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Short communication

New heterocyclic compounds from 1,2,4-triazole and 1,3,4-thiadiazole class bearing diphenylsulfone moieties. Synthesis, characterization and antimicrobial activity evaluation☆

Stefania-Felicia Barbuceanu [a], Gabriel Saramet [b,*], Gabriela Laura Almajan [a], Constantin Draghici [c], Florica Barbuceanu [d], Gabriela Bancescu [e]

[a] Organic Chemistry Department, Faculty of Pharmacy, Traian Vuia Street 6, 020956 Bucharest, Romania
[b] Pharmaceutical Techniques Department, Faculty of Pharmacy, Traian Vuia Street 6, 020956 Bucharest, Romania
[c] C.D. Nenitescu Institute of Organic Chemistry, Romanian Academy, Splaiul Independentei 202B, 060023 Bucharest, Romania
[d] Institute for Diagnosis and Animal Health, Dr. Staicovici Street 63, Bucharest, Romania
[e] Microbiology Department, Faculty of Dentistry, Calea Plevnei Street 19, 050051 Bucharest, Romania

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ABSTRACT

Some new 5-(4-(4-X-phenylsulfonyl)phenyl)-4-(R)-2H-1,2,4-triazol-3(4H)-thiones **4a**,**b**; **5a**,**b** and 5-(4-(4-X-phenylsulfonyl)phenyl)-N-(R)-1,3,4-thiadiazol-2-amines **6a**,**b**; **7a**,**b** were obtained by cyclization of new N^1-[4-(4-X-phenylsulfonyl)benzoyl]-N^4-(R)-thiosemicarbazides **2a**,**b**; **3a**,**b** (X = H, Br). The 1,2,4-triazoles were synthesized by intramolecular cyclization of acylthiosemicarbazides, in basic media. On the other hand, 1,3,4-thiadiazoles were obtained from same acylthiosemicarbazides, in acidic media. These new intermediates from thiosemicarbazide class were afforded by the reaction of 4-(4-X-phenylsulfonyl)benzoic acids hydrazides (X = H, Br) **1a**,**b** with 4-trifluoromethoxyphenyl or 3,4,5-trimethoxyphenyl isothiocyanate. The newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. All the new compounds were screened for their antimicrobial activity against some bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 13061, *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 49141, *Acinetobacter baumannii* ATCC 19606 and *Pseudomonas aeruginosa* ATCC 27853) and yeasts (*Candida albicans* ATCC 90028 and *Candida parapsilosis* ATCC 22019).

1. Introduction

During the past decades, the incidence of microbial infection has increased to alarming levels all over the world as a result of antimicrobial resistance. Thus, in recent years, much attention has been focused on addressing the problem of multi-drug resistant (MDR) bacteria and fungi resulting from the widespread use and misuse of classical antimicrobial agents. Due to this reason, discovering of new classes of antimicrobial agents with novel mechanisms is crucial to combat multi-drug resistant infections.

Organic compounds incorporating heterocyclic ring systems continue to attract considerable interest due to their wide range of biological activities. Among different five-membered heterocyclic systems 1,2,4-triazoles and 1,3,4-thiadiazoles and their derivatives have gained importance as they constitute the structural features of many bioactive compounds. It is known that triazole and thiadiazole rings are included in the structure of various drugs [1–4]. From these classes of heterocyclic compounds, the synthesis of new derivatives of 1,2,4-triazole-3-thiones and 2-amino-1,3,4-thiadiazoles has been attracting considerable attention because of various biological properties such as: antibacterial [5–10], antifungal [5,11,12], anti-tubercular [5,8,13], antiviral [6,7,13], antioxidant [14,15], antitumoral [16–18], anti-inflammatory [19–21], anticonvulsant [22–24] etc.

On the other hand, literature survey revealed that diphenylsulfone derivatives have antibacterial activity [25,26].

Motivated by these findings and in continuation of our ongoing efforts on the synthesis of heterocycles with potential antimicrobial activities [27–32], we are purposed to synthesize and investigate the antimicrobial activity of a new series from 1,2,4-triazole and 1,3,4-thiadiazole class having diarylsulfone moiety as pharmacophore centre in 5 position and different radicals linked in 4 position of triazole or to amino group from 2 position of thiadiazole nucleus.

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* Corresponding author. Tel.: +40 723 492067.
E-mail address: saramet.gabriel@yahoo.com (G. Saramet).

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2. Chemistry

The synthetic route for the newly synthesized compounds, 1,2,4-triazoles, 1,3,4-thiadiazoles and their acylthiosemicarbazides intermediary, is illustrated and outlined in Scheme 1.

The precursors, 4-(4-X-phenylsulfonyl)benzoic acid hydrazides **1a**,**b** (X = H, Br), were prepared, in several stages, according to the literature method [33]. Thus, diarylsulfones (X = H, Br), obtained by tosylation of benzene or bromobenzene with 4-methylbenzene-1-sulfonyl chloride, by oxidation with chromic anhydride and acetic acid, led to 4-(4-X-phenylsulfonyl)benzoic acids. Ethyl 4-(4-X-phenylsulfonyl)benzoates were synthesized by esterification reaction of 4-(4-X-phenylsulfonyl)benzoic acids with ethanol, in the presence of catalytic amount of sulphuric acid. In the final stage, the 4-(4-X-phenylsulfonyl)benzoic acid hydrazides **1a**,**b** were obtained by hydrazinolysis of ethyl 4-(4-X-phenylsulfonyl)benzoate with hydrazine hydrate.

The new N^1-[4-(4-X-phenylsulfonyl)benzoyl]-N^4-(R)-thiosemicarbazides **2a**,**b**; **3a**,**b** used as the key intermediates for the synthesis of 1,2,4-triazole and 1,3,4-thiadiazole derivatives were synthesized by nucleophilic addition of 4-(4-X-phenylsulfonyl) benzoic acid hydrazides **1a**,**b** to 4-trifluoromethoxyphenyl or 3,4,5-trimethoxyphenyl isothiocyanate.

