30^{a}	6.74±0.30^{a}	6.74±0.20^{a}
3b	**MIC**	1.45±0.20^{a}	1.45±0.00^{a}	5.80±0.20^{a}	5.80±0.20^{a}	5.80±0.00^{a}
	MFC	5.80±0.30^{a}	9.00±0.20^{c}	29.00±0.30^{b}	40.60±0.20^{d}	34.80±0.30^{e}
4a	**MIC**	14.3±0.10^{c}	14.30±0.10^{c}	nt	14.30±0.10^{b}	14.30±0.10^{b}
	MFC	17.2±0.07^{c}	23.00±0.00^{b}	nt	28.70±0.20^{b}	23.00±0.00^{d}
4b	**MIC**	2.76±0.00^{a}	5.52±0.20^{b}	11.04±0.01^{b}	30.90±0.10^{d}	11.04±0.01^{b}
	MFC	11.04±0.10^{b}	27.60±0.20^{bc}	27.60±0.2^{b}	44.16±0.20^{d}	44.16±0.05^{f}
4c	**MIC**	12.60±0.20^{c}	12.60±0.20^{c}	nt	25.30±0.10^{c}	12.60±0.20^{b}
	MFC	20.20±0.07^{c}	15.20±0.07^{a}	nt	32.20±0.07^{c}	15.20±0.07^{c}
4d	**MIC**	9.60±0.10^{b}	5.52±0.07^{b}	11.04±0.00^{b}	44.16±0.05^{e}	27.60±0.10^{c}
	MFC	11.04±0.00^{b}	27.60±0.20^{bc}	27.60±0.20^{b}	58.60±0.20^{e}	44.16±0.05^{f}
4e	**MIC**	13.20±0.07^{c}	13.20±0.07^{c}	nt	13.20±0.07^{b}	26.40±0.10^{c}
	MFC	13.20±0.07^{b}	21.10±0.03^{b}	nt	26.40±0.10^{b}	37.00±0.00^{e}
4f	**MIC**	12.70±0.20^{c}	25.40±0.00^{d}	nt	25.40±0.10^{c}	12.70±0.20^{b}
	MFC	20.30±0.10^{c}	32.20±0.07^{c}	nt	32.20±0.07^{c}	15.20±0.07^{c}
4g	**MIC**	5.46±0.20^{ab}	5.46±0.02^{b}	5.46±0.05^{a}	47.80±0.00^{e}	5.46±0.20^{a}
	MFC	27.30±0.10^{c}	21.40±0.10^{b}	47.80±0.20^{c}	95.50±0.20^{g}	10.92±0.30^{b}
4h	**MIC**	13.10±0.03^{c}	13.10±0.03^{c}	nt	13.10±0.03^{b}	13.10±0.03^{b}
	MFC	15.70±0.20^{bc}	21.00±0.00^{b}	nt	26.20±0.07^{b}	26.20±0.07^{c}
Ketoco-nazole	**MIC**	38.00±0.10^{d}	285.00±1.60^{f}	285.00±1.70^{d}	38.00±0.20^{c}	38.00±0.00^{d}
	MFC	95.00±0.00^{e}	380.00±1.70^{e}	380.00±1.70^{e}	95.00±0.00^{g}	95.00±0.03^{h}
Bifona-zole	**MIC**	48.00±0.00^{e}	48.00±0.20^{e}	48.00±0.20^{c}	48.00±0.20^{e}	48.00±0.00^{e}
	MFC	80.00±1.60^{d}	64.00±0.30^{d}	64.00±0.00^{d}	64.00±0.20^{f}	64.00±0.30^{g}

A.o.-Aspergillus ochraceus (ATCC 12066); *A. v.-Aspergillus versicolor* (ATCC 11730); *A. fl.-Aspergillus flavus* (ATCC 9643);); *A. n.- Aspergillus niger* (ATCC 6275), *A. fum.-Aspergillus fumigatus* (human isolate);

