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Synthesis of novel sulfonamide-1,2,4-triazoles, 1,3,4-thiadiazoles and 1,3,4-oxadiazoles, as potential antibacterial and antifungal agents. Biological evaluation and conformational analysis studies

P. Zoumpoulakis [a,*], Ch. Camoutsis [b], G. Pairas [b], M. Soković [c], J. Glamočlija [c], C. Potamitis [a], A. Pitsas [b]

[a] Laboratory of Molecular Analysis, Institute of Organic and Pharmaceutical Chemistry, National Hellenic Research Foundation, 48 Vas. Constantinou Ave., 11635 Athens, Greece
[b] Laboratory of Pharmaceutical Chemistry, Department of Pharmacy, University of Patras, 26500 Patras, Greece
[c] Department of Plant Physiology, Mycological Laboratory, Institute of Biological Research, University of Belgrade, Bulevar Despota Stefana 142, 11000 Belgrade, Serbia

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ABSTRACT

The significant antifungal activity of a series of sulfonamide-1,2,4-triazole and 1,3,4-thiazole derivatives against a series of micromycetes, compared to the commercial fungicide bifonazole has been reported. These compounds have also shown a comparable bactericidal effect to that of streptomycin and better activity than chloramphenicol against various bacteria.

In view of the potential biological activity of members of the 1,2,4-triazole, 1,3,4-thiadiazole and 1,3,4-oxadiazole ring systems and in continuation of our search for bioactive molecules, we designed the synthesis of a series of novel sulfonamide-1,2,4-triazoles, -1,3,4-thiadiazoles and -1,3,4-oxadiazoles emphasizing, in particular, on the strategy of combining two chemically different but pharmacologically compatible molecules (the sulfomamide nucleus and the five member) heterocycles in one frame. Synthesized compounds were tested in vitro for antibacterial and antifungal activity and some analogues exhibited very promising results especially as antifungal agents.

In order to explain structure–activity relationships, conformational analysis was performed for active and less active analogues using NMR spectroscopy and molecular modeling techniques. Furthermore, molecular properties which can be further used as descriptors for SAR studies, were predicted for the synthesized analogues. In general, antifungal activity seems to depend more on the triazol-3-thione moiety rather than the different length of the alkyl chain substitutions.

1. Introduction

Bacterial infection such as food poisoning, rheumatic, salmonellosis and diarrhea are caused by multidrug-resistant Gram-positive and Gram-negative pathogens. Principal players among these problematic organisms are isolates of methicillin-resistant *Staphylococcus aureus*, *Staphylococcus pyogenes*, *Salmonella typhimurium* and *Escherichia coli*.[1]

In contrast to the large number of antibacterial antibiotics, there are very few antifungal agents that can be used for life-threatening fungal infections. These drugs are amphotericin B,[2] 5-fluorocytosine, azoles (such as fluconazole and itraconazole[2]) and echinocandins (such as caspofungin and micafungin).[3] Because of their high therapeutic index, azoles are first-line drugs for the treatment of invasive fungal infections. Unfortunately, the broad use of azoles has led to development of severe resistance,[4,5] which significantly reduced the efficacy of them. This situation has led to an ongoing search for new azoles. Several novel triazole antifungal agents such as voriconazole,[6] posaconazole,[7] ravuconazole[8] and albaconazole,[9] are marketed or are at the late stages of clinical trials.

In general, azoles have been the leading agents used to treat fungal infections of plants, animals and humans. These drugs act by competitive inhibition of the lanosterol 14a-demethylase (CYP51),[10] which is the key enzyme in sterol biosynthesis of fungi. Selective inhibition of CYP51 would cause depletion of ergosterol and accumulation of lanosterol and other 14-methyl sterols resulting in the growth inhibition of fungal cells.[11] Being an essential enzyme for the fungal life cycle, CYP51 is the target for azole antifungals. Several in silico docking studies have been performed to explore the mechanism of action of azole antifungals.[12–18] These studies have been performed on homologous 3D models of CYP51 based on the crystal structures of known prokaryotic P450. The majority of these studies indicate that apart from the coordination of the heme iron from the azole ring nitrogen atom, crucial interactions for azole binding into the putative active site of CYP51 are hydrophobic interactions including p–p stacking.[13,18]

* Corresponding author. Tel.: +30 210 7273869; fax: +30 210 7273872.
E-mail address: pzoump@eie.gr (P. Zoumpoulakis).