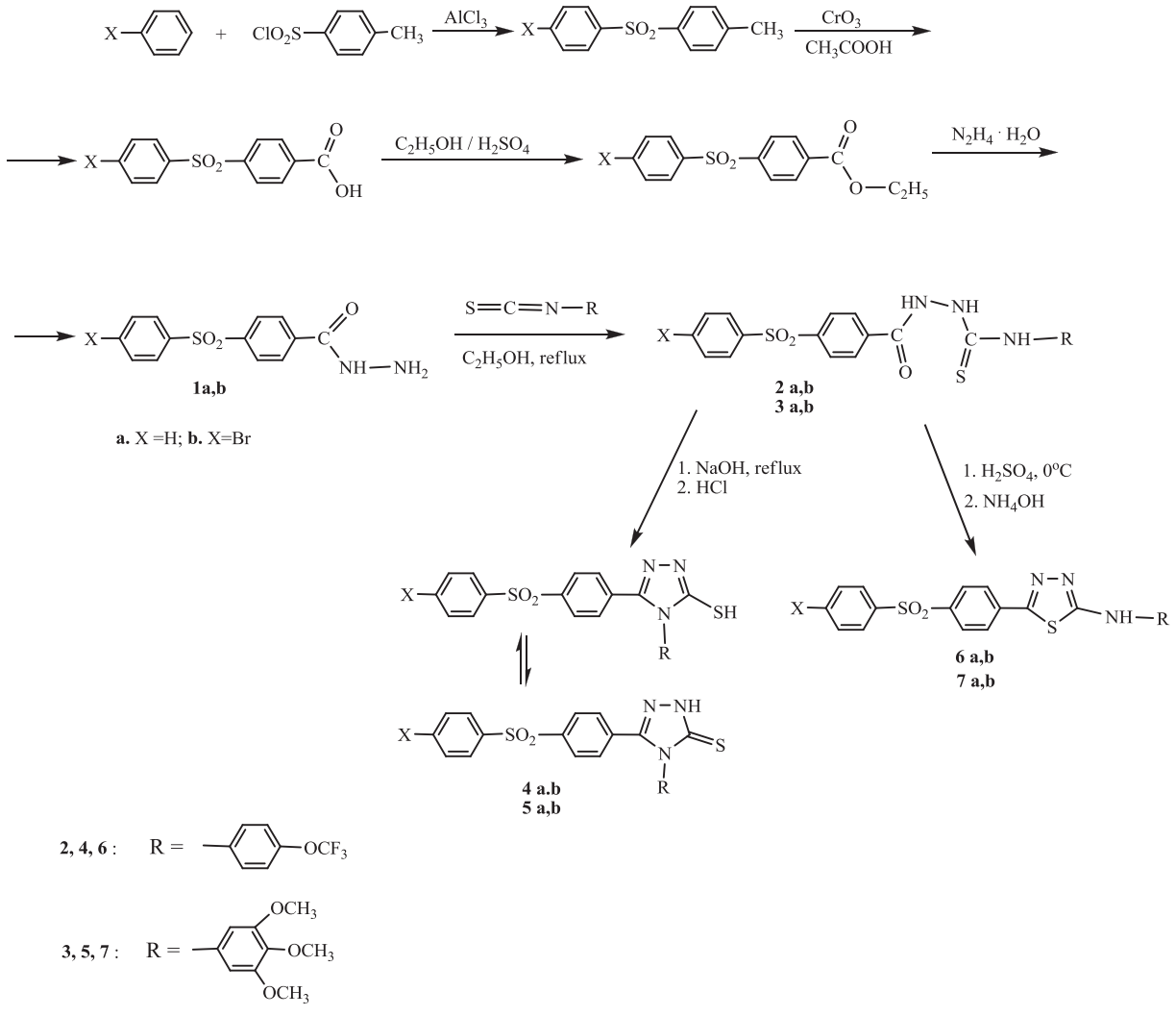
The 5-(4-(4-X-phenylsulfonyl)phenyl)-4-(R)-2H-1,2,4-triazol-3(4H)-thiones **4a**,**b** and **5a**,**b**, in equilibrium with thiole tautomer, were obtained by dehydrative intramolecular cyclization of acylthiosemicarbazides when refluxed in 8% sodium hydroxide solution, followed by treatment with 1% hydrochloric acid solution.

Intramolecular cyclization of same acylthiosemicarbazides in the presence of concentrated sulphuric acid and followed by treatment with a ammonia solution led to 5-(4-(4-X-phenylsulfonyl)phenyl)-N-(R)-1,3,4-thiadiazol-2-amines **6a**,**b**; **7a**,**b**.

The structures elucidation of the newly synthesized compounds was carried out by different spectroscopic techniques like IR, ^{1}H NMR and ^{13}C NMR. Further confirmations of the compounds were carried out by mass spectrometry and elemental analysis.

3. Antimicrobial activity

The new synthesized compounds were tested for their in vitro antimicrobial activity against the Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 13061, the Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 49141, *Acinetobacter baumannii ATCC 19606*, *Pseudomonas aeruginosa* ATCC 27853 and the yeasts *Candida albicans* ATCC 90028



Scheme 1.

and *Candida parapsilosis* ATCC 22019, by using the broth microdilution method for determination of MIC.

4. Results and discussions

4.1. Chemistry

The structural assignments of new compounds were based on their elemental analysis and spectral data (IR, ^{1}H NMR, ^{13}C NMR and MS). The elemental analysis data and some physical properties of these new compounds are reported in Table 1.

The structure of thiosemicarbazides **2** and **3** was confirmed by their IR spectra which displayed absorption peaks at 3150–3438 cm^{-1} for NH, 1673–1686 cm^{-1} for C=O and 1209–1236 cm^{-1} corresponding to C=S stretching vibrations. In the ^{1}H NMR spectra of these new compounds, the NH protons were observed as singlets at chemical shift δ 9.75–10.89 ppm [14,19]. The ^{13}C NMR spectra showed two signals at $\delta \sim 181$ ppm and ~ 165 ppm characteristic to C=S and C=O carbons, respectively, which confirm the formation of thiosemicarbazides [14].

In the IR spectra of the new heterocyclic compounds **4**–**7** the absorption band C=O from acylthiosemicarbazides disappeared, which confirms that their cyclization reaction took place.

According to the IR spectroscopic data of the compounds **4** and **5** which have triazole-3-thione structure, the presence of C=S and NH stretching bands at 1216–1235 cm^{-1} and 3413–3431 cm^{-1}, respectively [34,35] and the absence of an absorption about in 2600–2550 cm^{-1} region for SH group [34,36,37] have proved that these compounds predominantly exist, in solid state, in the tautomeric thione form.

The structure of compounds which contain thiadiazole nucleus is confirmed by the presence in their IR spectra of a single stretching band in 3241–3348 cm^{-1} region due to NH group and by disappearance of νC=S absorption band from thiosemicarbazides.

Also, the IR spectra of triazoles and thiadiazoles showed new absorption bands in region 1600–1620 cm^{-1} due to C=N stretching vibrations that is an evidence for ring closure [19].

Transformation of acylthiosemicarbazides in heterocyclic compounds from 1,2,4-triazole and 1,3,4-thiadiazole class is supported, from ^{13}C NMR spectra, by the absence of carbonyl and thiocarbonyl carbons signals characteristic to thiosemicarbazide. In the spectra of compounds **6** and **7** appear two new signals at δ 164.73–165.35 and 155.40–156.28 ppm due to quaternary carbon atoms C-2 and C-5 of the 1,3,4-thiadiazolic nucleus [14,38]. On the other hand, ^{13}C NMR spectra of compounds **4** and **5** show two new signals characteristic to C-5 and C-3 quaternary carbon atoms from 1,2,4-triazole nucleus which appear in the region 148.97–149.07 and 168.92–168.98 ppm, respectively. The appearance of the C-3 carbon atom at $\delta \sim 169$ ppm indicates the presence of C=S [13,14,27,37,38].

The assignments of the signals ^{13}C NMR of these new compounds **2**–**7** resulted from the ^{2}D-HETCOR spectra.

The ^{1}H NMR spectra of compounds **4** and **5** show a downfield singlet resonating at 14.26–14.38 ppm characteristic for the NH proton from 1,2,4-triazole-3-thione [13,14,36,37,39]. This strong deshielding of NH is probably explained by the strong intermolecular hydrogen bonding [40]. In compounds **6** and **7** appeared one singlet signal typical of the NH group in the δ range 10.64–10.90 ppm [19,23].

These findings in IR and NMR data of 1,2,4-triazole **4** and **5** clearly provide that these compounds predominantly exist, both in solid state and in solution, in the thione form rather than the tautomeric thiol form.