The best antifungal potential was obtained for compound **3a** which exhibited the strongest antifungal activity with MIC of 1.45-5.05 μmol x 10^{-2}/ml and MFC of 3.37-6.74 μmol x 10^{-2}/ml. This compound did not influenced *A. versicolor* et al. Compound **4g** showed the lowest antifungal activity with MIC 5.46-47.8 μmol x 10^{-2}/ml and fungicidal potential MFC at 10.92-95.5 μmol x 10^{-2}/ml. All the compounds exhibited the best activity against *A. ochraceus* and *A. versicolor,* while *A. niger* and *C. albicans* were the most resistant species. It should be mentioned that all the title compounds were much more potent than commercial antifungals, ketoconazole with fungistatic activity at 38.0-475.0 x 10^{-2} μmol/ml and fungicidal effect at 38.0-475.0 x 10^{-2} μmol/ml, while MIC of bifonazole was in range at 32.0-.64.0 x 10^{-2} μmol/ml and MFC at 95.0-570.0 x 10^{-2} μmol/ml.

Compound **3b** exhibited the best activity against *A. ochraceus* (MIC at 1.45 μmol x10^{-2} /ml and MFC at 5.8 μmol x10^{-2} /ml), while the highest potential against *A.versicolor* was observed for compound **4g** followed by **4d** and **4b.** As far as *A. flavus* is concerned, the most active appeared to be compound **3a** followed by **4d** and **4b**, while **3a** was the best against *A. fumigatus*. Compounds **4a, 4c** and **4f** were also potent against previously mentioned fungus.

The relationship between structure and antifungal activity revealed that for compounds **4a-c** the order of their activity is in agreement with that observed for antibacterial potential (**4c>4a>4b**). It was found that the presence of methoxy- and chloro- substituents at the benzene ring is endowed with higher potential for methylamino derivatives. On the other hand, 4-Me, 4-NO_2 and 4-F (**4d, 4f and 4g**) groups had negative effect compared to parent compound (**4a).**

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Table 4 Antifungal activity of title compounds (MIC and MFC in μmol/ml x 10^{-2})

Compounds		*T.v.*	*P.o.*	*P.f.*	*C.a.*
3a	**MIC**	2.52±0.20^{a}	3.37±0.10^{a}	3.37±0.02^{a}	3.37±0.10^{a}
	MFC	3.37±0.10^{a}	5.05±0.20^{a}	6.74±0.20^{a}	6.74±0.20^{a}
3b	**MIC**	1.45±0.00^{a}	34.80±0.30^{d}	34.80±0.30^{d}	nt
	MFC	29.00±0.03^{cd}	40.60±0.7^{e}	34.80±0.30^{c}	nt
4a	**MIC**	28.70±0.20^{d}	14.30±0.10^{b}	28.70±0.10^{c}	17.20±0.07^{b}
	MFC	40.20±0.07^{e}	28.70±0.20^{c}	40.20±0.07^{d}	23.00±0.00^{b}
4b	**MIC**	27.60±0.20^{d}	30.90±0.30^{d}	11.04±0.10^{b}	nt
	MFC	30.90±0.03^{d}	44.16±0.05^{e}	30.90±0.30^{c}	nt
4c	**MIC**	12.60±0.10^{c}	25.30±0.10^{c}	25.30±0.10^{c}	25.30±0.10^{c}
	MFC	25.30±0.10^{c}	32.20±0.07^{d}	32.20±0.07^{c}	30.40±0.10^{c}
4d	**MIC**	5.52±0.20^{b}	27.60±0.00^{c}	27.60±0.20^{c}	nt
	MFC	11.04±0.01^{b}	44.60±0.10^{e}	44.16±0.050^{d}	nt
4e	**MIC**	13.20±0.07^{c}	26.4±0.10^{c}	26.40±0.00^{c}	26.40±0.10^{c}
	MFC	13.20±0.07^{b}	37.00±0.00^{d}	37.00±0.00^{cd}	37.00±0.30^{cd}
4f	**MIC**	12.70±0.00^{c}	25.40±0.10^{c}	25.40±0.10^{c}	30.50±0.20^{cd}
	MFC	15.20±0.07^{b}	32.20±0.07^{d}	32.20±0.07^{c}	40.60±0.20^{d}
4g	**MIC**	10.92±0.03^{c}	21.40±0.10^{c}	10.92±0.30^{b}	nt
	MFC	47.80±0.03^{e}	47.80±0.03^{e}	27.30±0.10^{b}	nt
4h	**MIC**	13.10±0.03^{c}	13.10±0.00^{b}	26.20±0.07^{c}	15.70±0.10^{b}
	MFC	21.00±0.03^{c}	15.70±0.20^{b}	36.70±0.20^{c}	26.2±0.07^{bc}
Ketoco-nazole	**MIC**	475.00±1.70^{g}	380.0±1.70^{f}	38.00±0.00^{d}	37.60±0.00^{d}
	MFC	570.00±1.70^{g}	380.0±1.70^{g}	95.00±0.30^{f}	94.00±0.30^{e}
Bifona-zole	**MIC**	64.0±0.00^{e}	48.0±0.00^{e}	64.00±0.20^{e}	32.20±0.07^{cd}
	MFC	80.0±0.03^{f}	64.0±0.03^{f}	80.00±1.00^{e}	48.30±0.10^{d}

