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Sulfonamide-1,2,4-triazole derivatives as antifungal and antibacterial agents: Synthesis, biological evaluation, lipophilicity, and conformational studies

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Abstract—A series of 10 new 5-[2-(substituted sulfamoyl)-4,5-dimethoxy-benzyl]-4aryl-*s*-triazole-3-thiones were synthesized and evaluated for in vitro antifungal and antibacterial activity. All compounds tested showed significant antifungal activity against all the micromycetes, compared to the commercial fungicide bifonazole. Differences in their activity depend on the substitution of different reactive groups. More specifically, best antifungal activity among synthetic analogues was shown with *N*-dimethylsulfamoyl group. All the compounds tested against bacteria showed the same activity as the commercial agent streptomycin, except for *Enterobacter cloacce* and *Salmonella* species. Chloramphenicol showed lower bactericidal effect than the synthetic compounds. Furthermore, it is apparent that different compounds reacted in different ways against bacteria. Gram (−) bacteria seem to be more sensitive to these compounds than Gram (+) species. An effort was made to correlate the above-mentioned differences in activity with lipophilicity studies. Furthermore, molecular modeling was used to obtain the main conformational features of this class of molecules for future structure–activity relationship studies.

1. Introduction

Systemic fungal infections are important problems in phytopathology and especially in medicine, the care of patient's immunosuppressed by infectious diseases, chemotherapy, or age. Infections caused by fungal species are common in immunocompromised patients and carry significant treatment costs and mortality.[1] Standard systemic antibiotic therapy alone is frequently unsatisfactory in certain circumstances. Also, more attention is being focused on addressing the problem of multidrug-resistant bacteria and the staggering costs and consequences resulting from this. The emerging resistance of microorganisms to some synthetic antimicrobial agents makes it necessary to continue the search for new antimicrobial substances.

The aim of this paper is to investigate the antibacterial and antifungal activity of various triazole derivatives against food poisoning, plant, animal, and human pathogens. Triazole moiety may be considered as a bioisostere of imidazole, which is a part of the azole group of antifungal drugs (i.e., fluconazole). The biological activities of various triazole derivatives have been extensively studied. It is known from the literature that the *s*-triazole moiety has great versatility in fusing to various ring systems and possesses a broad spectrum of biological activities. Among the most important effects, triazole derivatives have been reported to exhibit antibacterial,[2–16] antifungal,[2–4,17–20] and antimycobacterial[21–25] properties.

Prompted by these observations and in continuation of our search for bioactive molecules, we designed the syn-

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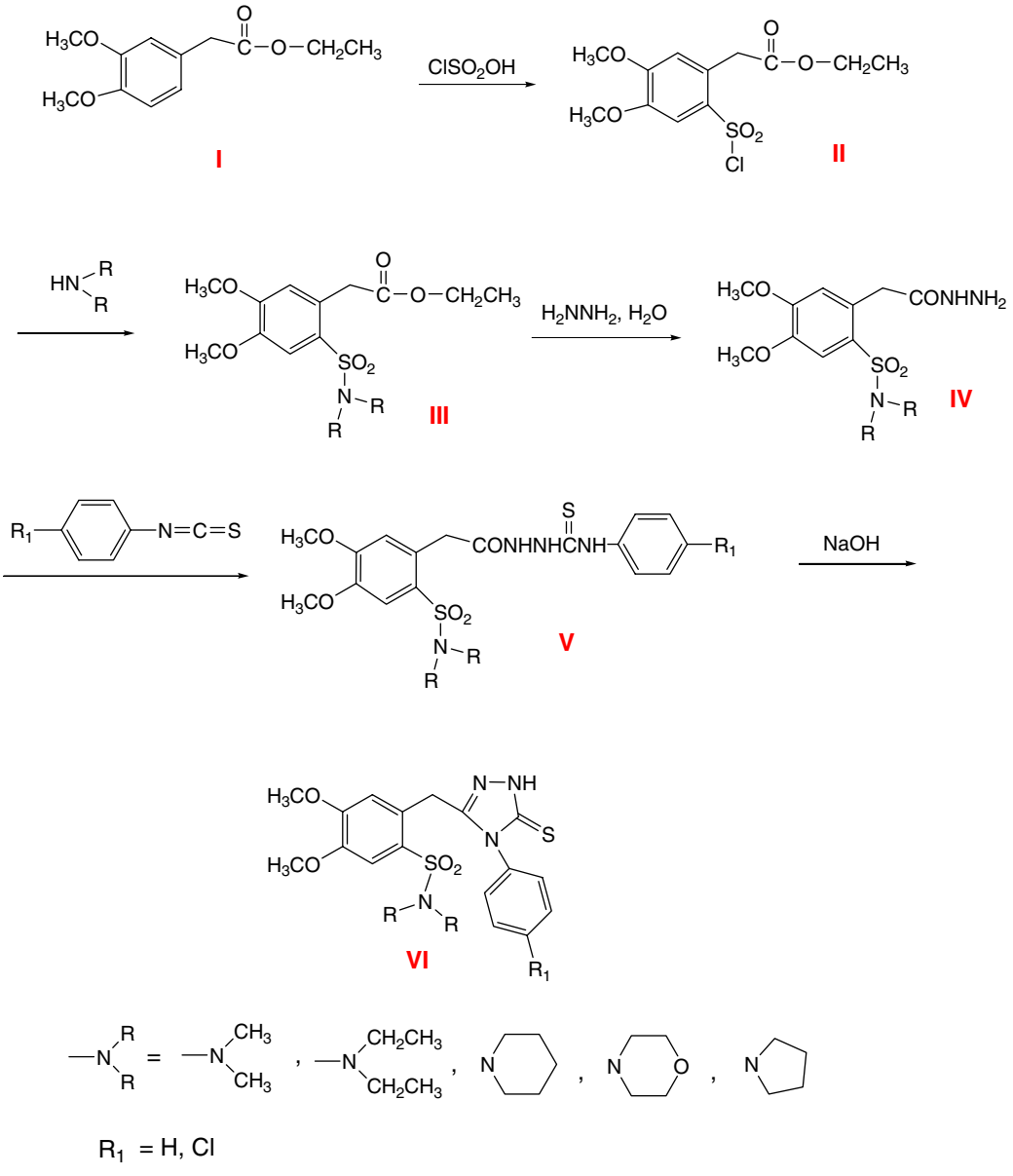
thesis of a series of novel sulfonamide-*s*-triazole. In particular, we emphasized in the strategy of combining two chemically different but pharmacologically compatible molecules (the sulfonamide nucleus and the *s*-triazole nucleus) in one frame, in order to study their antifungal and antibacterial activities.

2. Chemistry

The synthetic pathway followed for the preparation of the title compounds was accomplished as shown in Scheme 1.

Since the para position to one of the methoxy groups in ethyl 3,4-dimethoxyphenylacetate (**I**) is activated, direct chlorosulfonation at low temperature afforded ethyl-[2-(chlorosulfonyl)-4,5-dimethoxy-phenyl]acetate (**II**).[26]

We found that **II** reacts readily with secondary amines in anhydrous benzene to give excellent yields of the corresponding sulfonamides (**IIIb–e**). In the contrary to the preparation of the above sulfonamides, the *N*-dimethyl-sulfonamide **IIa** was prepared by treatment of sulfonyl chloride **II** with 3 equiv of a 40% aqueous solution of dimethylamine in chloroform at 0 °C.[27] The latter were converted to the desired 2-(*N*-substituted sulfamoyl)-4,5-dimethoxy-phenylacetylhydrazides **IV**(**a–e**) by treatment with hydrazine hydrate in xylol. The hitherto unknown 1-[2-(*N*-substituted sulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-aryl-thiosemicarbazides (**Va–j**) were synthesized by condensing of acid hydrazides **IVa–e** with suitable aryl isothiocyanates.[28] These thiosemicarbazides were cyclized into their corresponding 5-[2-(*N*-substituted sulfamoyl)-4,5-dimethoxy-benzyl]-4-aryl-triazole-3-thiones (**VIa–j**) in the presence of aqueous 2 N sodium hydroxide solution.[28]



Scheme 1.

It is interesting to note that compounds **VIa–j** were present in the solid state in the C=S form as indicated by their IR and ^{1}H NMR spectra. In the IR spectra, the presence of two absorption maxima at 1342 and 1320 cm^{-1}, characteristic of the C=S group in this type of compounds, and the lack of absorption bands at around 2500–2600 cm^{-1} for the S–H stretching support the thione form of **VIa–j**.[29] In addition N–H stretching due to *s*-triazoline-3-thiones was detected within the 3015–3300 cm^{-1} region. Furthermore in the ^{1}H NMR spectra, the broad signal between 13.38 and 13.70 ppm due to triazole–NH indicates that the above compounds exist in the C=S form.