In ^{1}H NMR spectra of compounds **3**, **5** and **7** the protons of three methoxy substituents resonated as two singlets: at $\delta = 3.70$–3.78 ppm for 4-OCH_3 group and at $\delta = 3.60$–3.63 ppm for 3-OCH_3 and 5-OCH_3 which are equivalent [41]. Also, in ^{13}C NMR spectra of these compounds, the carbon atoms of methoxy substituents appear at 60.07–60.23 ppm (4-OCH_3) and 55.81–56.23 ppm (3-OCH_3 and 5-OCH_3), respectively. The signal of OCF_3 substituent from compounds **2**, **4** and **6** appears in ^{13}C NMR spectra as quartet at 119.91–121.85 ppm with $J_{C\text{-}F} = 254.3$–256.9 Hz.

The mass spectra of all compounds displayed molecular ions which confirmed their molecular weights (see Section 6). The compounds which have bromine atom in their molecule show in the mass spectrum the characteristic peaks corresponding to isotopic distribution (^{79}Br and ^{81}Br isotopes).

4.2. Antimicrobial activity

The results of antimicrobial screening of newly prepared compounds from 1,2,4-triazole, 1,3,4-thiadiazole and acylthiosemicarbazide class expressed as the MIC values, are summarized in Table 2. Amicakin and fluconazole were used as standard drugs.

The investigations of antimicrobial screening data revealed that all newly synthesized compounds exhibited poor antimicrobial activity compared to that of the control drugs.

The results obtained on Gram-positive bacteria showed a stronger action of all compounds tested against *B. cereus* compared to *S. aureus*. Triazole **5b** which has in position 4 on diphenylsulfone moiety a bromine atom and at the nitrogen atom N-4 of triazole ring the 3,4,5-trimethoxyphenyl fragment, presented the strongest action against *B. cereus* (MIC = 8 μg/mL). Also compounds **3a**, **3b**, **5a**, **7a** and **7b** showed good inhibitory activities against *B. cereus* (MIC = 16 μg/mL). So, all triazoles, thiadiazoles and thiosemicarbazides containing 3,4,5-trimethoxyphenyl fragment have better action against *B. cereus* group compared with derivatives containing 4-trifluoromethoxyphenyl. Both thiosemicarbazides and triazoles containing 3,4,5-trimethoxyphenyl fragment at the nitrogen atom N-4 show lower MIC values (128 μg/mL) than those

Table 1
Characterization data of compounds **2**–**7**.

Compd	X	R	Molecular formula	Molecular mass	M.p. (°C)	Yield (%)	Elemental analysis found (calc)		
							C	H	N
2a	H	4-F_3CO-C_6H_4	$C_{21}H_{16}F_3N_3O_4S_2$	495.49	190–192	98.1	50.86 (50.90)	3.19 (3.25)	8.51 (8.48)
2b	Br	4-F_3CO-C_6H_4	$C_{21}H_{15}BrF_3N_3O_4S_2$	574.39	199–201	95.2	43.86 (43.91)	2.57 (2.63)	7.36 (7.32)
3a	H	3,4,5-$(H_3CO)_3$-C_6H_2	$C_{23}H_{23}N_3O_6S_2$	501.58	178–181	96.2	55.01 (55.07)	4.57 (4.62)	8.43 (8.38)
3b	Br	3,4,5-$(H_3CO)_3$-C_6H_2	$C_{23}H_{22}BrN_3O_6S_2$	580.47	184–186	87	47.52 (47.59)	3.75 (3.82)	7.18 (7.24)
4a	H	4-F_3CO-C_6H_4	$C_{21}H_{14}F_3N_3O_3S_2$	477.48	259–261	63.5	52.90 (52.82)	2.91 (2.96)	8.74 (8.80)
4b	Br	4-F_3CO-C_6H_4	$C_{21}H_{13}BrF_3N_3O_3S_2$	556.38	237–240	80	45.29 (45.33)	2.30 (2.36)	7.49 (7.55)
5a	H	3,4,5-$(H_3CO)_3$-C_6H_2	$C_{23}H_{21}N_3O_5S_2$	483.56	264–266	70	57.07 (57.12)	4.33 (4.38)	8.63 (8.69)
5b	Br	3,4,5-$(H_3CO)_3$-C_6H_2	$C_{23}H_{20}BrN_3O_5S_2$	562.46	156–158	62	49.02 (49.11)	3.51 (3.58)	7.42 (7.47)
6a	H	4-F_3CO-C_6H_4	$C_{21}H_{14}F_3N_3O_3S_2$	477.48	235–236	89.2	52.79 (52.82)	3.01 (2.96)	8.74 (8.80)
6b	Br	4-F_3CO-C_6H_4	$C_{21}H_{13}BrF_3N_3O_3S_2$	556.38	249–250	91.6	45.40 (45.33)	2.30 (2.36)	7.59 (7.55)
7a	H	3,4,5-$(H_3CO)_3$-C_6H_2	$C_{23}H_{21}N_3O_5S_2$	483.56	272–274	75.5	57.08 (57.13)	4.42 (4.38)	8.72 (8.69)
7b	Br	3,4,5-$(H_3CO)_3$-C_6H_2	$C_{23}H_{20}BrN_3O_5S_2$	562.46	300–302	74.0	49.18 (49.11)	3.62 (3.58)	7.41 (7.47)

Table 2
Antimicrobial activities of compounds **2**–**7** as MIC values (μg/mL).

Compd.	Gram-positive bacteria[a]		Gram-negative bacteria[b]				Yeasts[c]	
	Sa	*Bc*	*Ec*	*Ecl*	*Ab*	*Pa*	*Ca*	*Cp*
2a	256	32	128	128	64	64	64	64
2b	512	32	128	128	64	64	64	64
3a	128	16	128	128	32	64	64	64
3b	128	16	128	128	32	64	64	64
4a	256	32	128	128	32	64	128	64
4b	512	32	256	256	32	64	128	64
5a	128	16	128	128	32	64	64	32
5b	128	8	128	128	32	64	64	16
6a	512	32	128	128	32	64	64	64
6b	512	32	128	128	32	64	128	64
7a	512	16	128	128	32	64	64	64
7b	512	16	64	128	32	64	64	64
Control (AM)	2	–	2	–	–	2	–	–
Control (FL)	–	–	–	–	–	–	–	2

Control: AM = amikacin; FL = fluconazole.
[a] *Sa* (*Staphylococcus aureus* ATCC 25923); *Bc* (*Bacillus cereus* ATCC 13061).
[b] *Ec* (*Escherichia coli* ATCC 25922); *Ecl* (*Enterobacter cloacae* ATCC 49141); *Ab* (*Acinetobacter baumannii* ATCC 19606); *Pa* (*Pseudomonas aeruginosa* ATCC 27853).
[c] *Ca* (*Candida albicans* ATCC 90028); *Cp* (*Candida parapsilosis* ATCC 22019).

containing 4-trifluoromethoxyphenyl against *S. aureus*. All thiadiazoles showed the weakest action on *S. aureus* (MIC = 512 μg/mL). This finding suggests that presence of OCH_3 substituent in the positions 3, 4 and 5 on the phenyl fragment, would be beneficial for the activity against *B. cereus* and *S. aureus* (except thiadiazoles in case of last strain) comparing to the situation when OCF_3 substituent is linked in the para position of the phenyl fragment.