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Triazole antifungals which act predominantly by inhibition of lanosterol to ergosterol through the cytochrome P450, are compounds containing 1,2,4-triazole and 1,2,4-triazolone ring.[19] Moreover, our group has previously reported the significant antifungal activity of a series of sulfonamide-1,2,4-triazole and 1,3,4-thiazole derivatives against a series of micromycetes, compared to the commercial fungicide bifonazole. These compounds have exerted similar bactericidal effect to that of streptomycin and higher activity than chloramphenicol against various bacteria.[20]

Prompted by these observations and in continuation of search for bioactive molecules, we designed and synthesized a series of novel sulfonamide-1,2,4-triazoles, sulfonamide-1,3,4-thiadiazoles and sulfonamide-1,3,4-oxadiazoles. On our design we emphasized on the strategy of combining two chemically different but pharmacologically compatible molecules, the sulfomamide nucleus and the five member heterocycles in one frame.[20–28] Furthermore, the designed molecules contain different aliphatic side chains (methyl, ethyl, propyl, isopropyl, butyl and *tert*-butyl), aiming to increase the hydrophobic interactions and to determine the optimum chain length for increased activity.

2. Materials and methods

2.1. Synthesis

Melting points were taken in glass capillary tubes on a Haake Bucher apparatus. IR spectra were recorded on a FT-IR Jasco spectrophotometer in solid phase KBr. All proton NMR spectra were recorded on a Varian 300 MHz spectrometer using deuterated dimethylsulfoxide (DMSO-d_6) and are reported in δ units (ppm) relative to tetramethylsilane (TMS). Thin layer chromatography (TLC) 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 analysis (C, H, N) of synthesized compounds are within the range of experimental error (±0.4% of the calculated values). Accurate mass spectrometric data were obtained using LTQ Orbitrap Velos™ spectrometer (Thermo Scientific). Ionization was performed with heated electrospray source operated in the positive mode (HESI+). Full scan mass spectral data were acquired from *m/z* 100 to 1000 at a resolving power of 60,000. The molecular formulas of the compounds were confirmed by analysis with a mass spectrometer that provided accurate mass data. In all cases, the protonated molecules ($[M+H]^+$) and sodiated adducts ($[M+Na]^+$) were detected with mass error less than 1.5 ppm.

2.1.1. General procedure for the preparation of the 1-[2-(*N*-dimethylsulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-alkyl-thiosemicarbazide/semicarbazide (2)

Equimolar quantities of hydrazide (1 mmol) and alkyl isothiocyanate (1 mmol) in 5 ml of absolute ethanol were refluxed on a steam bath for 1 h. The formed precipitate was filtered and recrystallized from the appropriate solvent. The following compounds were prepared by an analogous procedure.

2.1.1.1. 1-[2-(*N*-Dimethylsulfamoyl)4,5-dimethoxy-phenylacetyl]-4-methyl-thiosemicarbazide (2_a). Yield: 95%. Mp 210–211 °C (ethanol). IR cm^{-1}: 3300 (NH), 1700 (CONH), 1560, 1520 (C═S), 1325 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^{1}H NMR (DMSO-d_6) δ(ppm): 2.66 (s, 6H, N(CH_3)$_2$), 2.87 (d, J = 4.35 Hz, 3H, CH_3), 3.81 (s, 3H, CH_3O), 3.83 (s, 3H, CH_3O), 3.85 (s, 2H, CH_2), 7.03 (s, 1H, ArH), 7.21 (s, 1H, ArH), 7.63 (m, 1H, NH), 9.32 (s, 1H, NH), 9.86 (s, 1H, NH).

Anal. Calcd for $C_{14}H_{22}N_4O_5S_2$: C, 43.07; H, 5.64; N, 14,35. Found: C, 43.11; H, 5.67; N, 14.31.

2.1.1.2. 1-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-ethyl-thiosemicarbazide (2_b). Yield: 90%. Mp 191–193 °C (ethanol). IR cm^{-1}: 3305, 3195 (NH), 1700 (CONH), 1550, 1520 (C═S), 1325 (S-$O_{antisym}$), 1150 (S-O_{sym}).