3. Results and discussion

3.1. Biological evaluation

Antifungal activity of compounds tested is presented in Table 1. All the compounds tested showed significant antifungal activity against all the micromycetes. It can be seen that compounds **VIa** and **VIb** showed the best inhibitory activity with lowest MIC (50.0 μg/ml) against mycotoxic species *Aspergillus flavus* and *Trichoderma viride*. Compounds **VIc** and **VId** also possessed great inhibitory effect with MIC of 50.0 μg/ml against *T. viride*. Compounds **VIg**, **VIh**, **VIi**, and **VIj** exhibited the lowest inhibitory activity with MIC of 100.0–150.0 μg/ml, but still greater than commercial fungicide bifonazole which showed MIC of 150.0–200.0 μg/ml (Fig. 1). Among the compounds tested, **VIa** and **VIb** showed the highest fungicidal potential with MFC of 100.0 μg/ml against *A. flavus*, *Penicillium funiculosum*, and *T. viride*. Compounds **VIc** and **VId** also possessed great fungicidal effect against *T. viride* with MFC of 100.0 μg/ml. All the other compounds showed fungicidal effect at 150.0 μg/ml which is lower than MFC for bifonazole (200.0-250.0 μg/ml) (Fig. 2). It is interesting that all the compounds exhibited the best antifungal activity against *T. viride*. Previous results of antifungal activities of both natural and synthetic antifungal agents against numerous fungal strains showed that *T. viride* is the most resistant species tested.[30,31]

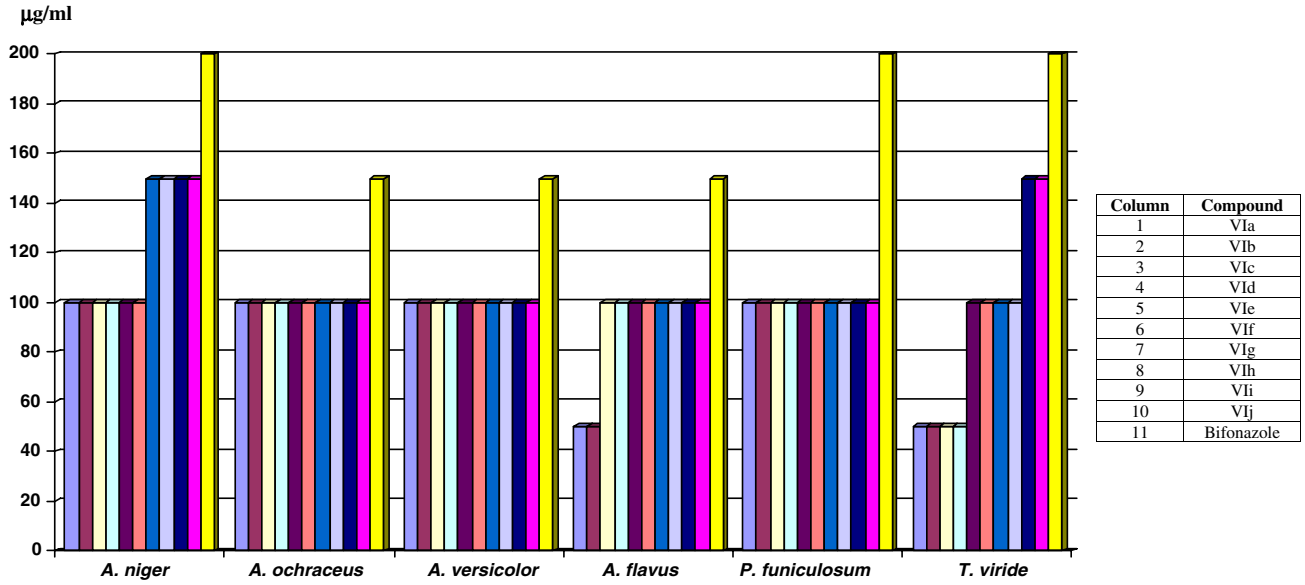
It can be concluded that there is a connection between antifungal activity and chemical structure of these compounds. Compounds **VIa** and **VIb** showed the best antifungal activity against micromycetes investigated, while **VIi** and **VIj** possessed the lowest antifungal effect. These better effects for compounds **VIa** and **VIb** could be explained as a consequence of the presence of an *N*-$(CH_3)_2$ group in their structure, and in the contrary compounds **VIi** and **VIj** do not possess this reactive group in their structure. However, all the compounds exhibited much higher antifungal activity than commercial fungicide bifonazole which is the imidazole-type fungicide.

Antibacterial activity of compounds tested is presented in Table 2. Compounds **VIa**, **VIb**, **VIc**, **VIb**, **VIe**, **VIf**, and **VIg** showed inhibitory effect at 100.0 μg/ml against *Escherichia coli*, the same MIC as antibiotic streptomycin, but much better than chloramphenicol (MIC at 250.0 μg/ml). Compounds **VIi** and **VIj** showed greater MIC at 150.0 μg/ml against *E. coli*. The highest inhibitory effect against *Salmonella typhimurium* can be seen for compounds **VIe**, **VIf**, **VIh**, and **VIj** with MIC at 100.0 μg/ml, these compounds showed lower activity than streptomycin but much better than chloramphenicol. Compound **VIj** exhibited inhibitory effect at 100.0 μg/ml against *Enterobacter cloacce*, better than streptomycin but lower than chloramphenicol. The rest of the compounds showed MIC at 150.0 μg/ml (Fig. 3). The values for minimal bactericidal concentrations of compounds tested are the same for all of them (150.0 μg/ml), except against *E. cloacce* where the MBC is lower (100.0 μg/ml) and same as for streptomycin but higher than that for chloramphenicol. All the compounds tested showed the same bactericidal activity as streptomycin, except for *E. cloacce* and *Salmonella* species. Chloramphenicol showed lower bactericidal effect than compounds tested. Demirayak et al.[32] and Ulusoy et al.[33] showed in their results that triazole derivates possessed antibacterial and antifungal activities with MIC of 6.25–250 μg/ml. The most resistant species was *E. coli*, while the most sensitive was *Salmonella aureus*. Our results showed that *E. coli* was the most susceptible species to triazole derivates tested in this work.

It is obvious that different compounds reacted in different ways against bacteria. Gram (−) bacteria seem to be more sensitive in these compounds than Gram (+) species. Chloramphenicol and streptomycin also showed differences in their activities (Figs. 3 and 4).

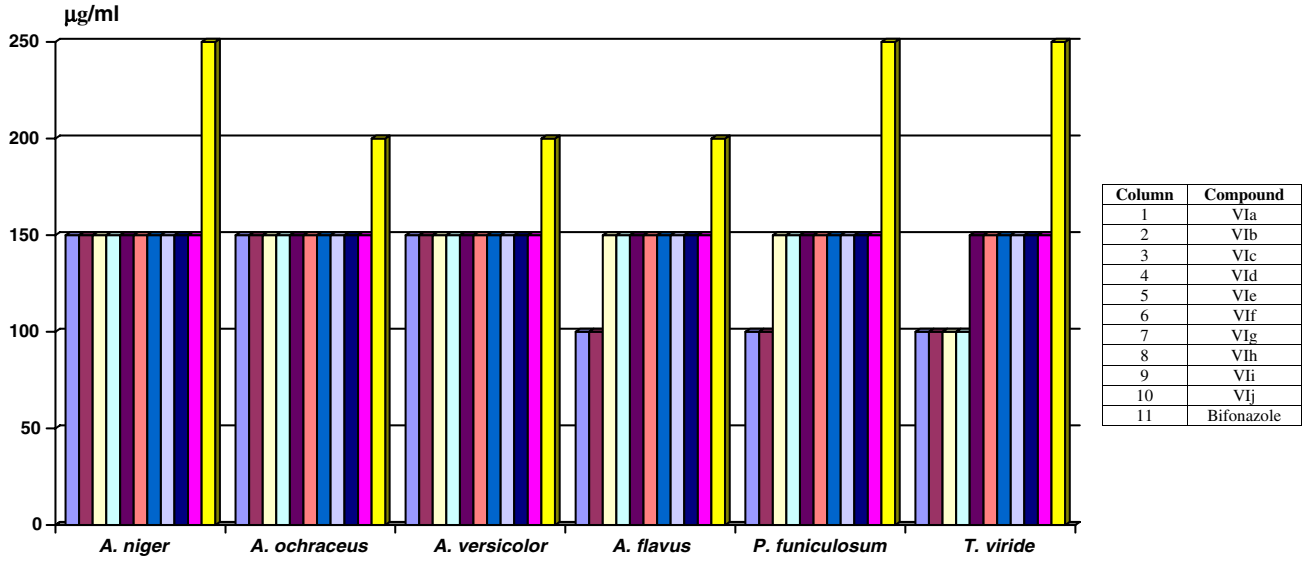
Table 1. Minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs-μg/ml)