The investigation of the biological action on Gram-negative bacteria revealed that almost all compounds, especially those of triazole and thiadiazole class, were active against *A. baumannii* (MIC = 32 μg/mL) and that only two thiosemicarbazides which contain 4-trifluoromethoxyphenyl substituent at N-4 nitrogen atom had a lower action (MIC = 64 μg/mL).

The action on *P. aeruginosa* could be characterized as a medium one, MIC being of 64 μg/mL, and was not influenced by the presence of the functional groups of the substitutes upon diphenylsulfone fragment or on the nitrogen atoms, as in the previous case.

The compounds showed a uniform weaker action against *E. coli* and *E. cloacae*, especially the triazole **4b**, substituted at the nitrogen atom N-4 with trifluoromethoxyphenyl fragment, which proved to be less active against both strains (MIC = 256 μg/mL). The thiadiazole **7b** substituted at the amino group with the trimethoxyphenyl fragment manifested the best action against *E. coli* (MIC = 64 μg/mL).

The antifungal activity tested against *C. albicans* and *C. parapsilosis* was characterized by medium and equal MIC values, of 64 μg/mL, excepting the triazoles **4a** and **4b**, and thiadiazole **6b**, all containing trifluoromethoxyphenyl fragment, which showed weaker action (MIC = 128 μg/mL). Also, the results mentioned in Table 2 are indicating that the triazoles **5a** and **5b** have shown a promising antifungal activity against *C. parapsilosis*, with MIC values of 32 μg/mL and 16 μg/mL, respectively. These compounds also contained a trimethoxyphenyl fragment in their structure.

In order to analyze the relationship: structure–the activity of these compounds, the value of the logarithm of the partition coefficient (log *P*) was determinated. The obtained data are presented in Table S.1 (in the Supplementary data section).

The lipophilicity (estimated by the n-octanol/water partition coefficient, log *P*), varies between 2.79 for **3a** and 5.0 for **6b**. As previously reported [10], no clear correlation could be established between estimated values for log *P* and the antimicrobial activity. Although p-Br substitution determines a considerable increase in log *P* values (mean 0.65), this fact seems to have no correspondent into the antimicrobial activity.

5. Conclusions

In this study we report the synthesis, characterization and antimicrobial activity evaluation of new compounds from 1,2,4-triazole and 1,3,4-thiadiazole class and their acylthiosemicarbazide intermediates bearing diphenylsulfone moiety. The target compounds from 1,2,4-triazole and 1,3,4-thiadiazole class were obtained from intramolecular cyclization of some new acylthiosemicarbazides in basic/acidic media. The intermediates from thiosemicarbazide class were synthesized by reaction of 4-(4-X-phenylsulfonyl)benzoic acids hydrazides with 4-trifluoro-methoxy/3,4,5-trimethoxyphenyl isothiocyanate. The most antibacterial activity was presented by 1,2,4-triazole **5b** against *B. cereus* with MIC = 8 μg/mL. Also, 1,2,4-triazole **5a**, 1,3,4-thiadiazoles **7a**,**b** and acylthiosemicarbazides **3a**,**b** display good activity against *B. cereus* (MIC = 16 μg/mL). The most antifungal activity was presented by the same 1,2,4-triazole **5b** against *C. parapsilosis* (MIC = 16 μg/mL).

Making a general appreciation of the relationship between the molecular structure and biological activity of the above mentioned compounds, it might be concluded that in most cases:

- the presence of OCH_3 substituent in molecule increases, in the most cases, the antimicrobial efficiency of corresponding compounds;
- the presence of OCF_3 substituent has decreased, generally, the efficiency of studied compounds;
- the presence of bromine on the diphenylsulfone fragment has little influence on the biological activity of tested compounds;
- the cyclization of the thiosemicarbazides to triazoles and thiadiazole, in most cases, has not influenced too much the action against the Gram-positive bacteria, Gram-negative bacteria and fungi.

None of these compounds is effective against the tested microorganisms comparable with used drugs.

6. Experimental protocols

6.1. Chemistry

Melting points were determined with Boetius apparatus and are uncorrected. The IR spectra (KBr pellets) were recorded on a Vertex 70 Bruker spectrometer. The NMR spectra were recorded on a Varian

Gemini 300 BB spectrometer working at 300 MHz for a ^{1}H and 75 MHz for ^{13}C in DMSO-d_6 solutions using TMS as an internal standard. The mass spectra of the new compounds were registered on a triple quadrupole mass spectrometer Varian 1200 L/MS/MS with electrospray interface (ESI), coupled with a high performance liquid chromatograph with Varian ProStar 240 SDM ternar pump and a Varian ProStar 410 automatic injector. The instrument was operated in positive ions mode. The sample solution (2 μg/mL in $CHCl_3$/CH_3OH 1/1, v/v) was introduced in the ESI interface by direct infusion, after a tenth dilution with methanol, at a flow rate of 20 μL0/min. Elemental analyses were performed on ECS-40-10-Costeh micro-dosimeter, after drying the compounds at 105 °C.

*6.1.1. General procedure for synthesis of N^1-[4-(4-X-phenylsulfonyl) benzoyl]-N^4-(R)-thiosemicarbazides **2a**,**b**; **3b***

An equimolar mixture (1 mmol) of 4-(4-X-phenylsulfonyl)-benzoic acid hydrazide (X = H, Br) **1** and arylisothiocyanate in ethanol (5 mL) was refluxed for 16–19 h. The reaction mixture was cooled to room temperature and the obtained precipitated was filtered, washed with cold ethanol, dried and recrystallized from ethanol.

*6.1.1.1. N^1-[4-(Phenylsulfonyl)benzoyl]-N^4-(4-trifluoromethoxyphenyl)-thiosemicarbazide **2a**.* IR (KBr, ν, cm^{-1}): 3367, 3297 (NH), 3086, 3069, 3043 (aromatic C–H), 1681 (C=O), 1579, 1531, 1488 (C=C), 1322, 1295, 1158 (SO_2), 1261 (C–O–C), 1209 (C=S); ^{1}H NMR (DMSO-d_6, δ ppm): 10.89 (bs, 1H, NH); 9.97 (bs, 1H, NH); 9.82 (bs, 1H, NH); 8.14 (d, 2H, J = 8.9 Hz, aromatic protons); 8.09 (d, 2H, J = 8.9 Hz, aromatic protons); 8.00 (dd, 2H, J = 7.3, 1.6 Hz, aromatic protons); 7.72 (tt, 1H, J = 7.3, 1.6 Hz, aromatic proton); 7.64 (bt, 2H, J = 7.3 Hz, aromatic protons); 7.55 (bd, 2H, J = 8.2 Hz, aromatic protons); 7.33 (d, 2H, J = 8.2 Hz, aromatic protons); ^{13}C NMR (DMSO-d_6, δ ppm): 181.17 (C=S), 164.60 (C=O), 145.15, 143.78, 140.60, 138.32, 137.03, 133.99, 129.83, 129.19, 128.30, 127.48, 127.41, 120.69 (aromatic ring carbons); 120.52 (q, J = 256.1 Hz, OCF_3); (ESI–MS) m/z: 496 $[M + H]^+$.