^{1}H NMR (DMSO-d_6) δ(ppm): 1.06 (t, J = 7.19 Hz, 3H, CH_3), 2.66 (s, 6H, N(CH_3)$_2$), 3.44 (pentaplet, J = 6.88 Hz, 2H, CH_2), 3.81 (s, 3H, CH_3O), 3.83 (s, 3H, CH_3O), 3.83 (s, 2H, CH_2), 7.05 (s, 1H, ArH), 7.20 (s, 1H, ArH), 7.65 (m, 1H, NH), 9.27 (s, 1H, NH), 9.86 (s, 1H, NH).

Anal. Calcd for $C_{15}H_{24}N_4O_5S_2$: C, 44.51; H, 5.60; N, 13.06. Found: C, 44.55; H, 5.62; N, 13.10.

2.1.1.3. 1-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-propyl-thiosemicarbazide (2_c). Yield: 87%. Mp 235–236 °C (ethanol). IR cm^{-1}: 3325, 3180 (NH), 1720 (CONH), 1605 (C═N), 1555, 1520 (C═S), 1325 (S-$O_{antisym}$), 1145 (S-O_{sym}).

^{1}H NMR (DMSO-d_6) δ(ppm): 0.80 (t, J = 7.42 Hz, 3H, CH_3), 1.49 (hexaplet, J = 7.07 Hz, 2H, CH_2), 2.64 (s, 6H, N(CH_3)$_2$), 3.34 (m, 2H, CH_2), 3.79 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 3.81 (m, 2H, CH_2), 7.04 (s, 1H, ArH), 7.18 (s, 1H, ArH), 7.65 (s, 1H, NH), 9.27 (s, 1H, NH), 9.87 (s, 1H, NH).

Anal. Calcd for $C_{16}H_{26}N_4O_5S_2$: C, 45.93; H, 6.22; N, 13.39. Found: C, 45.90; H, 6.27; N, 13.36.

2.1.1.4. 1-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-isopropyl- thiosemicarbazide (2_d). Yield: 74%. Mp 214–216 °C (ethanol). IR cm^{-1}: 3335, 3255 (NH), 1690 (C0NH), 1550, 1510 (C═S), 1330 (S-O $_{antisym}$), 1140(S-O_{sym}).

^{1}H NMR (DMSO-d_6) δ(ppm): 1.07 (d, J = 6.59 Hz, 6H, 2CH_3), 2.66 (s, 6H, N(CH_3)$_2$), 3.77 (s, 2H, CH_2), 3.80 (s, 3H, CH_3O), 3.82 (s, 3H, CH_3O), 4.37 (m, 1H, CH), 7.08 (s, 1H, ArH), 7.16 (s, 1H, ArH), 7.27 (m, 1H, NH), 9.21 (s, 1H, NH), 9.85 (s, 1H, NH).

Anal. Calcd for $C_{16}H_{26}N_4O_5S_2$: C, 45.93; H, 6.22; N, 13.39. Found: C, 45.97; H, 6.18; N, 13.42.

2.1.1.5. 1-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-butyl-thiosemicarbazide (2_e). Yield: 89%. Mp 210–211 °C (ethanol). IR cm^{-1}: 3335, 3255 (NH), 1690 (C0NH), 1550, 1510 (C═S), 1330 (S-$O_{antisym}$), 1140(S-O_{sym}).

^{1}H NMR (DMSO-d_6) δ(ppm): 0.84 (t, J = 6.78 Hz, 3H, CH_3), 1.22 (hexaplet, J = 7.22 Hz, 2H, CH_2), 1.45 (pentaplet, J = 6.51 Hz, 2H, CH_2), 2.64 (s, 6H, N(CH_3)$_2$), 3.37 (m, 2H, CH_2), 3.81 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 3.81 (m, 2H, CH_2), 7.04 (s, 1H, ArH), 7.18 (s, 1H, ArH), 7.60 (s, 1H, NH), 9.25 (s, 1H, NH), 9.86 (s, 1H, NH).

Anal. Calcd for $C_{17}H_{28}N_4O_5S_2$: C, 47.22; H, 6.48; N, 12.96. Found: C, 47.26; H, 6.44; N, 13.00.

2.1.1.6. 1-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-*tert*-butyl-thiosemicarbazide (2_f). Yield: 74%. Mp 187–188 °C (ethanol). IR cm^{-1}: 3355, 3315, 3240 (NH), 1670 (C0NH), 1550, 1520 (C═S), 1335 (S-$O_{antisym}$), 1150 (S-O_{sym}).