Micromycetes	**VIa** Mic (Mfc)	**VIb** Mic (Mfc)	**VIc** Mic (Mfc)	**VId** Mic (Mfc)	**VIe** Mic (Mfc)	**VIf** Mic (Mfc)	**VIg** Mic (Mfc)	**VIh** Mic (Mfc)	**VIi** Mic (Mfc)	**VIj** Mic (Mfc)	Bifonazole Mic (Mfc)
A. niger	100	100	100	100	100	100	150	150	150	150	200
	150	150	150	150	150	150	150	150	150	150	250
A. ochraceus	100	100	100	100	100	100	100	100	100	100	150
	150	150	150	150	150	150	150	150	150	150	200
A. versicolor	100	100	100	100	100	100	100	100	100	100	150
	150	150	150	150	150	150	150	150	150	150	200
A. flavus	50	50	100	100	100	100	100	100	100	100	150
	100	100	150	150	150	150	150	150	150	150	200
P. funiculosum	100	100	100	100	100	100	100	100	100	100	200
	100	100	150	150	150	150	150	150	150	150	250
T. viride	50	50	50	50	100	100	100	100	150	150	200
	100	100	100	100	150	150	150	150	150	150	250



Column	Compound
1	VIa
2	VIb
3	VIc
4	VId
5	VIe
6	VIf
7	VIg
8	VIh
9	VIi
10	VIj
11	Bifonazole

Figure 1. Minimal inhibitory concentrations (µg/ml) of compounds tested and bifonazole against micromycetes. The table on the right provides the correlation between columns and compounds.



Column	Compound
1	VIa
2	VIb
3	VIc
4	VId
5	VIe
6	VIf
7	VIg
8	VIh
9	VIi
10	VIj
11	Bifonazole

Figure 2. Minimal fungicidal concentrations (µg/ml) of compounds tested and bifonazole against micromycetes.

The susceptibility of a microorganism to some agents depends, first of all, on the properties of the agents and the microorganism itself. It is known that Gram (+) bacteria are more susceptible to antimicrobial agents and Gram (−) bacteria are less sensitive. Fungi are more susceptible than bacteria in general.[34] These observations are confirmed with our results. The results obtained clearly demonstrate that compounds tested present a great potential for medical procedures and for food, cosmetics, and pharmaceutical industries.

3.2. Lipophilicity studies

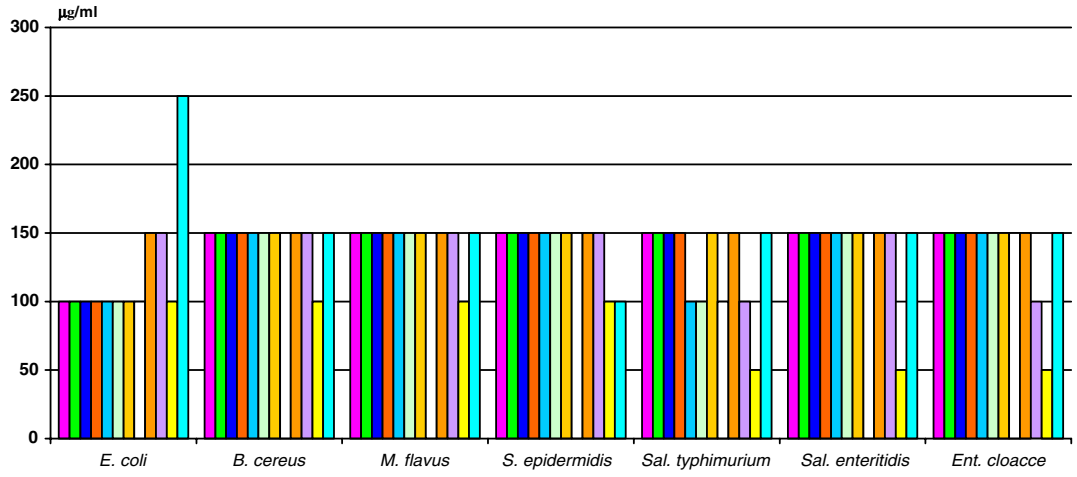
The examined compounds have the same skeleton with variation only in the amino moiety $N(R)_2$ and the substituent in the 4-position of aromatic ring substituted in the C1 position with triazole cycle. The attempt to correlate antibacterial and antifungal activities of the synthesized compounds with lipophilicity was unsuccessful. It is obvious from the obtained results (Table 3) that lipophilicity does not affect so much the antibacterial/antifungal activities of synthesized compounds. Probably the structural characteristics of the synthesized molecules are more important for this kind of activity.

3.3. Molecular modeling studies

As a first step for future structure–activity relationship studies, we performed conformational analysis using molecular modeling. In this paper, we demonstrate the conformational properties of two of the synthesized compounds. For comparison reasons we selected **VIa** as one of the most active and **VIe** from the less active molecules.

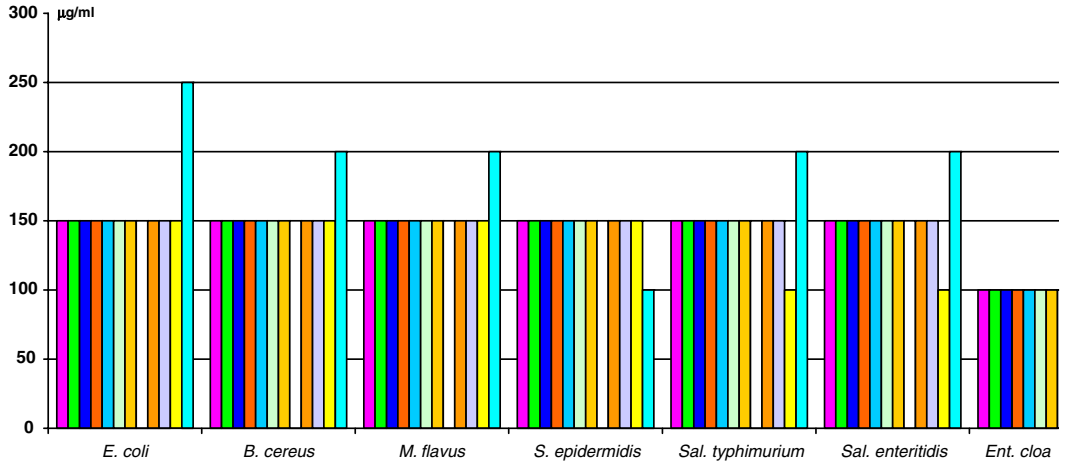
Table 2. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) in μg/ml of compounds tested in micro-dilution method

Bacteria	**VIa** Mic (Mfc)	**VIb** Mic (Mfc)	**VIc** Mic (Mfc)	**VId** Mic (Mfc)	**VIe** Mic (Mfc)	**VIf** Mic (Mfc)	**VIg** Mic (Mfc)	**VIh** Mic (Mfc)	**VIi** Mic (Mfc)	**VIj** Mic (Mfc)	Streptomycin Mic (Mfc)	Chloramphenicol Mic (Mfc)
E. coli	100	100	100	100	100	100	100	100	150	150	100	250
	150	150	150	150	150	150	150	150	150	150	150	250
B. cereus	150	150	150	150	150	150	150	150	150	150	100	150
	150	150	150	150	150	150	150	150	150	150	150	200
M. flavus	150	150	150	150	150	150	150	150	150	150	100	150
	150	150	150	150	150	150	150	150	150	150	150	200
S. epidermidis	150	150	150	150	150	150	150	150	150	150	100	100
	150	150	150	150	150	150	150	150	150	150	150	100
S. typhimurium	100	150	150	150	100	100	150	100	150	150	50	150
	150	150	150	150	150	150	150	150	150	150	100	200
S. enteritidis	150	150	150	150	150	150	150	150	150	150	50	150
	150	150	150	150	150	150	150	150	150	150	100	200
E. cloacce	100	100	100	100	100	100	100	100	100	100	50	150
	100	100	100	100	100	100	100	100	100	100	100	200



Column	Compound
1	VIa
2	VIb
3	VIc
4	VId
5	VIe
6	VIf
7	VIg
8	VIh
9	VIi
10	VIj
11	Str
12	Chlo

Figure 3. Minimal inhibitory concentrations (μg/ml) of compounds tested and antibiotics against bacteria.



Column	Compound
1	VIa
2	VIb
3	VIc
4	VId
5	VIe
6	VIf
7	VIg
8	VIh
9	VIi
10	VIj
11	Str
12	Chlo

Figure 4. Minimal bactericidal concentrations (μg/ml) of compounds tested and streptomycin against bacteria.