*6.1.1.2. N^1-[4-(4-Bromophenylsulfonyl)benzoyl]-N^4-(4-trifluoro-methoxyphenyl)-thiosemicarbazide **2b**.* IR (KBr, ν, cm^{-1}): 3327, 3305, 3205 (NH), 3093, 3071, 3013 (aromatic C–H), 1686 (C=O), 1575, 1543, 1524, 1483 (C=C), 1321, 1293, 1159 (SO_2), 1263 (C–O–C), 1210 (C=S), 573 (C–Br); ^{1}H NMR (DMSO-d_6, δ ppm): 10.84 (bs, 1H, NH); 9.99 (bs, 1H, NH); 9.80 (bs, 1H, NH); 8.15 (d, 2H, J = 9.0 Hz, aromatic protons); 8.12 (d, 2H, J = 9.0 Hz, aromatic protons); 7.93 (d, 2H, J = 8.8 Hz, aromatic protons); 7.86 (d, 2H, J = 8.8 Hz, aromatic protons); 7.52 (bd, 2H, J = 8.8, 1.6 Hz, aromatic protons); 7.33 (d, 2H, J = 8.8 Hz, aromatic protons); ^{13}C NMR (DMSO-d_6, δ ppm): 181.15 (C=S), 164.56 (C=O), 145.18, 143.25, 139.82, 138.29, 137.20, 132.94, 129.50, 128.50, 128.26, 127.50, 127.35, 120.70 (aromatic ring carbons); 120.86 (q, J = 256.9 Hz, OCF_3); (ESI–MS) m/z: 574 $[M + H]^+$; m/z: 576 $[M + H]^+$.

*6.1.1.3. N^1-[4-(Phenylsulfonyl)benzoyl]-N^4-(3,4,5-trimethoxyphenyl)-thiosemicarbazide **3a**.* IR (KBr, ν, cm^{-1}): 3390, 3254, 3150 (NH), 3094, 3066 (aromatic C–H), 2837 (CH_3), 1673 (C=O), 1531, 1507, 1449 (C=C), 1320, 1295, 1156 (SO_2), 1235 (C=S + C–O–C), 1127 (C–O–C); ^{1}H NMR (DMSO-d_6, δ ppm): 10.78 (bs, 1H, NH); 9.75 (s, 2H, NH); 8.12 (s, 4H, aromatic protons); 8.00 (dd, 2H, J = 7.2, 1.4 Hz, aromatic protons); 7.72 (tt, 1H, J = 7.2, 1.4 Hz; aromatic proton); 7.64 (t, 2H, J = 7.2 Hz, aromatic protons); 6.81 (s, 2H, aromatic protons); 3.73 (s, 3H, 4-OCH_3); 3.63 (s, 6H, 3,5-di-OCH_3); ^{13}C NMR (DMSO-d_6, δ ppm): 181.14 (C=S), 164.67 (C=O), 152.18, 143.85, 143.76, 140.62, 137.13, 134.78, 134.02, 129.86, 129.22, 128.31, 127.43, 103.40 (aromatic ring carbons); 60.07 (OCH_3), 55.86 (3,5-di-OCH_3); (ESI–MS) m/z: 502 $[M + H]^+$.

*6.1.1.4. N^1-[4-(4-Bromophenylsulfonyl)benzoyl]-N^4-(3,4,5-trimethoxyphenyl)-thiosemicarbazide **3b**.* IR (KBr, ν, cm^{-1}): 3438, 3303, 3150 (NH), 3087, 3003 (aromatic C–H), 2838 (CH_3), 1680 (C=O), 1573, 1532, 1507, 1464 (C=C), 1322, 1300, 1159 (SO_2), 1236 (C=S+ C–O–C), 1128 (C–O–C), 576 (C–Br); ^{1}H NMR (DMSO-d_6, δ ppm): 10.64 (bs, 1H, NH); 9.79 (bs, 2H, NH); 8.12 (s, 4H; aromatic protons); 7.95 (d, 2H, J = 8.8 Hz, aromatic protons); 7.75 (d, 2H, J = 8.8 Hz, aromatic protons); 6.82 (s, 2H, aromatic protons); 3.73 (s, 3H, 4-OCH_3); 3.63 (s, 6H, 3,5-di-OCH_3); ^{13}C NMR (DMSO-d_6, δ ppm): 181.14 (C=S), 164.70 (C=O), 152.25, 143.32, 140.06, 139.88, 137.30, 134.67, 133.05, 129.59, 129.36, 128.37, 127.58, 103.36 (aromatic ring carbons), 60.14 (4-OCH_3), 55.92 (3,5-di-OCH_3); (ESI–MS) m/z: 580 $[M + H]^+$; m/z: 582 $[M + H]^+$.

*6.1.2. General procedure for synthesis of 5-(4-(4-X-phenylsulfonyl) phenyl)-4-(R)-2H-1,2,4-triazol-3(4H)-thiones **4a**,**b**; **5a**,**b***

A mixture of acylthiosemicarbazide **2** or **3** (1mmol) and 8% sodium hydroxide solution (15 mL) was heated under reflux for 5 h. The reaction mixture was filtered and the filtrate was cooled and acidified with a diluted solution of hydrochloric acid (1%) to pH ~ 5. The precipitate obtained was filtered, washed with water, dried and finally recrystallized from $CHCl_3$/petroleum ether (1:2, v/v).

*6.1.2.1. 5-(4-(Phenylsulfonyl)phenyl)-4-(4-trifluoromethoxyphenyl)-2H-1,2,4-triazol-3(4H)-thione **4a**.* IR (KBr, ν, cm^{-1}): 3413 (NH), 3086, 3024 (aromatic C–H), 1600 (C=N), 1578, 1512, 1474 (C=C), 1323, 1298, 1159 (SO_2), 1261 (C–O–C), 1216 (C=S); ^{1}H NMR (DMSO-d_6, δ ppm): 14.38 (s, 1H, NH); 7.80 (d, 2H, J = 8.6 Hz, aromatic protons); 7.75 (d, 2H, J = 7.5 Hz, aromatic protons); 7.70 (tt, 1H, J = 7.5, 1.5 Hz, aromatic proton); 7.61 (t, 2H, J = 7.5 Hz, aromatic protons); 7.56 (d, 2H, J = 7.9 Hz, aromatic protons); 7.52 (d, 2H, J = 8.6 Hz, aromatic protons); 7.50 (d, 2H, J = 7.9 Hz, aromatic protons); ^{13}C NMR (DMSO-d_6, δ ppm): 168.91 (C3-triazolic ring), 149.01 (C5-triazolic ring), 148.65, 142.50, 140.33, 134.07, 133.05, 130.96, 130.34, 129.85, 129.50, 127.62, 127.53, 121.83 (aromatic ring carbons), 119.93 (q, J = 255.0 Hz, OCF_3) (ESI–MS) m/z: 478 $[M + H]^+$.

*6.1.2.2. 5-(4-(4-Bromophenylsulfonyl)phenyl)-4-(4-trifluoro-methoxyphenyl)-2H-1,2,4-triazol-3(4H)-thione **4b**.* IR (KBr, ν, cm^{-1}): 3423 (NH), 3088, 3020 (aromatic C–H), 1600 (C=N), 1574, 1512, 1470 (C=C), 1329, 1290, 1161 (SO_2), 1264 (C–O–C), 1217 (C=S), 574 (C–Br); ^{1}H NMR (DMSO-d_6, δ ppm): 14.35 (s, 1H, NH); 7.96 (d, 2H, J = 8.6 Hz, aromatic protons); 7.87 (d, 2H, J = 8.4 Hz, aromatic protons); 7.82 (d, 2H, J = 8.4 Hz, aromatic protons); 7.56 (d, 2H, J = 9.8 Hz, aromatic proton); 7.53 (d, 2H, J = 8.6 Hz, aromatic protons); 7.51 (d, 2H, J = 9.8 Hz, aromatic protons); ^{13}C NMR (DMSO-d_6, δ ppm): 168.92 (C3-triazolic ring), 148.97 (C5-triazolic ring), 148.64, 141.99, 139.56, 132.96, 130.95, 130.85, 130.55, 129.54, 129.44, 128.35, 127.69, 121.82 (aromatic ring carbons), 119.91 (q, J = 254.5 Hz, OCF_3); (ESI–MS) m/z: 556 $[M + H]^+$; m/z: 558 $[M + H]^+$.