^{1}H NMR (DMSO-d_6) δ(ppm): 1.42 (s, 9H, 3CH_3), 2.65 (s, 6H, N(CH_3)$_2$), 3.75 (s, 2H, CH_2), 3.80 (s, 3H, CH_3O), 3.82 (s, 3H, CH_3O), 6.89 (s, 1H, NH), 7.08 (s, 1H, ArH), 7.15 (s, 1H, ArH), 9.04 (s, 1H, NH), 9.83 (s, 1H, NH).

Anal. Calcd for $C_{17}H_{28}N_4O_5S_2$: C, 47.22; H, 6.48; N, 12.96. Found: C, 47.27; H, 6.52; N, 12.89.

2.1.1.7. 1-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-ethyl-semicarbazide (2_g). Yield: 74%. Mp 133–135 °C (ethanol). IR cm^{-1}: 3350, 3270 (NH), 1685 (C0NH), 1665 (NHCO NH), 1325 (S-$O_{antisym}$), 1145 (S-O_{sym}).

^{1}H NMR (DMSO-d_6) δ(ppm): 0.97 (t, J = 7.20 Hz, 3H, CH_3), 2.64 (s, 6H, N(CH_3)$_2$), 3.00 (m, 2H, CH_2), 3.79 (s, 3H, CH_3O), 3.80 (s, 2H, CH_2), 3.82 (s, 3H, CH_3O), 6.22 (m, 1H, NH), 7.08 (s, 1H, ArH), 7.19 (s, 1H, ArH), 7.74 (s, 1H, NH), 9.59 (s, 1H, NH).

Anal. Calcd for $C_{15}H_{24}N_4O_6S$: C, 46.39; H, 6.18; N, 14.43. Found: C, 46.45; H, 6.23; N, 14.38.

2.1.1.8. 1-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-propyl-semicarbazide (2_h). Yield: 70%. Mp 124–126 °C (ethanol). IR cm^{-1}: 3350 (NH), 1685 (C0NH), 1660 (NHCONH), 1330 (S-$O_{antisym}$), 1145(S-O_{sym}).

^1H NMR (DMSO-d_6) δ(ppm): 0.80 (t, J = 7.30 Hz, 3H, CH_3), 1.60 (hexaplet, J = 6.90 Hz, 2H, CH_2), 2.62 (s, 6H, $N(CH_3)_2$), 2.94 (t, J = 6.40 Hz, 2H, CH_2), 3.79 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 3.80 (s, 2H, CH_2), 6.23 (m, 1H, NH), 7.08 (s, 1H, ArH), 7.21 (s, 1H, ArH), 7.72 (s, 1H, NH), 9.59 (s, 1H, NH).

Anal. Calcd for $C_{16}H_{26}N_4O_6S$: C, 47.76; H, 6.46; N, 13.93. Found: C, 47.81; H, 6.41; N, 13.88.

2.1.1.9. 1-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-isopropyl-semicarbazide (2_i). Yield: 76%. Mp 178–179 °C (ethanol). IR cm^{-1}: 3405, 3370, 3215, 3090 (NH), 1695 (CONH), 1670 (NHCONH), 1330 (S-$O_{antisym}$), 1140(S-O_{sym}).

^1H NMR (DMSO-d_6) δ(ppm): 1.00 (d, J = 6.50 Hz, 6H, $2CH_3$), 2.64 (s, 6H, $N(CH_3)_2$), 3.68 (m, 1H, CH), 3.77 (s, 2H, CH_2), 3.79 (s, 3H, CH_3O), 3.82 (s, 3H, CH_3O), 6.01 (m,1H, NH), 7.09 (s, 1H, ArH), 7.18 (s, 1H, ArH), 7.65 (s, 1H, NH), 9.59 (s, 1H, NH).

Anal. Calcd for $C_{16}H_{26}N_4O_6S$: C, 47.76; H, 6.46; N, 13.93. Found: C, 47.71; H, 6.49; N, 13.97.