3.3.1. Conformational analysis of VIa and VIe. The most important conformational properties of both compounds are related to the dihedral angles τ_1–τ_7 which are presented in Figure 5.

The two molecules were designed and subjected to energy minimization procedures using first and second order algorithms. The last were input as initial conformations for the generation of low energy con-

Table 3. Calculated lipophilicity of tested compounds

Compound	$N(R)_2$	R_1	*A*log*P*	IAlog*P*	*C*log*P*	Cosmo log*P*	*m*log*P*	Kowwin	*X*log*P*	Average
VIa	$N(Me)_2$	H	2.59	2.31	3.687	4.25	1.72	3.56	2.39	2.93
VIb	$N(Me)_2$	Cl	3.30	2.96	4.40	4.55	2.40	4.20	3.02	3.55
VIc	$N(Et)_2$	H	3.32	2.49	4.745	5.58	2.48	4.54	3.24	3.77
VId	$N(Et)_2$	Cl	3.96	2.65	5.458	5.88	3.15	5.18	3.86	4.31
VIe		H	3.08	3.43	4.880	5.53	2.63	4.92	3.26	3.96
VIf		Cl	3.68	3.83	5.593	5.84	3.31	5.56	3.88	4.53
VIg		H	2.10	3.16	3.587	4.28	1.57	3.17	1.99	2.98
VIh		Cl	2.82	2.73	4.297	4.58	2.25	3.81	2.62	3.30
VIi		H	2.78	2.55	4.321	4.81	2.13	4.42	2.90	3.42
VIj		Cl	3.37	3.69	5.034	510	2.11	5.07	3.52	3.98

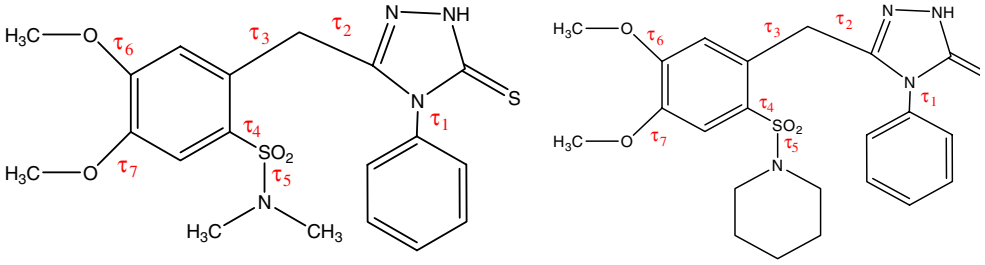


Figure 5. Chemical structures of **VIa** (left) and **VIe** (right).

formers using Random Sampling algorithm. The conformers are produced by random modifications of the dihedral angles followed by minimization procedure. Consequently, the minimized conformers are representatives of the potential energy surface.

Compund **VIa** produced 1000 low energy conformers which were grouped into eight different classes according to their atom coordinates with an RMSD value equal to 2.38. The four lowest energy conformers are displayed in Figure 6 and their dihedral values are shown in Table 4. Differences among **VIa** conformers are mainly due to τ_2 and τ_3 dihedrals resulting in two main classes. The first one includes **VIa**_1 and **VIa**_2 and favors a cluster between the two phenyl rings. The second includes **VIa**_3 and **VIa**_4 and extends the two phenyl systems away from each other. Interestingly, the four low energy conformers have a mirror image relationship forming two pairs of enantiomers. This is also apparent from the identical energy values of each pair (**IVa**_1 and **IVa**_2 have an energy of 26.85 kcal/mol, and **IVa**_3 and **IVa**_4 have an energy of 27.34 kcal/mol).

Respectively, **VIe** produced 1000 low energy conformers which were grouped into 8 different classes according to their atom coordinates with an RMSD value equal to 2.68. The four lowest energy conformers are displayed in Figure 7 and their dihedral values are shown in Table 5. **VIe**_1 and **VIe**_2 favor a cluster between the two phenyl rings as in case of **VIa**. Furthermore, these two conformers have a mirror image relationship and an energy equal to 37.47 kcal/mol. **VIe**_3 (38.65 kcal/mol) and **VIe**_4 (40.40 kcal/mol) differ in τ_1, τ_2, and τ_3 dihedral angles and favor a cluster between the phenyl ring attached to the triazole system and the pyridine ring. Grid Scan analysis on τ_2 and τ_3 has proved that also **VIe**_3 and **VIe**_4 can have enantiomeric conformations which were not produced with simple use of Random Sampling method. A more detailed conformational analysis study of all series of synthesized molecules is still in progress.

4. Experimental

4.1. General experimental considerations

Melting points were taken in glass capillary tubes on a Haake Bucher apparatus and are uncorrected. IR spectra were recorded on a FT-IR Jasco spectrophotometer in solid phase KBr. All proton NMR spectra were determined with a Bruker AC300 spectrometer using deuterated dimethylsulfoxide (DMSO-d_6) and are reported in δ units (ppm) relative to tetramethylsilane (TMS) as an internal standard. Thin layer chromatography (TLC)

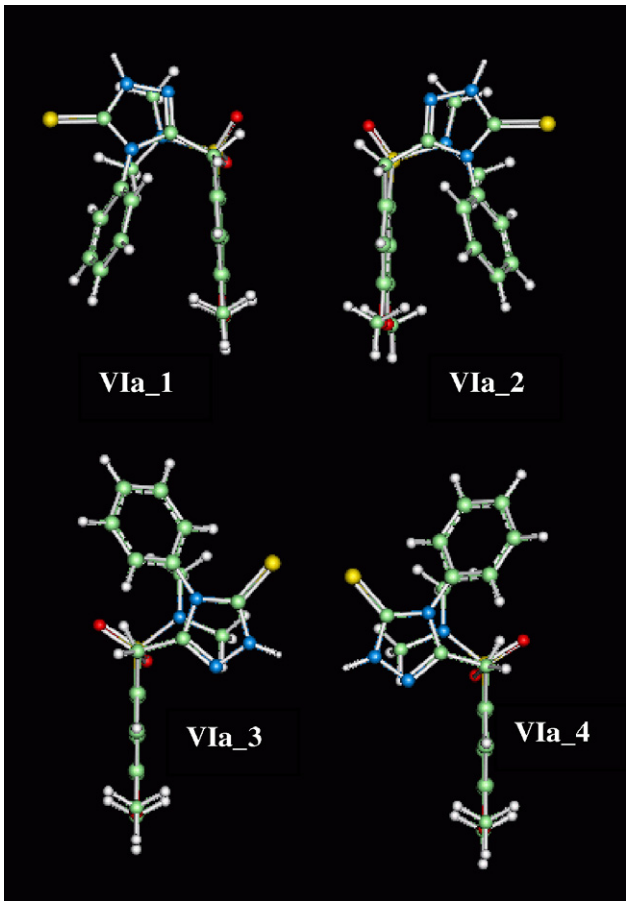


Figure 6. Low energy representative conformers of **VIa**.

Table 4. Dihedral angles for the low energy conformers of compound **VIa**

Conformer	VIa_1	VIa_2	VIa_3	VIa_4
Dihedral				
τ_1	−65.9	66.1	125.2	−125.0
τ_2	−55.9	55.4	−158.3	158.4
τ_3	−67.3	67.3	96.3	−96.1
τ_4	−98.0	98.1	126.8	−126.6
τ_5	−171.8	−53.7	169.5	57.3
τ_6	−178.4	178.3	−179.2	179.4
τ_7	1.2	−1.1	−0.3	0.3

was performed in E. Merck precoated silica gel plates (Kieselgel 60F_{254}). Visualization was obtained by exposure to iodine vapors and/or under UV light (254 nm). The elemental analyses (C, H, N) of all compounds were performed by the Center of Instrumental Analysis of the University of Patras and are within the range of experimental error (±0.4% of the calculated values).