*6.1.2.3. 5-(4-(Phenylsulfonyl)phenyl)-4-(3,4,5-trimethoxyphenyl)-2H-1,2,4-triazol-3(4H)-thione **5a**.* IR (KBr, ν, cm^{-1}): 3418 (NH), 3086, 3065, 3028 (aromatic C–H), 2958, 2838 (CH_3), 1600 (C=N), 1543, 1505, 1465 (C=C), 1325, 1277, 1160 (SO_2), 1234 (C=S + C–O–C), 1128 (C–O–C); ^{1}H NMR (DMSO-d_6, δ ppm): 14.26 (s, 1H, NH); 7.97 (d, 2H, J = 8.6 Hz, aromatic protons); 7.94 (dd, 2H, J = 7.4, 1.5 Hz, aromatic protons); 7.93 (t, 2H, J = 7.4 Hz, aromatic protons); 7.70 (tt, 1H, J = 7.4, 1.5 Hz, aromatic proton); 7.60 (d, 2H, J = 8.6 Hz, aromatic protons); 6.75 (s, 2H, aromatic protons); 3.70 (s, 3H, 4-OCH_3); 3.60 (s, 6H, 3,5-di-OCH_3); ^{13}C NMR (DMSO-d_6, δ ppm): 168.98 (C3-triazolic ring), 149.05 (C5-triazolic ring), 153.04, 142.27, 140.46, 138.04, 133.98, 130.58, 129.80, 129.57, 129.19, 127.55, 127.43, 106.69 (aromatic ring carbons), 60.15 (4-OCH_3), 56.17 (3,5-di-OCH_3); (ESI–MS) m/z: 484 $[M + H]^+$.

6.1.2.4. 5-(4-(4-Bromophenylsulfonyl)phenyl)-4-(3,4,5-trimethoxyphenyl)-2H-1,2,4-triazol-3(4H)-thione ***5b***. IR (KBr, ν, cm^{-1}): 3431 (NH), 3088, 3002 (aromatic C–H), 2940, 2836 (CH_3), 1600 (C=N), 1573, 1506, 1465 (C=C), 1324, 1280, 1160 (SO_2), 1235 (C=S + C–O–C), 1128 (C–O–C), 585 (C–Br); ^{1}H NMR (DMSO-d_6, δ ppm): 14.26 (s, 1H, NH); 7.99 (d, 2H, $J = 8.7$ Hz, aromatic protons); 7.88 (d, 2H, $J = 8.4$ Hz, aromatic protons); 7.82 (d, 2H, $J = 8.4$ Hz, aromatic protons); 7.61 (d, 2H, $J = 8.7$ Hz, aromatic protons); 6.75 (s, 2H, aromatic protons); 3.70 (s, 3H, 4-OCH_3); 3.61 (s, 6H, 3,5-di-OCH_3); ^{13}C NMR (DMSO-d_6, δ ppm): 168.93 (C3-triazolic ring), 149.07 (C5-triazolic ring), 153.11, 141.84, 139.72, 138.07, 132.99, 130.83, 129.62, 129.32, 128.56, 128.36, 127.69, 106.72 (aromatic ring carbons), 60.23 (4-OCH_3), 56.23 (3,5-di-OCH_3); (ESI–MS) m/z: 562 $[M + H]^+$; m/z: 564 $[M + H]^+$.

6.1.3. General procedure for synthesis of 5-(4-(4-X-phenylsulfonyl) phenyl)-N-(R)-1,3,4-thiadiazol-2-amines ***6a,b; 7a,b***

Appropriate acylthiosemicarbazide **2** or **3** (1 mmol) in 40 mL of concentrated sulphuric acid was stirred at 0 °C for 3 h and then another 4 h at room temperature. To the resulting solution was added, on ice bath, an aqueous ammonia till pH ~ 8 and then the obtained precipitated was filtered, washed with water and recrystallized from $CHCl_3$/petroleum ether (1:2, v/v).

6.1.3.1. 5-(4-(Phenylsulfonyl)phenyl)-N-(4-trifluoromethoxyphenyl)-1,3,4-thiadiazol-2-amine ***6a***. IR (KBr, ν, cm^{-1}): 3348 (NH), 3060, 3017 (aromatic C–H), 1614 (C=N), 1579, 1555, 1510, 1480 (C=C), 1323, 1308, 1156 (SO_2), 1264 (C–O–C); ^{1}H NMR (DMSO-d_6, δ ppm): 10.88 (s, 1H, NH); 8.09 (d, 2H, $J = 9.0$ Hz, aromatic protons); 8.06 (d, 2H, $J = 9.0$ Hz, aromatic protons); 7.99 (dd, 2H, $J = 7.5$, 1.4 Hz, aromatic protons); 7.77 (d, 2H, $J = 8.8$ Hz, aromatic protons); 7.71 (tt, 1H, $J = 7.5$, 1.4 Hz, aromatic proton); 7.64 (t, 2H, $J = 7.5$ Hz, aromatic protons); 7.37 (d, 2H, $J = 8.8$ Hz, aromatic protons); ^{13}C NMR (DMSO-d_6, δ ppm): 164.73 (C2-thiadiazolic ring), 156.28 (C5-thiadiazolic ring), 142.82, 141.91, 140.71, 139.29, 134.69, 133.94, 129.86, 128.35, 127.86, 127.42, 122.08, 118.90 (aromatic ring carbons), 121.85 (q, $J = 254.3$ Hz, OCF_3); (ESI–MS) m/z: 478 $[M + H]^+$.

6.1.3.2. 5-(4-(4-Bromophenylsulfonyl)phenyl)-N-(4-trifluoromethoxyphenyl)-1,3,4-thiadiazol-2-amine ***6b***. IR (KBr, ν, cm^{-1}): 3241 (NH), 3087, 3057, 3015 (aromatic C–H), 1612 (C=N), 1573, 1556, 1510, 1493 (C=C), 1323, 1307, 1158 (SO_2), 1264 (C–O–C), 571 (C–Br); ^{1}H NMR (DMSO-d_6, δ ppm): 10.90 (s, 1H, NH); 8.08 (d, 2H, $J = 9.1$ Hz, aromatic protons); 8.04 (d, 2H, $J = 9.1$ Hz, aromatic protons); 7.92 (d, 2H, $J = 8.8$ Hz, aromatic protons); 7.85 (d, 2H, $J = 8.8$ Hz, aromatic protons); 7.77 (d, 2H, $J = 9.1$ Hz, aromatic protons); 7.38 (d, 2H, $J = 9.1$ Hz, aromatic protons); ^{13}C NMR (DMSO-d_6, δ ppm): 164.81 (C2-thiadiazolic ring), 156.23 (C5-thiadiazolic ring), 142.82, 141.37, 139.96, 139.28, 134.89, 132.97, 129.44, 128.43, 128.19, 127.92, 122.08, 118.91 (aromatic ring carbons), 120.16 (q, $J = 255.7$ Hz, OCF_3); (ESI–MS) m/z: 556 $[M + H]^+$; m/z: 558 $[M + H]^+$.