2.1.1.10. 1-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-butyl-semicarbazide (2_j). Yield: 77%. Mp 128–130 °C (ethanol). IR cm^{-1}: 3355 (NH), 1685 (C0NH), 1660 (NHCONH), 1330 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^1H NMR (DMSO-d_6) δ(ppm): 0.83 (t, J = 7.0 Hz, 3H, CH_3), 1.22 (m, 2H, CH_2), 1.31 (m, 2H, CH_2), 2.64 (s, 6H, $N(CH_3)_2$), 2.95 (m, 2H, CH_2), 3.71 (s, 2H, CH_2), 3.79 (s, 3H, CH_3O), 3.80 (s, 3H, CH_3O), 6.22 (m, 1H, NH), 7.08 (s, 1H, ArH), 7.20 (s, 1H, ArH), 7.72 (s, 1H, NH), 9.59 (s, 1H, NH).

Anal. Calcd for $C_{17}H_{28}N_4O_6S$: C, 49.03; H, 6.73; N, 13.46. Found: C, 49.07; H, 6.69; N, 13.51.

2.1.1.11.1-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-*tert*-butyl-semicarbazide (2_k). Yield: 81%. Mp 125–127 °C (ethanol). IR cm^{-1}: 3530, 3350 (NH), 1685 (C0NH), 1665 (NHCONH), 1325 (S-$O_{antisym}$), 1145 (S-O_{sym}).

^1H NMR (DMSO-d_6) δ(ppm): 1.21 (s, 9H, $3CH_3$), 2.63 (s, 6H, $N(CH_3)_2$), 3.76 (s, 2H, CH_2), 3.81 (s, 3H, CH_3O), 3.83 (s, 3H, CH_3O), 5.90 (s, 1H, NH), 7.13 (s, 1H, ArH), 7.19 (s, 1H, ArH), 7.50 (s, 1H, NH), 9.58 (s, 1H, NH).

Anal. Calcd for $C_{17}H_{28}N_4O_6S$: C, 49.03; H, 6.73; N, 13.46. Found: C, 49.01; H, 6.76; N, 13.50.

2.1.2. General procedure for the preparation of the 5-[2-(*N*-dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-4-alkyl-s-triazole-3-thiones/3-ones (3)

A suspension of thiosemicarbazide/semicarbazide (1 mmol) in sodium hydroxide solution (5%, 5 ml) was refluxed for 1 h. The reaction mixture was allowed to cool and then adjusted to pH 6 with 10% hydrochloric acid. The formed precipitate was filtered, washed with water, dried and recrystallized from the appropriate solvent.

The following compounds were prepared by an analogous procedure.

2.1.2.1. 5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-4-methyl-s-triazole-3-thione (3_a). Yield: 76%. Mp 194–196 °C (methanol). IR cm^{-1}: 3100, 3050 (NH), 1600 (C=N), 1575, 1520 (C=S), 1330 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^1H NMR (DMSO-d_6) δ(ppm): 2.59 (s, 6H, $N(CH_3)_2$), 3.41 (s, 3H, NCH_3), 3.77 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 4.28 (s, 2H, CH_2), 7.02 (s, 1H, ArH), 7.22 (s, 1H, ArH), 13.44 (s, 1H, NH).

Anal. Calcd for $C_{14}H_{20}N_4O_4S_2$: C, 45.16; H, 5.37; N, 15.05. Found: C, 45.21; H, 5.41; N, 15.00.

2.1.2.2. 5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-4-ethyl-s-triazole-3-thione (3_b). Yield: 86%. Mp 193–194 °C (methanol). IR cm^{-1}: 3100, 3050 (NH), 1600 (C=N), 1570, 1510 (C=S), 1330 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^1H NMR (DMSO-d_6) δ(ppm): 1.18 (t, J = 7.12 Hz, 3H, CH_3), 2.61 (s, 6H, $N(CH_3)_2$), 3.79 (s, 3H, CH_3O), 3.83 (s, 3H, CH_3O), 3.98 (q, J = 7.05 Hz, 2H, NCH_2), 4.35 (s, 2H, CH_2), 7.07 (s, 1H, ArH), 7.24 (s, 1H, ArH), 13.43 (s, 1H, NH).

Anal. Calcd for $C_{16}H_{22}N_4O_4S_2$: C, 46.63; H, 5.70; N, 14.50. Found: C, 46.58; H, 5.73; N, 14.54.

MS (HESI+). (m/z) 387.1159 (100%, $[M+H]^+$), 409.0979 (20%), $[M+Na]^+$).