4.1.1. General procedure for the preparation of the ethyl-[2-(*N*-substituted sulfamoyl)-4,5-dimethoxy-phenyl]acetate (III). To a flask containing 0.01 mol of ethyl-(2-chlorosulfonyl-4,5-dimethoxy-phenyl)acetate in 30 ml of anhydrous benzene was added 0.02 mol of aliphatic amine. The mixture was heated under reflux for 2 h. Then the solvent was evaporated under reduced pressure and ice-water was added to yield the corresponding sul-

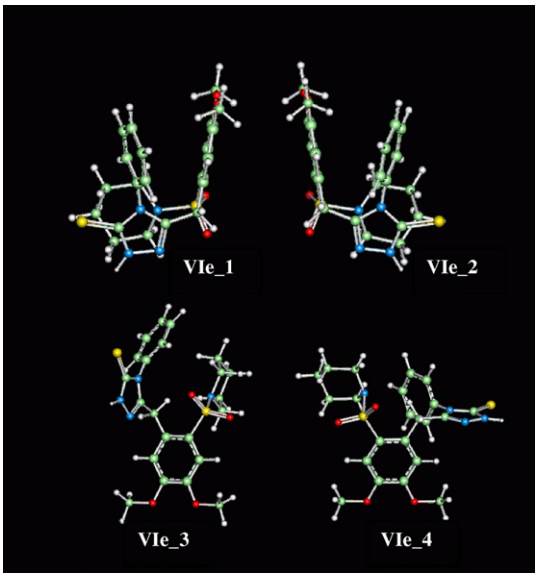


Figure 7. Low energy representative conformers of **VIe**.

Table 5. Dihedral angles for the low energy conformers of compound **VIe**

Conformer	VIe_1	VIe_2	VIe_3	VIe_4
Dihedral				
τ_1	−71.3	−108.6	60.3	−93.6
τ_2	−56.8	56.7	147.1	74.5
τ_3	−68.0	68.0	−86.0	−158.5
τ_4	−94.8	94.7	119.4	−105.8
τ_5	52.7	175.2	170.7	−171.4
τ_6	−177.7	177.5	179.5	176.1
τ_7	1.3	−1.4	−0.3	0.9

fonamides. The title compounds prepared are reported below.

4.1.1.1. Ethyl-[2-(*N*-diethylsulfamoyl)-4,5-dimethoxy-phenyl]acetate (IIIb). Mp 68–69 °C (*n*-hexane-ethyl acetate) (Ref. 35; 67–68 °C).

4.1.1.2. Ethyl-[2-(1-peperidinesulfamoyl)-4,5-dimethoxy-phenyl]acetate (IIIc). Mp 101 °C (methanol) (Ref. 26; 100–101 °C).

4.1.1.3. Ethyl-[2-(4-morpholinesulfamoyl)-4,5-dimethoxy-phenyl]acetate (IIId). Mp 104–105 °C (methanol) (Ref. 26; 105–106 °C).

4.1.1.4. Ethyl-[2-(1-pyrrolidinesulfamoyl)-4,5-dimethoxy-phenyl]acetate (IIIe). Yield: 95%, Mp 107–108 °C (ethyl acetate). IR ν cm^{-1}: 1745 (COO), 1335 ($S–O_{anti-sym}$), 1140 ($S–O_{sym}$). Anal. calc for $C_{16}H_{23}NO_6S$: C, 53.78; H, 6.44; N, 3.92. Found: C, 53.45; H, 6.55; N, 3.80.

4.1.1.5. Ethyl-[2-(*N*-dimethylsulfamoyl)-4,5-dimethoxy-phenyl]acetate (IIIa). To a solution of 3.225 g (0.01 mol) of ethyl-[2-(chlorosulfonyl)-4,5-dimethoxy-phenyl]acetate in 30 ml of chloroform was added 3.8 ml (0.034 mol) of a 40% aqueous solution of

dimethylamine. The reaction mixture was stirred for 3 h at 0°C and then 30 ml of chloroform was added. The organic layer was separated and washed with a solution of dilute hydrochloric acid (30 ml) and water (30 ml). The solvent was next dried and evaporated and the resulting solid was recrystallized from ethyl acetate to give 2.95 g (89%) of the *N*-dimethyllsulfonamide. Mp 109–110 °C, IR ν cm^{-1}: 1735 (COO), 1330 (S–$O_{antisym}$), 1135 (S–O_{sym}). Anal. calc for $C_{14}H_{21}NO_6S$: C, 50.75; H, 6.34; N, 4.23. Found: C, 51.03; H, 6.28; N, 4.42.

4.1.2. General procedure for the preparation of the 2-(*N*-substituted sulfamoyl)-4,5-dimethoxy-phenylacetylhydrazides (IV). To a flask containing 0.01 mol of ethyl-[2-(*N*-substituted sulfamoyl)-4,5-dimethoxy-phenyl]acetate in 20 ml of xylol was added 0.02 mol of 80% hydrazine monohydrate. The mixture was refluxed for 20 h. The solvent was then removed under reduced pressure and the solid residue after crystallization from the appropriate solvent was collected by filtration.

The following compounds were prepared by an analogous procedure.

4.1.2.1. 2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-phenylacetylhydrazide (IVa). Yield: 78%. Mp 147–149 °C (ethanol). IR ν cm^{-1}: 3295, 3375 (NH, NH_2), 1655, 1615 (CONH), 1370 (S–$O_{antisym}$), 1120 (S–O_{sym}). Anal. calc for $C_{12}H_{19}N_3O_5S$: C, 45.42; H, 5.99; N, 13.24. Found: C, 45.31; H, 5.80; N, 13.11.

4.1.2.2. 2-(*N*-Diethylsulfamoyl)-4,5-dimethoxy-phenylacetylhydrazide (IVb). Yield: 95%. Mp 160–161 °C (ethanol). IR ν cm^{-1}: 3180, 3290, 3375 (NH, NH_2), 1660, 1615 (CONH), 1345 (S–$O_{antisym}$), 1120 (S–O_{sym}). Anal. calc for $C_{14}H_{23}N_3O_5S$: C, 48.69; H, 6.66; N, 12.17. Found: C, 48.81; H, 6.78; N, 12.05.

4.1.2.3. 2-(1-Piperidinesulfamoyl)-4,5-dimethoxy-phenylacetylhydrazide (IVc). Yield: 82%. Mp 134–136 °C (ethanol). IR ν cm^{-1}: 3200, 3320 (NH, NH_2), 1630 (CONH), 1330 (S–$O_{antisym}$), 1140 (S–O_{sym}). Anal. calc for $C_{15}H_{23}N_3O_5S$: C, 50.42; H, 6.44; N, 11.76. Found: C, 50.58; H, 6.70; N, 11.50.

4.1.2.4. 2-(4-Morpholinesulfamoyl)-4,5-dimethoxy-phenylacetylhydrazide (IVd). Yield: 62%. Mp 144–145 °C (ethanol). IR ν cm^{-1}: 3230, 3390 (NH, NH_2), 1640 (CONH), 1340 (S–$O_{antisym}$), 1155 (S–O_{sym}). Anal. calc for $C_{14}H_{21}N_3O_6S$: C, 46.79; H, 5.84; N, 11.70. Found: C, 46.58; H, 5.95; N, 11.63.

4.1.2.5. 2-(1-Pyrrolidinesulfamoyl)-4,5-dimethoxy-phenylacetylhydrazide (IVe). Yield: 81%. Mp 139–141 °C (ethanol). IR ν cm^{-1}: 3185, 3380, 3395 (NH, NH_2), 1660, 1615 (CONH), 1320 (S–$O_{antisym}$), 1140 (S–O_{sym}). Anal. calc for $C_{14}H_{21}N_3O_5S$: C, 48.97; H, 6.12; N, 12.24. Found: C, 49.05; H, 6.38; N, 12.07.

4.1.3. General procedure for the preparation of the 1-[2-(substituted sulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-aryl-thiosemicarbazides (V). Equimolar quantities of hydrazide (1 mmol) and aryl isothiocyanate (1 mmol) in 3 ml of absolute ethanol were refluxed on a steam bath for 1 h. The resulting solid was filtered and recrystallized from the appropriate solvent.

The following compounds were prepared by an analogous procedure.

4.1.3.1. 1-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-phenyl-thiosemicarbazide (Va). Yield: 85%. Mp 218–220 °C (methanol–dichloromethane). IR ν cm^{-1}: 3035, 3270, 3315 (3NH), 1620 (CONH), 1525 (C=S), 1325 (S–$O_{antisym}$), 1135 (S–O_{sym}). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.67 [s, 6H, $N(CH_3)_2$], 3.82 (d, 6H, $2CH_3O$, J = 5.58 Hz), 3.90 (s, 2H, CH_2CO), 7.10 (s, 1H, C_6), 7.12–7.18 (m, 1H, ArH), 7.21(s, 1H, C_3), 7.30–7.35 (m, 2H, ArH), 7.50 (d, 2H, ArH, J = 7.68 Hz), 9.29 (b, 1H, NH), 9.75 (s, 1H, NH), 10.13 (s, 1H, NH) Anal. calc for $C_{19}H_{24}N_4O_5S_2$: C, 50.44; H, 5.30; N, 12.39. Found: C, 50.42; H, 5.15; N, 12.31.