T. v.-Trichoderma viride (IAM 5061); *P. o.-Penicillium ochrochloron* (ATCC 9112); *P. f.- Penicillium funiculosum* (ATCC 36839); *C. a.-Candida albicans* (human isolate).

3. 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. Wave numbers in the IR spectra are given in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra of the newly synthesized compounds, in DMSO-d_6 or $CDCl_3$ 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). 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).

3.1. General synthesis of 1-(4-methyl-2-(alkylamino)thiazol-5-yl)ethanones[22]

1-Alkylthiourea (0.02 mol) was dissolved in acetone (50 ml). 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 re-crystallized from ethanol.

3.2. General synthesis of 1-(4-methyl-2-phenylthiazol-5-yl)ethanone [21, 22]

Thiobenzamide (2.74 g, 0.02 mol) was dissolved in absolute ethanol (15 ml). 3-chloroacetylacetone (2.26 ml, 0.02 mol), diluted in absolute ethanol (2 ml) was added dropwise and mixture was refluxed for 3 h. After cooling at room temperature, solution was transferred to an ice bath and H_2O (15 ml) was added under continuous stirring. The precipitate was filtered under vacuum and re-crystallized from petroleum ether/ethanol.

3.3. General procedure for the synthesis of chalcone precursors[21, 22]

1-(4-Methyl-2-(alkylamino/phenyl)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 ºC 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, washed with H_2O and re-crystallized from dioxane to give the corresponding chalcones.

3.4. General procedure for the synthesis of aminopyrimidine derivatives (3a-b) [18, 19]

Mixture of corresponding chalcones (0.01 mol) and guanidine hydrochloride (1.433 g, 0.015 mol) was dissolved under stirring in the minimum quantity of hot absolute ethanol. Solution 10% NaOH was added dropwise until pH became basic and reaction mixture was refluxed for 27-32 h. After evaporation of solvent under vacuum, the residue was extracted with chloroform. The organic phases were collected, dried with anhydrous Na_2SO_4, filtered and the solvent was evaporated under vacuum. The crude product was purified by flash column chromatography (petroleum ether/ethyl acetate) and the yield of compounds was between (**3a-b**) 15–27%.

3.5. General procedure for the synthesis of N-phenylpyrazoline derivatives (4a-h) [20]

0.01 Mol of corresponding chalcones was dissolved under stirring in the minimum quantity of hot absolute ethanol. Phenylhydrazine hydrochloride (2.168 g, 0.015 mol) was added and mixture was refluxed for 35-40 h. The precipitate was filtered under vacuum, washed with water and recrystallized from ethanol to give the corresponding pyrazolines. The yield range after recrystallization of pyrazolines (**4a-h**) was 31–74%.

3.4.1. 5-(2-amino-6-phenylpyrimidin-4-yl)-N,4-dimethylthiazol-2-amine (3a)

Yield: 15%, mp: 195-196 °C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 2.83 (s, 3H, thiazole 4-CH_3), 3.26 (s, 3H, thiazole NCH_3), 7.09 (s, 1H, ArH), 7.49-8.06 (m, 5H, ArH). Anal. Calcd for $C_{15}H_{15}N_5S$ (MW 297): C, 60.58; H, 5.08; N, 23.55. Found: C, 60.56; H, 5.09; N, 23.56.