2.1.2.3. 5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-4-propyl-s-triazole-3-thione (3_c). Yield: 96%. Mp 189–190 °C (methanol). IR cm^{-1}: 3100, 3050 (NH), 1605 (C=N), 1580, 1510 (C=S), 1330 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^1H NMR (DMSO-d_6) δ(ppm): 0.87 (t, J = 7.45 Hz, 3H, CH_3), 1.60 (hexaplet, J = 7.73 Hz, 2H, CH_2), 2.59 (s, 6H, $N(CH_3)_2$), 3.77 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 3.87 (t, J = 7.88 Hz, 2H, NCH_2), 4.33 (s, 2H, CH_2), 7.05 (s, 1H, ArH), 7.22 (s, 1H, ArH), 13.43 (s, 1H, NH).

Anal. Calcd for $C_{16}H_{24}N_4O_4S_2$: C, 48.00; H, 6.00; N, 14.00. Found: C, 48.07; H, 5.96; N, 14.03.

2.1.2.4. 5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-4-isopropyl-s-triazole-3-thione (3_d). Yield: 78%. Mp 228–230 °C (methanol–chloroform). IR cm^{-1}: 3295, 3050 (NH), 1600 (C=N), 1560, 1520 (C=S), 1330 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^1H NMR (DMSO-d_6) δ(ppm): 1.46 (d, J = 7.02 Hz, 6H, $2CH_3$), 2.59 (s, 6H, $N(CH_3)_2$), 3.78 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 4.38 (s, 2H, CH_2), 4.91 (m, 1H, NCH), 7.07 (s, 1H, ArH), 7.21 (s, 1H, ArH), 13.38 (s, 1H, NH).

Anal. Calcd for $C_{16}H_{24}N_4O_4S_2$: C, 48.00; H, 6.00; N, 14.00. Found: C, 47.96; H, 6.04; N, 14.05.

MS (HESI+). (m/z) 401.1315 (100%, $[M+H]^+$), 423.1135 (20%), $[M+Na]^+$).

2.1.2.5. 5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-4-butyl-s-triazole-3-thione. (3_e). Yield: 59%. Mp 190–191 °C (methanol). IR cm^{-1}: 3100, 3050 (NH), 1605 (C=N), 1575, 1510 (C=S), 1330 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^1H NMR (DMSO-d_6) δ(ppm): 0.87 (t, J = 7.38 Hz, 3H, CH_3), 1.28 (hexaplet, J = 7.35 Hz, 2H, CH_2), 1.54 (m, 2H, CH_2), 2.59 (s, 6H, $N(CH_3)_2$), 3.77 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 3.91 (t, J = 7.51 Hz, 2H, NCH_2), 4.33 (s, 2H, CH_2), 7.05 (s, 1H, ArH), 7.22 (s, 1H, ArH), 13.41 (s, 1H, NH).

Anal. Calcd for $C_{17}H_{26}N_4O_4S_2$: C, 49.27; H, 6.28; N, 13.52. Found: C, 49.33; H, 6.23; N, 13.55.

2.1.2.6. 5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-4-*tert*-butyl-s-triazole-3-thione (3_f). Yield: 53%. Mp 194–196 °C (methanol–chloroform). IR cm^{-1}: 3355, 3320 (NH), 1600 (C=N), 1550, 1520 (C=S), 1335 (S-$O_{antisym}$), 1150 (S-O_{sym}).

^1H NMR (DMSO-d_6) δ(ppm): 1.84 (s, 9H, $3CH_3$), 2.59 (s, 6H, $N(CH_3)_2$), 3.77 (s, 3H, CH_3O), 3.82 (s, 3H, CH_3O), 4.48 (s, 2H, CH_2), 6.91 (s, 1H, ArH), 7.23 (s, 1H, ArH), 13.34 (s, 1H, NH).

Anal. Calcd for $C_{17}H_{26}N_4O_4S_2$: C, 49.27; H, 6.28; N, 13.52. Found: C, 49.23; H, 6.33; N, 13.48.