4.1.3.2. 1-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-(*p*-chlorophenyl)-thiosemicarbazide (Vb). Yield: 82%. Mp 196–198 °C (methanol–dichloromethane). IR ν cm^{-1}: 3170, 3320 (3NH), 1680 (CONH), 1520 (C=S), 1313 (S–$O_{antisym}$), 1155 (S–O_{sym}). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.67 [s, 6H, $N(CH_3)_2$], 3.82 (d, 6H, $2CH_3O$, J = 5.13 Hz), 3.90 (s, 2H, CH_2CO), 7.09 (s, 1H, C_6), 7.21 (s, 1H, C_3) 7.39 (d, 2H, ArH, J = 8.61 Hz), 7.53 (d, 2H, ArH, J = 8.85 Hz) 9.36 (b, 1H, NH), 9.85 (br s, 1H, NH), 10.15 (s, 1H, NH) Anal. calc for $C_{19}H_{23}ClN_4O_5S_2$: C, 46.86; H, 4.72; N, 11.51. Found: C, 46.75; H, 4.80; N, 11.38.

4.1.3.3. 1-[2-(*N*-Diethylsulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-phenyl-thiosemicarbazide (Vc). Yield: 81%. Mp 202–203 °C (ethanol-dichloromethane). IR ν cm^{-1}: 3185, 3335 (3NH), 1690 (CONH), 1525 (C=S), 1325 (S–$O_{antisym}$), 1140 (S–O_{sym}). ^{1}H NMR (DMSO-d_6) δ (ppm): 0.96–1.05 (m, 6H, $2CH_2CH_3$), 3.09–3.45 (m, 4H, $2CH_2CH_3$), 3.82 (d, 6H, $2CH_3O$, J = 5.34 Hz), 3.89 (s, 2H, CH_2CO), 7.10 (s, 1H, C_6), 7.12–7.17 (m, 1H, ArH), 7.21 (s, 1H, C_3), 7.30-7.35 (m, 3H, ArH), 7.49 (d, 2H, ArH, J = 12Hz), 9.29 (b, 1H, NH), 9.75 (s, 1H, NH), 10.13 (s, 1H, NH) Anal. calc for $C_{21}H_{28}N_4O_5S_2$: C, 52.50; H, 5.83; N, 11.66. Found: C, 52.38; H, 5.75; N, 11.53.

4.1.3.4. 1-[2-(*N*-Diethylsulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-(*p*-chlorophenyl)-thiosemicarbazide (Vd). Yield: 77%. Mp 199–200 °C (methanol–dichloromethane). IR ν cm^{-1}: 3280, 3320 (3NH), 1690 (CONH), 1535 (C=S), 1335 (S–$O_{antisym}$), 1140 (S–O_{sym}). ^{1}H NMR (DMSO-d_6) δ (ppm): 0.98–1.05 (m, 6H, $2CH_2CH_3$), 3.12-3.40 (m, 4H, $2CH_2CH_3$), 3.82 (d, 6H, $2CH_3O$, J = 5.1 Hz), 3.90 (s, 2H, CH_2CO), 7.09 (s, 1H, C_6), 7.21 (s, 1H, C_3), 7.39 (d, 2H, ArH, J = 8.85 Hz), 7.53 (d, 2H, ArH, J = 8.85 Hz), 9.35 (b, 1H, NH), 9.85 (s, 1H, NH), 10.15 (s, 1H, NH). Anal. calc for $C_{21}H_{27}ClN_4O_5S_2$: C, 48.98; H, 5.24; N, 10.88. Found: C, 48.87; H, 5.30; N, 10.77.

4.1.3.5. 1-[2-(1-Piperidinesulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-phenyl-thiosemicarbazide (Ve). Yield: 66%. Mp 193–195 °C (ethanol-dichloromethane). IR ν cm^{-1}: 3180, 3335, 3400 (3NH), 1650 (CONH), 1525 (C═S), 1320 ($S–O_{antisym}$), 1135 ($S–O_{sym}$). ^1H NMR (DMSO-d_6) δ (ppm): 1.42 (br s, 2H, piperidine), 1.51 (br s, 4H, piperidine), 3.01 (br s, 4H, piperidine), 3.82 (d, 6H, $2CH_3O$, J = 5.67 Hz), 3.89 (s, 2H, CO CH_2), 7.11 (s, 1H, C_6), 7.13–7.18 (m, 1H, ArH), 7.21 (s, 1H, C_3), 7.30–7.36 (m, 2H, ArH), 7.52 (d, 2H, ArH, J = 7.8 Hz), 9.27 (br s, 1H, NH), 9.77 (s, 1H, NH), 10.16 (s, 1H, NH). Anal. calc for $C_{22}H_{28}N_4O_5S_2$: C, 53.65; H, 5.69; N, 11.38. Found: C, 53.17; H, 5.65; N, 11.43.

4.1.3.6. [2-(1-Piperidinesulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-(*p*-chlorophenyl)-thiosemicarbazide (Vf). Yield: 72%. Mp 212–213 °C (methanol–dichloromethane). IR ν cm^{-1}: 3085, 3310, (3NH), 1690 (CONH), 1515 (C═S), 1315 ($S–O_{antisym}$), 1135 ($S–O_{sym}$). ^1H NMR (DMSO-d_6) δ (ppm): 1.41–1.42 (m, 2H, piperidine), 1.51 (br s, 4H, piperidine), 2.99–3.00 (m, 4H, piperidine), 3.82 (d, 6H, $2CH_3O$, J = 4.89 Hz), 3.89 (s, 2H, $COCH_2$), 7.09 (s, 1H, C_6), 7.21 (s, 1H, C_3), 7.39 (d, 2H, ArH, J = 8.61 Hz), 7.54 (d, 2H, ArH, J = 8.61 Hz), 9.32 (br s, 1H, NH), 9.86 (s, 1H, NH), 10.17 (s, 1H, NH). Anal. calc for $C_{22}H_{27}ClN_4O_5S_2$: C, 50.14; H, 5.12; N, 10.63. Found: C, 50.20; H, 5.17; N, 10.55.

4.1.3.7. [2-(4-Morpholinesulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-phenyl-thiosemicarbazide (Vg). Yield: 92%. Mp 213–214 °C (methanol–dichloromethane). IR ν cm^{-1}: 3070, 3255, 3315 (3NH), 1680 (CONH), 1530 (C═S), 1340 ($S–O_{antisym}$), 1120 ($S–O_{sym}$). ^1H NMR (DMSO-d_6) δ (ppm): 2.87–2.99 (m, 4H, morpholine), 3.59–3.60 (m, 4H, morpholine), 3.83 (d, 6H, $2CH_3O$, J = 5.1 Hz), 3.91 (s, 2H, CH_2CO), 7.13 (s, 1H, C_6), 7.15–7.18 (m, 1H, ArH), 7.22 (s, 1H, C_3), 7.31–7.36 (m, 2H, ArH), 7.50 (d, 2H, ArH, J = 7.68 Hz), 9.29 (b, 1H, NH), 9.77 (br s, 1H, NH), 10.16 (s, 1H, NH). Anal. calc for $C_{21}H_{26}N_4O_6S_2$: C, 51.01; H, 5.26; N, 11.33. Found: C, 51.09; H, 5.20; N, 11.41.

4.1.3.8. [2-(4-Morpholinesulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-(*p*-chlorophenyl)-thiosemicarbazide (Vh). Yield: 71%. Mp 230–231 °C (methanol–dichloromethane). IR ν cm^{-1}: 3095, 3280, 3315 (3NH), 1680 (CONH), 1530 (C═S), 1335 ($S–O_{antisym}$), 1155 ($S–O_{sym}$). ^1H NMR (DMSO-d_6) δ (ppm): 2.99 (m, 4H, morpholine), 3.59–3.60 (m, 4H, morpholine), 3.83 (d, 6H, $2CH_3O$, J = 5.1 Hz), 3.92 (s, 2H, CH_2CO), 7.12 (s, 1H, C_6), 7.23 (s, 1H, C_3), 7.40 (d, 2H, ArH, J = 8.61 Hz), 7.53 (d, 2H, ArH, J = 8.85 Hz), 9.35 (b, 1H, NH), 9.88 (br s, 1H, NH), 10.18 (s, 1H, NH). Anal. calc for $C_{21}H_{25}ClN_4O_6S_2$: C, 47.68; H, 4.73; N, 10.59. Found: C, 47.75; H, 4.80; N, 10.51.