6.1.3.3. 5-(4-(Phenylsulfonyl)phenyl)-N-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-amine ***7a***. IR (KBr, ν, cm^{-1}): 3264 (NH), 3088, 3062 (aromatic C–H), 2966, 2840 (CH_3), 1606 (C=N), 1580, 1564, 1509, 1462 (C=C), 1319, 1309, 1157 (SO_2), 1240, 1129 (C–O–C); ^{1}H NMR (DMSO-d_6, δ ppm): 10.64 (s, 1H, NH); 8.08 (d, 2H, $J = 8.8$ Hz, aromatic protons); 8.05 (d, 2H, $J = 8.8$ Hz, aromatic protons); 8.00 (dd, 2H, $J = 7.2$, 1.9 Hz, aromatic protons); 7.72 (tt, 1H, $J = 7.2$, 1.9 Hz, aromatic proton); 7.65 (t, 2H, $J = 7.2$ Hz, aromatic protons); 6.99 (s, 2H, aromatic protons); 3.78 (s, 3H, 4-OCH_3); 3.63 (s, 6H, 3,5-di-OCH_3); ^{13}C NMR (DMSO-d_6, δ ppm): 165.34 (C2-thiadiazolic ring), 155.53 (C5-thiadiazolic ring), 153.20, 141.80, 140.78, 139.51, 136.51, 134.90, 134.04, 129.15, 128.41, 127.82, 127.57, 95.85 (aromatic ring carbons), 60.22 (4-OCH_3), 55.85 (3,5-di-OCH_3); (ESI–MS) m/z: 484 $[M + H]^+$.

6.1.3.4. 5-(4-(4-Bromophenylsulfonyl)phenyl)-N-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-amine ***7b***. IR (KBr, ν, cm^{-1}): 3270 (NH), 3087, 3062 (aromatic C–H), 2940, 2840 (CH_3), 1620 (C=N), 1573, 1509, 1463 (C=C), 1325, 1291, 1159 (SO_2), 1237, 1128 (C–O–C); 573 (C–Br); ^{1}H NMR (DMSO-d_6, δ ppm): 10.68 (s, 1H, NH); 8.08 (d, 2H, $J = 8.8$ Hz, aromatic protons); 8.04 (d, 2H, $J = 8.8$ Hz, aromatic protons); 7.93 (d, 2H, $J = 8.8$ Hz, aromatic protons); 7.86 (d, 2H, $J = 8.8$ Hz, aromatic protons); 7.00 (s, 2H, aromatic protons); 3.78 (s, 3H, 4-OCH_3); 3.62 (s, 6H, 3,5-di-OCH_3); ^{13}C NMR (DMSO-d_6, δ ppm): 165.35 (C2-thiadiazolic ring), 155.40 (C5-thiadiazolic ring), 153.15, 141.21, 139.99, 139.50, 136.38, 135.06, 133.00, 129.48, 128.43, 127.81, 126.64, 95.83 (aromatic ring carbons), 60.18 (4-OCH_3), 55.81 (3,5-di-OCH_3); (ESI–MS) m/z: 562 $[M + H]^+$; m/z: 564 $[M + H]^+$.

6.2. Antibacterial activity

The stock solution (2048 μg/mL) of each compound was made using dimethyl sulfoxide (DMSO) as solvent. The minimum inhibitory concentrations (MIC) of the new compounds were determined by the broth microdilution method carried out in 96-well microplates (Nunc, Denmark). Series of two-fold dilutions (from 1/2 to 1/1024) of the 12 compounds were made in cation-adjusted Műller-Hinton broth when tested against the bacterial strains and in Sabouraud broth when tested against the yeast strains.

The inoculum of the bacterial strains was obtained by suspending 5 colonies from a 24 h culture obtained on Columbia blood agar, in 5 mL of Műller-Hinton broth. After vigorous shaking of the microbial suspensions on a vortex-mixer for 15″, the turbidity was adjusted at 0.5 McFarland standard with a densitometer (DEN-1, BioSan), in order to achieve a concentration of 1.5×10^8 bacterial colony forming units (CFU)/mL. Afterwards, 0.1 mL of the above prepared bacterial inoculum was added in a tube with 9.9 mL Műller-Hinton broth and the concentration achieved after vortex mixing was of 1×10^6 CFU/mL. An aliquot of 50 μL of the final inoculum was added to all wells with 50 μL broth with compound, except for the negative growth control well (the sterility control), which contained only compound-free broth (100 μL). The final liquid volume in every well was of 100 μL, including the positive growth control (containing 50 μL compound-free broth and 50 μL bacterial inoculum). In addition, an inoculum control was performed by transferring 10 μL from each bacterial growth control well (just after the inoculum was added) into a tube with 10 mL Műller-Hinton broth, followed by vortex mixing and spreading of 100 μL of this dilution onto a plate with Columbia blood agar, with overnight incubation.

In case of the *Candida* reference strains, the inoculum was obtained by suspending 5 colonies (obtained from a 24 h culture on a Sabouraud dextrose agar) into 5 mL of sterile distilled water. After vortex mixing the suspension for 15″, the turbidity was adjusted at 0.5 McFarland, and afterwards, the inoculum was diluted in sterile distilled water in order to obtain a working suspension of 10^5 CFU/mL. The series of two-fold dilutions of the compounds (from 1/2 to 1/1024) were made in Sabouraud broth, and the inoculum was added into the microplates wells in the same amount as the broth. In addition, the positive and negative growth controls were prepared too. An inoculum control was performed by transferring 10 μL from the growth control well (just after the inoculum was added) into a tube with 2 mL Sabouraud broth, followed by vortex mixing. Afterwards, 100 μL of this dilution was spread on Sabouraud dextrose agar plates, which were incubated for 24–48 h.

The inoculum control plates and the microplates sealed with sterile adhesive sheets and covered with proper lids were incubated at 37 °C for 20 h for the bacterial strains and for 24–48 h for the yeasts strains (24 h for *C. albicans* and 48 h for *C. parapsilosis*).

After the incubation period, the microbial growth in the wells containing the tested compounds was examined macroscopically and compared to the positive and negative growth controls. The MIC value was considered to be the lowest concentration of the compound which could inhibit the microbial growth, traduced by the absence of any turbidity or growth button.

The investigation of the antimicrobial activity of the compounds was done in duplicate and as control, *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were tested against amikacin, and *C. parapsilosis* ATCC 22019 against fluconazole by the broth microdilution method [42,43]. The MIC value of amikacin was 2 μg/mL in case of all tested strains and the MIC value of fluconazole was also 2 μg/mL, in case of *C. parapsilosis* reference strain.

Because the MIC values are not spectacular, no statistical calculations were made.

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Appendix. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2012.01.031.