3.4.2. 4-(4-methyl-2-phenylthiazol-5-yl)-6-phenylpyrimidin-2-amine (3b)

Yield: 27%, mp: 180-181 °C. ^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 2.88 (s, 3H, thiazole 4-CH_3), 7.36-8.03 (m, 11H, ArH). ^{13}C NMR (δ ppm, $CDCl_3$, 75 MHz): 18.58, 104.98, 126.58, 127.15, 128.88, 129.02, 130.45, 130.72, 131.75, 133.35, 137.44, 153.43, 159.99, 163.22, 166.25, 167.55. MS : (m/z): 346 (M^++2, 66%), 345 (M^++1, 100%), 163 (18%). Anal. Calcd for $C_{20}H_{16}N_4S$ (MW 344): C, 69.74; H, 4.68; N, 16.27. Found: C, 69.76; H, 4.66; N, 16.28.

3.5.1. 5-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)-N,4-dimethylthiazol-2-amine (4a)

Yield: 58%, mp: 255-257 °C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 2.36 (s, 3H, thiazole 4-CH_3), 3.06 (s, 3H, thiazole NCH_3), 3.14 (dd, J_1 = 17.1, J_2 = 6.9 Hz, 1H, C$\underline{H}_A$H$_B$C=N), 4.02 (dd, J_1 = 17.1, J_2 = 12.3 Hz, 1H, CH$_A\underline{H}_B$C=N), 5.47 (dd, J_1 = 12.3, J_2 = 6.9 Hz, 1H, ArCHN), 6.71-7.39 (m, 10H, ArH). ^{13}C NMR (δ ppm, DMSO-d_6, 75 MHz): 13.62, 32.57, 44.28, 64.40, 111.75, 113.43, 119.52, 126.39, 128.07, 129.39, 129.52, 141.49, 142.38, 144.12, 166.88. MS : (m/z): 350 (M^++2, 31%), 349 (M^++1, 100%), 348 (M^+, 58%), 259 (18%).Anal. Calcd for $C_{20}H_{20}N_4S$ (MW 348): C, 68.93; H, 5.79; N, 16.08. Found: C, 68.95; H, 5.78; N, 16.07.

3.5.2. 5-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)-N-ethyl-4-methylthiazol-2-amine (4b)

Yield: 54%, mp: 223-225 °C. ^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 1.42 (t, J = 6.9 Hz, 3H, NCH$_2$C$\underline{H}_3$), 2.39 (s, 3H, thiazole 4-CH_3), 3.12 (dd, J_1 = 17.1, J_2 = 7.5 Hz, 1H, C$\underline{H}_A$H$_B$C=N), 3.41 (q, J = 6.9 Hz, 2H, NC$\underline{H}_2$CH$_3$), 3.82 (dd, J_1 = 17.1, J_2 = 12.3 Hz, 1H, CH$_A\underline{H}_B$C=N), 5.32 (dd, J_1 = 12.3, J_2 = 7.5 Hz, 1H, ArCHN), 6.79-7.38 (m, 10H, ArH), 9.54 (s, 1H, NH). Anal. Calcd for $C_{21}H_{22}N_4S$ (MW 362): C, 69.58; H, 6.12; N,15.46. Found: C, 69.59; H, 6.14; N, 15.48.

3.5.3. 5-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)-4-methyl-2-phenylthiazole (4c)

Yield: 74%, mp: 151-152 °C. ^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 2.76 (s, 3H, thiazole 4-CH_3), 3.21 (dd, J_1 = 17.1, J_2 = 7.5 Hz, 1H, C$\underline{H}_A$H$_B$C=N), 3.90 (dd, J_1 = 17.1, J_2 = 12.3 Hz, 1H, CH$_A\underline{H}_B$C=N), 5.30 (dd, J_1 = 12.3, J_2 = 7.5 Hz, 1H, ArCHN), 6.80-7.98 (m, 15H, ArH). Anal. Calcd for $C_{25}H_{21}N_3S$ (MW 395): C, 75.92; H, 5.35; N, 10.62. Found: C, 75.91; H, 5.34; N, 10.64.