4.1.3.9. [2-(1-Pyrrolidinesulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-phenyl-thiosemicarbazide (Vi). Yield: 80%. Mp 189–191 °C (ethanol-dichloromethane). IR ν cm^{-1}: 3200, 3335 3435 (3NH), 1675 (CONH), 1530 (C═S), 1320 ($S–O_{antisym}$), 1145 ($S–O_{sym}$). ^1H NMR (DMSO-d_6) δ (ppm): 1.74–1.78 (m, 4H, pyrrolidine), 3.13–3.18 (m, 4H, pyrrolidine), 3.82 (d, 6H, $2CH_3O$, J = 4.47 Hz), 3.92 (s, 2H, CH_2CO), 7.10 (s, 1H, C_6), 7.12–7.17 (m, 1H, ArH), 7.25 (s, 1H, C_3), 7.30–7.35 (m, 2H, ArH), 7.51 (d, 2H, ArH, J = 7.92 Hz), 9.29 (b, 1H, NH), 9.74 (s, 1H, ArH), 10.15 (s, 1H, NH). Anal. calc for $C_{21}H_{26}N_4O_5S_2$: C, 52.72; H, 5.44; N, 11.71. Found: C, 52.65; H, 5.54; N, 11.80.

4.1.3.10. 1-[2-(1-Pyrrolidinesulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-(*p*-chlorophenyl)-thiosemicarbazide (Vj). Yield: 76%. Mp 212–214 °C (ethanol). IR ν cm^{-1}: 3070, 3275, 3315 (3NH), 1685 (CONH), 1520 (C═S), 1335 ($S–O_{antisym}$), 1155 ($S–O_{sym}$). ^1H NMR (DMSO-d_6) δ (ppm): 1.74–1.78 (m, 4H, pyrrolidine), 3.14–3.18 (m, 4H, pyrrolidine), 3.82 (d, 6H, $2CH_3O$, J = 4.47 Hz), 3.92 (s, 2H, CH_2CO), 7.09 (s, 1H, C_6), 7.25 (s, 1H, C_3), 7.39 (d, 2H, ArH, J = 8.73 Hz), 7.54 (d, 2H, ArH, J = 8.97 Hz), 9.34 (b, 1H, NH), 9.85 (s, 1H, ArH), 10.17 (s, 1H, NH). Anal. calc for $C_{21}H_{25}ClN_4O_5S_2$: C, 49.17; H, 4.87; N, 10.92. Found: C, 49.22; H, 5.05; N, 10.85.

4.1.4. General procedure for the preparation of the 5-[2-(substituted sulfamoyl)-4,5-dimethoxy-benzyl]-4-aryl-*s*-triazole-3-thiones (VI). A suspension of thiosemicarbazide (1 mmol) in sodium hydroxide solution (5%, 5 ml) was heated under reflux for 1 h. The reaction mixture was allowed to cool and then adjusted to pH 6 with 10% hydrochloric acid. The precipitate formed was filtered, washed with water, dried, and recrystallized from the appropriate solvent.

The following compounds were prepared by an analogous procedure.

4.1.4.1. 5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-4-phenyl-*s*-triazole-3-thione (VIa). Yield: 70%. Mp 208–209 °C (methanol). IR ν cm^{-1}: 3085, 3050 (NH), 1590, 1570, 1515 (C═N), 1335 ($S–O_{antisym}$), 1135 ($S–O_{sym}$). ^1H NMR (DMSO-d_6) δ (ppm): 2.49 [s, 6H, $N(CH_3)_2$], 3.82 (s, 6H, $2CH_3O$), 4.03 (s, 2H, CH_2CO), 7.10 (s, 1H, C_6), 7.20 (s, 1H, C_3), 7.43 (d, 2H, ArH, J = 7.32 Hz), 7.55–7.64 (m, 3H, ArH), 13.67 (s, 1H, NH). Anal. calc for $C_{19}H_{22}N_4O_4S_2$: C, 52.53; H, 5.07; N, 12.90. Found: C, 52.41; H, 5.15; N, 12.82.

4.1.4.2. 5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-4-(*p*-chlorophenyl)-*s*-triazole-3-thione (VIb). Yield: 58%. Mp 246–247 °C (methanol). IR ν cm^{-1}: 3075, 3045 (NH), 1585, 1560, 1515 (C═N), 1325 ($S–O_{antisym}$), 1150 ($S–O_{sym}$). ^1H NMR (DMSO-d_6) δ (ppm): 2.49 [s, 6H, $N(CH_3)_2$], 3.80 (s, 6H, $2CH_3O$), 4.03 (s, 2H, CH_2CO), 7.04 (s, 1H, C_6), 7.17 (s, 1H, C_3), 7.43 (d, 2H, ArH, J = 8.55 Hz), 7.67 (d, 2H, ArH, J = 8.55 Hz), 13.68 (b, 1H, NH). Anal. calc for $C_{19}H_{21}ClN_4O_4S_2$: C, 48.66; H, 4.48; N, 11.95. Found: C, 48.72; H, 4.54; N, 11.88.

4.1.4.3. 5-[2-(*N*-Diethylsulfamoyl)-4,5-dimethoxy-benzyl]-4-phenyl-*s*-triazole-3-thione (VIc). Yield: 75%. Mp 205–206 °C (methanol–dichloromethane). IR ν cm^{-1}: 3105, 3050 (NH), 1595, 1570, 1515 (C═N), 1330 (S–

$O_{antisym}$), 1140 (S–O_{sym}). ^{1}H NMR (DMSO-d_6) δ (ppm): 0.98–1.10 (m, 6H, $2CH_2CH_3$), 3.11–3.43 [m, 4H, $2CH_2CH_3$], 2.95 (s, 6H, $2CH_3O$), 3.16 (s, 2H, CH_2CO), 6.23 (s, 1H, C_6), 6.33 (s, 1H, C_3), 6.56 (d, 2H, ArH, J = 6.69 Hz), 6.68–6.77 (m, 3H, ArH), 12.79 (b, 1H, NH). Anal. calc for $C_{21}H_{26}N_4O_4S_2$: C, 54.54; H, 5.62; N, 12.12. Found: C, 54.47; H, 5.56; N, 12.18.

4.1.4.4. 5-[2-(*N*-Diethylsulfamoyl)-4,5-dimethoxy-benzyl]-4-(*p*-chlorophenyl)-*s*-triazole-3-thione (VId). Yield: 69%. Mp 247–249 °C (methanol–dichloromethane). IR ν cm^{-1}: 3080, 3015 (NH), 1590, 1515 (C=N), 1330 (S–$O_{antisym}$), 1145 (S–O_{sym}). ^{1}H NMR (DMSO-d_6) δ (ppm): 0.98–1.10 (m, 6H, $2CH_2CH_3$), 3.11–3.43 [m, 4H, $2CH_2CH_3$], 3.80 (s, 6H, $2CH_3O$), 4.03 (s, 2H, CH_2CO), 7.05 (s, 1H, C_6), 7.17 (s, 1H, C_3), 7.44 (d, 2H, ArH, J = 8.55 Hz), 7.67 (d, 2H, ArH, J = 8.55 Hz), 13.70 (b, 1H, NH). Anal. calc for $C_{21}H_{25}ClN_4O_4S_2$: C, 50.75; H, 5.03; N, 11.27. Found: C, 50.68; H, 5.10; N, 11.21.

4.1.4.5. 5-[2-(1-Piperidinesulfamoyl)-4,5-dimethoxy-benzyl]-4-phenyl-*s*-triazole-3-thione (VIe). Yield: 44%. Mp 225–226 °C (methanol–dichloromethane). IR ν cm^{-1}: 3090, 3025 (NH), 1590, 1560, 1520 (C=N), 1330 (S–$O_{antisym}$), 1140 (S–O_{sym}). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.32 (s, 6H, piperidine), 2.76 (s, 4H, piperidine), 3.80 (d, 6H, $2CH_3O$, J = 1.83 Hz), 4.01 (s, 2H, CH_2CO), 7.07 (s, 1H, C_6), 7.21 (s, 1H, C_3), 7.40 (d, 2H, ArH, J = 7.32 Hz), 7.53–7.59 (m, 3H, ArH), 13.70 (b, 1H, NH). Anal. calc for $C_{22}H_{26}N_4O_4S_2$: C, 55.69; H, 5.48; N, 11.81. Found: C, 55.62; H, 5.52; N, 11.87.

4.1.4.6. 5-[2-(1-Piperidinesulfamoyl)-4,5-dimethoxy-benzyl]-4-(*p*-chlorophenyl)-*s*-triazole-3-thione (VIf). Yield: 48%. Mp 248–249 °C (methanol–dichloromethane). IR ν cm^{-1}: 3085, 3040 (NH), 1595, 1570, 1525 (C=N), 1330 (S–$O_{antisym}$), 1140 (S–O_{sym}). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.33 (s, 6H, piperidine), 2.76 (s, 4H, piperidine), 3.80 (d, 6H, $2CH_3O$, J = 1.23 Hz), 4.03 (s, 2H, CH_2CO), 7.03 (s, 1H, C_6), 7.20 (s, 1H, C_3), 7.43 (d, 2H, ArH, J = 9.15 Hz), 7.67 (d, 2H, ArH, J = 8.52 Hz), 13.69 (b, 1H, NH). Anal. calc for $C_{22}H_{25}ClN_4O_4S_2$: C, 51.91; H, 4.91; N, 11.01. Found: C, 51.85; H, 4.98; N, 10.97.