References

[1] F. Sabatelli, R. Patel, P.A. Mann, C.A. Mendrick, C.C. Norris, R. Hare, D. Loebenberg, T.A. Black, P.M. McNicholas, Antimicrob. Agents Chemother. 50 (2006) 2009–2015.
[2] T. El-Sayed Ali, A.M. El-Kazak, Eur. J. Chem. 1 (2010) 6–11.
[3] M. Serdar, N. Gümrükçüoğlu, Ş. Alpay Karaoğlu, N. Demirbaş, Turk. J. Chem. 31 (2007) 315–326.
[4] N. Siddiqui, P. Ahuja, W. Ahsan, S.N. Pandeya, M.S. Alam, J. Chem. Pharm. Res. 1 (2009) 19–30.
[5] N.U. Güzeldemirci, Ö. Küçükbasmaci, Eur. J. Med. Chem. 45 (2010) 63–68.
[6] Ş.G. Küçükgüzel, I. Küçükgüzel, E. Tatar, S. Rollas, F. Şahin, M. Güllüce, E. De Clercq, L. Kabasakal, Eur. J. Med. Chem. 42 (2007) 893–901.
[7] M.T. Abdel-Aal, W.A. El-Sayed, A.H. Abdel Aleem, E.S.H. El Ashry, Pharmazie 58 (2003) 788–792.
[8] I. Küçükgüzel, S.G. Küçükgüzel, S. Rollas, M. Kiraz, Bioorg. Med. Chem. Lett. 11 (2001) 1703–1707.
[9] A.A. Kadi, N.R. El-Brollosy, O.A. Al-Deeb, E.E. Habib, T.M. Ibrahim, A.A. El-Emam, Eur. J. Med. Chem. 42 (2007) 235–242.
[10] T. Plech, M. Wujec, A. Siwek, U. Kosikowska, A. Malm, Eur. J. Med. Chem. 46 (2011) 241–248.
[11] A.A. Siddiqui, A. Arora, N. Siddiqui, A. Misra, Indian J. Chem. 44B (2005) 838–841.
[12] C. Camoutsis, A. Geronikaki, A. Ciric, M. Soković, P. Zoumpoulakis, M. Zervou, Chem. Pharm. Bull. 58 (2010) 160–167.
[13] I. Küçükgüzel, E. Tatar, Ş.G. Küçükgüzel, S. Rollas, E. De Clercq, Eur. J. Med. Chem. 43 (2008) 381–392.
[14] I. Khan, S. Ali, S. Hameed, N.H. Rama, M.T. Hussain, A. Wadood, R. Uddin, Z. Ul-Haq, A. Khan, S. Ali, M.I. Choudhary, Eur. J. Med. Chem. 45 (2010) 5200–5207.
[15] G. Ayhan-Kilcigil, C. Kus, T. Çoban, B. Can-Eke, M. Iscan, J. Enz. Inhib. Med. Chem. 19 (2004) 129–135.
[16] A. Duran, H.N. Dogan, S. Rollas, Il Farmaco 57 (2002) 559–564.
[17] W. Rzeski, J. Matysiak, M. Kandefer-Szerszen, Bioorg. Med. Chem. 15 (2007) 3201–3207.
[18] A.T. Mavrova, D. Wesselinova, Y.A. Tsenov, P. Denkova, Eur. J. Med. Chem. 44 (2009) 63–69.
[19] H. Kumar, S.A. Javed, S.A. Khan, M. Amir, Eur. J. Med. Chem. 43 (2008) 2688–2698.
[20] M. Amir, K. Shikha, Eur. J. Med. Chem. 39 (2004) 535–545.
[21] S.M. El-Moghazy, F.F. Barsoum, H.M. Abdel-Rahman, A.A. Marzouk, Med. Chem. Res. (2011). doi:10.1007/s00044-011-9691-4.
[22] J.M. Kane, M.A. Staeger, C.R. Dalton, F.P. Miller, M.W. Dudley, A.M.L. Ogden, J.H. Kehne, H.J. Ketteler, T.C. McCloskey, Y. Senyah, P.A. Chmielewski, J.A. Miller, J. Med. Chem. 37 (1994) 125–132.
[23] H.N. Dogan, A. Duran, S. Rollas, G. Sener, M.K. Uysal, D. Gülen, Bioorg. Med. Chem. 10 (2002) 2893–2898.
[24] R. Sharma, G.P. Misra, J. Sainy, S.C. Chaturvedi, Med. Chem. Res. 20 (2011) 245–253.
[25] E.F. Elslager, Z.B. Gavrilis, A.A. Phillips, D.F. Worth, J. Med. Chem. 12 (1969) 357–363.
[26] R. Wolf, H. Matz, E. Orion, B. Tuzun, Y. Tuzun, Dermatol. Online J. 8 (2002) 2.
[27] S.-F. Barbuceanu, G. Bancescu, O.D. Cretu, C. Draghici, A. Bancescu, M. Radu-Popescu, Rev. Chim. (Bucharest) 61 (2010) 140–145.
[28] I. Şaramet, G.-L. Almăjan, Ş. Bărbuceanu, C. Drăghici, M.D. Banciu, Rev. Roum. Chim. 50 (2005) 19–27.
[29] S.F. Barbuceanu, G.L. Almajan, I. Saramet, C. Draghici, R. Socoteanu, F. Barbuceanu, J. Serb. Chem. Soc. 74 (2009) 1041–1049.
[30] S.-F. Barbuceanu, G.L. Almajan, I. Saramet, C. Draghici, A.I. Tarcomnicu, G. Bancescu, Eur. J. Med. Chem. 44 (2009) 4752–4757.
[31] G.L. Almajan, S.-F. Barbuceanu, G. Bancescu, I. Saramet, G. Saramet, C. Draghici, Eur. J. Med. Chem. 45 (2010) 6139–6146.
[32] G.L. Almajan, S.-F. Barbuceanu, E.-R. Almajan, C. Draghici, G. Saramet, Eur. J. Med. Chem. 44 (2009) 3083–3089.
[33] A. Mavrodin, V. Zotta, V.M. Stoenescu, D. Oteleanu, Pharm. Zentr. Deutsch. 95 (1956) 353–361.
[34] M.R. Banday, A. Rauf, Indian J. Chem. 48B (2009) 97–102.
[35] S. Shelke, G. Mhaske, S. Gadakh, C. Gill, Bioorg. Med. Chem. Lett. 20 (2010) 7200–7204.
[36] Z. Cesur, N. Cesur, S. Birteksöz, G. Ötük, Acta Chim. Slov. 57 (2010) 355–362.
[37] J. Tomaščiková, J. Imrich, I. Danihel, S. Böhm, P. Kristian, J. Pisarčíková, M. Sabol, K.D. Klika, Molecules 13 (2008) 501–518.
[38] U. Salgın-Gökşen, N. Gökhan-Kelekçi, Ö. Göktaş, Y. Köysal, E. Kiliç, Ş. Işik, G. Aktay, M. Özalp, Bioorg. Med. Chem. 15 (2007) 5738–5751.
[39] N.N. Gülerman, H.N. Doğan, S. Rollas, C. Johansson, C. Çelik, Il Farmaco 56 (2001) 953–958.
[40] G.A. Idrees, O.M. Aly, G. El-Din, A.A. Abuo-Rahma, M.F. Radwan, Eur. J. Med. Chem. 44 (2009) 3973–3980.
[41] W. Xu, B. Song, P. Bhadury, Y. Song, D. Hu, Molecules 15 (2010) 766–779.
[42] European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution, Clin. Microbiol. Infect. 9 (2003) 1–7.
[43] Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST-AFST) 2008, EUCAST definitive document EDef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts, Clin. Microbiol. Infect. 14 (2008) 398–405.