3.5.4. N-4-dimethyl-5-(1-phenyl-5-p-tolyl-4,5-dihydro-1H-pyrazol-3-yl)thiazol-2-amine (4d)

Yield: 48%, mp: 217-219 °C. ^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 2.35 (s, 3H, Ar-CH_3), 2.41 (s, 3H, thiazole 4-CH_3), 3.09 (dd, J_1 = 17.1, J_2 = 7.5 Hz, 1H, C$\underline{H}_A$H$_B$C=N), 3.15 (s, 3H, thiazole NCH_3), 3.82 (dd, J_1 = 17.1, J_2 = 12.3 Hz, 1H, CH$_A\underline{H}_B$C=N), 5.31 (dd, J_1 = 12.3, J_2 = 7.5 Hz, 1H, ArCHN), 6.81-7.21 (m, 9H, ArH), 9.76 (s, 1H, NH). Anal. Calcd for $C_{21}H_{22}N_4S$ (MW 362): C, 69.58; H, 6.12; N,15.46. Found: C, 69.57; H, 6.14; N, 15.47.

3.5.5. 5-(5-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-N,4-dimethylthiazol-2-amine (4e)

Yield: 31%, mp: 203-205 °C. ^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz):

2.42 (s, 3H, thiazole 4-CH_3), 3.15 (s, 3H, thiazole NCH_3), 3.73 (dd, J_1 = 17.1, J_2 = 7.5 Hz, 1H, $C\underline{H}_AH_BC{=}N$), 3.81 (s, 3H, OCH_3), 3.86 (dd, J_1 = 17.1, J_2 = 12.3 Hz, 1H, $CH_A\underline{H}_BC{=}N$), 5.31 (dd, J_1 = 12.3, J_2 = 7.5 Hz, 1H, ArCHN), 6.82-7.28 (m, 9H, ArH), 9.80 (s, 1H, NH). Anal. Calcd for $C_{21}H_{22}N_4OS$ (MW 378): C, 66.64; H, 5.86; N, 14.80. Found: C, 66.65; H, 5.88; N, 14.78.

3.5.6. N,4-dimethyl-5-(5-(4-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)thiazol-2-amine (4f)

Yield: 42%, mp: 239-240 °C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 2.35 (s, 3H, thiazole 4-CH_3), 3.04 (s, 3H, thiazole NCH_3), 3.21 (dd, J_1 = 17.1, J_2 = 6.9 Hz, 1H, $C\underline{H}_AH_BC{=}N$), 4.06 (dd, J_1 = 17.1, J_2 = 12.3 Hz, 1H, $CH_A\underline{H}_BC{=}N$), 5.67 (dd, J_1 = 12.3, J_2 = 6.9 Hz, 1H, ArCHN), 6.73-6.78 (m, 1H, ArH), 6.88 (d, J = 7.8 Hz, 2H, ArH), 7.14-7.19 (m, 2H, ArH), 7.57 (d, J = 8.7 Hz, 2H, ArH), 8.23 (d, J = 8.7 Hz, 2H, ArH), 9.81 (s, 1H, NH). Anal. Calcd for $C_{20}H_{19}N_5O_2S$ (MW 393): C, 61.05; H, 4.87; N, 17.80. Found: C, 61.06; H, 4.85; N, 17.82.

3.5.7. 5-(5-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-N,4-dimethylthiazol-2-amine (4g)

Yield: 58%, mp: 177-179 °C. ^{1}H NMR (δ ppm, DMSO-d_6, 300 MHz): 2.43 (s, 3H, thiazole 4-CH_3), 3.01-3.26 (m, 4H, thiazole NCH_3, $C\underline{H}_AH_BC{=}N$), 3.84 (dd, J_1 = 17.1, J_2 = 12.3 Hz, 1H, $CH_A\underline{H}_BC{=}N$), 5.34 (dd, J_1 = 12.3, J_2 = 6.9 Hz, 1H, ArCHN), 6.86-7.28 (m, 9H, ArH), 9.74 (s, 1H, NH). Anal. Calcd for $C_{20}H_{19}FN_4S$ (MW 366): C, 65.55; H, 5.23; N, 15.29. Found: C, 65.57; H, 5.22; N, 15.32.