4.1.4.7. 5-[2-(4-Morpholinesulfamoyl)-4,5-dimethoxy-benzyl]-4-phenyl-*s*-triazole-3-thione (VIg). Yield: 60%. Mp 241–242 °C (methanol–dichloromethane). IR ν cm^{-1}: 3105, 3070 (NH), 1590, 1575, 1515 (C=N), 1320 (S–$O_{antisym}$), 1145 (S–O_{sym}). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.76 (s, 4H, Morpholine), 3.43 (br s, 4H, Morpholine), 3.81 (s, 6H, $2CH_3O$, J = 1.23 Hz), 4.04 (s, 2H, CH_2CO), 7.11 (s, 1H, C_6), 7.22 (s, 1H, C_3), 7.41 (d, 2H, ArH, J = 10.32 Hz), 7.54–7.62 (m, 3H, ArH), 13.66 (b, 1H, NH). Anal. calc for $C_{21}H_{24}N_4O_5S_2$: C, 52.94; H, 5.04; N, 11.76. Found: C, 53.00; H, 5.09; N, 11.71.

4.1.4.8. 5-[2-(4-Morpholinesulfamoyl)-4,5-dimethoxy-benzyl]-4-(*p*-chlorophenyl)-*s*-triazole-3-thione (VIh). Yield: 55%. Mp 246–247 °C (methanol–dichloromethane). IR ν cm^{-1}: 3080, 3045 (NH), 1605, 1565, 1515 (C=N), 1350 (S–$O_{antisym}$), 1140 (S–O_{sym}). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.75 (s, 4H, Morpholine), 3.45 (br s, 4H, Morpholine), 3.84 (s, 6H, $2CH_3O$), 4.07 (s, 2H, CH_2CO), 7.08 (s, 1H, C_6), 7.22 (s, 1H, C_3), 7.43 (d, 2H, ArH, J = 7.2 Hz), 7.67 (d, 2H, ArH, J = 6.3 Hz). Anal. calc for $C_{21}H_{23}ClN_4O_5S_2$: C, 49.36; H, 4.50; N, 10.97. Found: C, 49.41; H, 4.47; N, 10.92.

4.1.4.9. 5-[2-(1-Pyrrolidinesulfamoyl)-4,5-dimethoxy-benzyl]-4-phenyl-*s*-triazole-3-thione (VIi). Yield: 70%. Mp 241–242 °C (methanol–dichloromethane). IR ν cm^{-1}: 3085, 3020 (NH), 1510, 1500 (C=N), 1315 (S–$O_{antisym}$), 1145 (S–O_{sym}). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.65 (m, 4H, Pyrrolidine), 2.92 (t, 4H, Pyrrolidine), 3.80 (s, 6H, $2CH_3O$), 4.04 (s, 2H, CH_2CO), 7.07 (s, 1H, C_6), 7.24 (s, 1H, C_3), 7.41 (d, 2H, ArH, J = 7.95 Hz), 7.57 (m, 3H, ArH), 13.64 (b, 1H, NH). Anal. calc for $C_{21}H_{24}N_4O_4S_2$: C, 54.78; H, 5.21; N, 12.17. Found: C, 54.82; H, 5.16; N, 12.21.

4.1.4.10. 5-[2-(1-Pyrrolidinesulfamoyl)-4,5-dimethoxy-benzyl]-4-(*p*-chlorophenyl)-*s*-triazole-3-thione (VIj). Yield: 56%. Mp 232–233 °C (methanol–dichloromethane). IR ν cm^{-1}: 3085, 3050 (NH), 1585, 1570, 1515 (C=N), 1340 (S–$O_{antisym}$), 1140 (S–O_{sym}). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.66 (m, 4H, Pyrrolidine), 2.93 (t, 4H, Pyrrolidine), 3.80 (s, 6H, $2CH_3O$), 4.06 (s, 2H, CH_2CO), 7.03 (s, 1H, C_6), 7.23 (s, 1H, C_3), 7.44 (d, 2H, ArH, J = 8.55 Hz), 7.67 (d, 2H, ArH, J = 8.55 Hz), 13.69 (b, 1H, NH). Anal. calc for $C_{21}H_{23}ClN_4O_4S_2$: C, 50.96; H, 4.65; N, 11.32. Found: C, 51.02; H, 4.59; N, 11.35.

4.2. Pharmacology

4.2.1. Test for antifungal activity

4.2.1.1. Microdilution test. In order to investigate the antifungal activity of compounds we used the following fungi: *Aspergillus niger* (ATCC 6275), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus versicolor* (ATCC 11730), *A. flavus* (ATCC 9643), *P. funiculosum* (ATCC 36839), and *T. viride* (IAM 5061).

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 (MA) and the cultures were stored at +4 °C and subcultured once a month.[36]

In order to investigate the antifungal activity of compounds the modified microdilution technique was used.[37,38] 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 MA to verify the absence of contamination and to check the validity of the inoculum.

Minimum inhibitory concentration (MIC) determination was performed by a serial dilution technique using 96-well microtiter plates. Compounds investigated were dissolved in DMSO and in concentrations of 50.0–150.0 μg/ml added in broth malt medium with fungal inoculum. The microplates were incubated for 72 h at 28 °C. The lowest concentration without visible growth (under the binocular microscope) was defined as MIC. The minimum 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 for 72 h at 28 °C. The lowest concentration with no visible growth was defined as the MFC, indicating = 99.5% killing of the original inoculum. The commercial fungicide bifonazole (imidazole-type fungicide) was used as a positive control (100.0–250.0 μg/ml).

4.2.2. Tests for antibacterial activity. The following bacteria were used: *E. coli* (ATCC 35210), *Bacillus subtilis* (ATCC 10907), *Micrococcus flavus* (ATCC 10240), *Staphylococcus epidermidis* (ATCC 12228), *S. typhimurium* (ATCC 13311), *Salmonella enteritidis* (ATCC 13076), and *E. cloacce* (human isolate).

The bacterial suspension was adjusted with sterile saline to a concentration of 1.0×10^5 CFU ml^{-1}. The inocula were daily prepared 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.

4.2.2.1. Microdilution test. The minimum inhibitory and bactericidal concentrations (MICs and MBCs) were determined using 96-well microtiter plates. The bacterial suspension was adjusted with sterile saline to a concentration of 1.0×10^5 CFU ml^{-1}. Compounds to be investigated were dissolved in broth LB medium (100 μl) with bacterial inoculum (1.0×10^4 CFU per well) to achieve the wanted concentrations (50.0–150.0 μg ml^{-1}). The microplates were incubated for 24 h at 28 °C. The lowest concentrations without visible growth (under the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The minimum bactericidal concentrations (MBCs) were determined by serial subcultivation of 2 μl into microtiter 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 540 nm by Microplate manager 4.0 (Bio-Rad Laboratories) and compared with a blank and the positive control. Commercial antibiotics streptomycin (50.0–200.0 μg/ml) and chloramphenicol (150–250 μg/ml) were used as a positive control and the solvent (DMSO) was used as a negative control. Two replicates were done for each compound and experiment was repeated two times.

4.3. Molecular modeling

Computer calculations were performed using Quanta software of Molecular Simulations on a Silicon Graphics O^2. Molecular Mechanics calculations were carried out using the CHARMm force field. Synthetic compounds were first minimized with Steepest Descents, Conjugate Gradients, and Newton–Raphson algorithms using an energy tolerance of 0.01 kcal/mol^{-1} A^{-1}, to reach a local minimum. The dielectric constant (e) was set to 45 during minimization simulating the amphiphilic environment. To generate random conformers, a systematic search procedure (Random Sampling) was applied. This method randomly changes all defined torsion angles within a predefined angular window. The range of the torsion angle varies during the search procedure and generates new conformations. CHARMm energy minimization is performed for each randomly altered conformation. In order to explore the preferred torsion angles that correspond to the lowest energy conformers and energy barriers of the molecules under study, stochastic search procedures (systematic grid scan) were used. This method initiates a grid scan search that generates conformations by varying specified torsion angles over a grid of equally spaced values. Intervals of 5° were applied for single bond rotation and 10° of two bond rotation. During these searches, the predetermined torsion angle remained constant while minimization using 1000 steps of conjugate gradient algorithm was applied to 'relax' the whole molecule.

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