3.5.8. 5-(5-(4-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-N,4-dimethylthiazol-2-amine (4h)

Yield: 51%, mp: 215-217 °C. ^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 2.43 (s, 3H, thiazole 4-CH_3), 3.08-3.15 (m, 4H, thiazole NCH_3, $C\underline{H}_AH_BC{=}N$), 3.85 (dd, J_1 = 17.1, J_2 = 12.3 Hz, 1H, $CH_A\underline{H}_BC{=}N$), 5.34 (dd, J_1 = 12.3, J_2 = 7.5 Hz, 1H, ArCHN), 6.85-7.38 (m, 9H, ArH), 9.81 (s, 1H, NH). Anal. Calcd for $C_{20}H_{19}ClN_4S$ (MW 382.5): C, 62.73; H, 5.00; N, 14.63. Found: C, 62.76; H, 5.01; N, 14.60.

3.6. Biological evaluation

3.6.1. Antibacterial activity

The following Gram-negative bacteria were used: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Enterocbacter cloacae* (ATCC 35030) and the following Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), *Listeria monocytogenes* (NCTC 7973) and *Enterococcus faecalis* (ATCC 7080). 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 microdilution method [29,30] 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 x 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.

3.6.2. 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 x 10^5 cfu/ml. The compounds investigated were dissolved in DMSO (1 mg/ml) and added in broth TSB medium (100 µl) with bacterial inoculum (1.0 x 10^4 cfu per well) to achieve the wanted concentrations (1 mg/ml). The microplates were incubated 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 72 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 negative control (DMSO). Streptomycin (Sigma P 7794) and Ampicillin (Panfarma, Belgrade, Serbia) were used as a positive control (1 mg/ml DMSO). All experiments were performed in duplicate and repeated three times.

3.6.3. Antifungal activity

For the antifungal bioassays, eight fungi were used: *Aspergillus ochraceus* (ATCC 12066), *Aspergillus flavus* (ATCC 9643), *Aspergillus fumigatus* (plant isolate), Aspergillus niger (ATCC 6275), Aspergillus versicolor (ATCC 11730), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112) *Trichoderma viride* (IAM 5061) and *Candida albicans* ATCC 10231. 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.[26] In order to investigate the antifungal activity of the compounds, a modified microdilution technique was used. [30-32]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 x 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 DMSO (1 mg/ml) and added in broth Malt medium with inoculum. The microplates were incubated for 72 h at 28 °C, respectively. 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 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.

3.6.4. Statistical analysis

All the assays were carried out in triplicate and the results are expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This treatment was carried out using SPSS v. 18.0 program.

4. Conclusions

The newly synthesized thiazole-based aminopyrimidines **3a-b** and N-phenylpyrazolines **4a-h** 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 or comparable antibacterial activity than reference drugs with the most potent the compound **3a** followed by **4f.** The most sensitive bacterial species on these compounds is *L. monocytogenes* and *S. aureus*. *En. faecalis* and *En. cloacae* are the more resistant ones.

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 **3a** followed by **4h**. The most sensitive fungi were *A. ochraceus* and *A. versicolor,* while *A. niger* and *C. albicans* were the most resistant ones.

It can be seen that the growth of tested bacteria (Gram (-) and Gram (+) bacteria) and fungi responded differently to the tested compounds, which indicates that different components may have different modes of action or that the metabolism of some bacteria/fungi is able to better overcome the effect of the compounds or adapt to it. Gram (-) bacteria and fungi are in general more resistant than Gram (+) bacteria.[33]

Notes and references

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1 F. C. Tenover and L. C. McDonald, *Curr. Opin. Infect. Dis.* 2005, **18**, 300.
2 R. F. Pfeltz and B. J. Wilkinson, *Curr. Drug Targets Infect.Disord.* 2004, **4**, 273.
3 M. C. Roberts, *Curr. Drug Targets Infect. Disord.* 2004, **4**, 207.
4 A. Dessen, A. M. Di Guilmi, T. Vernet and O. Dideberg, *Curr. Drug Targets Infect. Disord.* 2001, **1**, 63.
5 H. Muroi, K. Nihei, K. Tsujimoto and I. Kubo, *Bioorg. Med. Chem.* 2004, **12**, 583.
6 N. Y. Yount and M. R. Yeaman, *Proc. Natl. Acad. Sci. USA.* 2004, **101**, 7363.
7 M. W. Khan, M. J. Alam, M. A. Rashid and R. Chowdhury, *Bioorg. Med. Chem.* 2005, **13**, 4796.
8 R. M. E. Sydnor and M. T. Perl, *Clin. Microbiol. Rev.* 2011, **24 (1)**, 141.
9 C. Torres-Viera and L. M. Dembry, *Curr. Opin. Infect. Dis.* 2004, **17**, 541.
10 G. M. Anstead and A. D. Owens, *Curr. Opin. Infect. Dis.* 2004, **17**, 549.
11 C. W. Ford, G. E. Zurenko and M. R. Barbachyn, *Curr. Drug Targets Infect. Disord.,*2001, **1**, 181.
12 N. M. Abunada, H. M. Hassaneen, N. G. Kandile and O. A. Miqdad, *Molecules* 2008, **13**, 1011.
13 H. S. Shah and S. P. Patel, *Chem. Sci. Trans.* 2012, **1 (3)**, 632.
14 M. Ghannoum, K. Abu Elteen and N. R. el-Rayyes, *Microbios.* 1989, **60 (242)**, 23.
15 S. Chandrasekaran and S. Nagarajan, *Il Farmaco* 2005, **60**, 279.
16 I. G. Rathish, K. Javed, S. Ahmad, S. Bano, M. S. Alam, K. K. Pillai, S. Singh and V. Bagchi, *Bioorg. Med. Chem. Lett.* 2008, **21**, 212.
17 M. T. Chhabria, H. G. Bhatt, H. G. Raval and P. M. Oza, *Bioorg. Med. Chem. Lett.* 2007, **17 (4)**, 1022.
18 H. B. Shivarama, P. M. Akberali and M. K. Shivananda, *Il Farmaco* 2000, **55**, 256.
19 A. Agarwal, K. Srivastava, S. K. Puri and M. S. P. Chauhan, *Bioorg. Med. Chem.* 2005, **13**, 6226.
20 Y. R. Prasad, A. L. Rao, L. Prasoona, K. Murali and P. R. Kumar, *Bioorg. Med. Chem. Lett.* 2005, **15**, 5030.
21 K. Liaras, A. Geronikaki, J. Glamočlija, A. Ćirić and M. Soković, *Bioorg. Med. Chem.* 2011, **19 (10)**, 3135.
22 K. Liaras, A. Geronikaki, J. Glamočlija, A. Ćirić and M. Soković, *Bioorg. Med. Chem.* 2011, **19 (24)**, 7349.
23 I. Rahavi, Ch. Kamoutsis, P. Zoumpoulakis, A. Geronikaki, M. Soković, J. Glamočlija and A. Ćirić, *Bioorg. Med. Chem.* 2008, **16**, 1150.
24 O. Kouatli, P. Zoumpoulakis, Ch. Camoutsis, M. Soković, A. Ćirić , J. Glamočlija and A. Geronikaki, *Bioorg. Med. Chem.* 2010, **18(1)**, 426.
25 I. Apostolidis, K. Liaras, K. Geronikaki, D. Hadjipavlou-Litina, A. Gavalas, M. Soković, J. Glamočlija and A. Ćirić, *Bioorg. Med. Chem.* 2013, **21 (2)**, 532.
26 M. Soković, J. Glamočlija, P. D. Marin, D. Brkić and L. J. L. D. van Griensven, *Molecules*, 2010, **15,** 7532-7546.
27 M. Soković, A.Ćirić, J. Glamočlija, M.Nikolić, L. J. L. D. van Griensven, *Molecules,* 2014, **19**, 4189-4199.
28 M. Soković, J. Vukojević, P. D. Marin, D. Brkić, V. Vajs, L.J.L.D. van Griensven, *Molecules,* 2009, **14**, (1), 238-249.
29 C. Booth, Fungal Culture Media, *In Methods in Microbiology*, Norris, J. R., Ribbons, D. W., Eds.; Academic Press: London and New York, 1971; pp 49–94.
30 K. D. Daouk, M. S. Dagher and J. E. J. Sattout, *Food Protect.* 1995, **58**, 1147.
31 H. Hanel and W. Raether, *Mycoses* 1988, **31**, 148.
32 A. J. Espinel-Ingroff, *Clin. Microbiol*. 2001, **39**, 1360.
33 M. Soković, J. Glamočlija, P. Marin, D. Brkić and L.J.L.D. van Griensven, *Molecules*, 2010, **15**, 7532.