2.1.2.7. 5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-4-ethyl-s-triazole-3-one (3_g). Yield: 65%. Mp 192–193 °C (methanol). IR cm^{-1}: 3365, 3170 (NH), 1695 (NHCO), 1600 (C=N), 1330 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^{1}H NMR (DMSO-d_6) δ(ppm): 1.05 (t, J = 7.11 Hz, 3H, CH_3), 2.62 (s, 6H, $N(CH_3)_2$), 3.52 (q, J = 7.08 Hz, 2H, NCH_2), 3.77 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 4.19 (s, 2H, CH_2), 6.96 (s, 1H, ArH), 7.24 (s, 1H, ArH), 11.37 (s, 1H, NH).

Anal. Calcd for $C_{15}H_{22}N_4O_5S$: C, 48.64; H, 5.94; N, 15.13. Found: C, 48.59; H, 5.99; N, 15.09.

2.1.2.8. 5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-4-propyl-s-triazole-3-one (3_h). Yield: 26%. Mp 158–159 °C (methanol). IR cm^{-1}: 3170 (NH), 1710 (NHCO), 1600 (C=N), 1335 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^{1}H NMR (DMSO-d_6) δ(ppm): 0.80 (t, J = 7.51 Hz, 3H, CH_3), 1.46 (hexaplet, J = 7.45 Hz, 2H, CH_2), 2.61 (s, 6H, $N(CH_3)_2$), 3.45 (t, J = 7.32 Hz, 2H, NCH_2), 3.76 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 4.19 (s, 2H, CH_2), 6.96 (s, 1H, ArH), 7.23 (s, 1H, ArH), 11.37 (s, 1H, NH).

Anal. Calcd for $C_{16}H_{24}N_4O_5S$: C, 50.00; H, 6.25; N, 14.58. Found: C, 50.04; H, 6.31; N, 14.54.

2.1.2.9. 5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-4-isopropyl-s-triazole-3-one (3_i). Yield: 40%. Mp 223–225 °C (methanol). IR cm^{-1}: 3165 (NH), 1690 (NHCO), 1600 (C=N), 1330 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^{1}H NMR (DMSO-d_6) δ(ppm): 1.30 (d, J = 6.83 Hz, 6H, $2CH_3$), 2.62 (s, 6H, $N(CH_3)_2$), 3.76 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 4.08 (m, 1H, NCH), 4.18 (s, 2H, CH_2), 6.91 (s, 1H, ArH), 7.24 (s, 1H, ArH), 11.30 (s, 1H, NH).

Anal. Calcd for $C_{16}H_{24}N_4O_5S$: C, 50.00; H, 6.25; N, 14.58. Found: C, 49.95; H, 6.30; N, 14.63.

2.1.2.10. 5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-4-butyl-s-triazole-3-one (3_j). Yield: 28%. Mp 167–169 °C (me thanol). IR cm^{-1}: 3175 (NH), 1715 (C=O), 1600 (C=N), 1335 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^{1}H NMR (DMSO-d_6) δ(ppm): 0.82 (t, J = 7.34 Hz, 3H, CH_3), 1.20 (hexaplet, J = 7.61 Hz, 2H, CH_2), 1.38 (m, 2H, CH_2), 2.61 (s, 6H, $N(CH_3)_2$), 3.48 (t, J = 7.36 Hz, 2H, NCH_2), 3.76 (s, 3H, CH_3O), 3.80 (s, 3H, CH_3O), 4.19 (s, 2H, CH_2), 6.96 (s, 1H, ArH), 7.23 (s, 1H, ArH), 11.37 (s, 1H, NH).

Anal. Calcd for $C_{17}H_{26}N_4O_5S$: C, 51.25; H, 5.32; N, 14.07. Found: C, 51.31; H, 5.25; N, 14.01.

2.1.2.11. 5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-4-*tert*-butyl-s-triazole-3-one (3_k). Yield: 63%. Mp 137–138 °C (methanol). IR cm^{-1}: 3540, 3350 (NH), 1685, 1665 (C=O), 1605 (C=N), 1325 (S-$O_{antisym}$), 1145 (S-O_{sym}).

^{1}H NMR (DMSO-d_6) δ(ppm): 1.21 (s, 9H, $3CH_3$), 2.64 (s, 6H, $N(CH_3)_2$), 3.76 (s, 2H, CH_2), 3.79 (s, 3H, CH_3O), 3.83 (s, 3H, CH_3O), 5.91 (s, 1H, NH), 7.14 (s, 1H, ArH), 7.19 (s, 1H, ArH), 7.51 (s, 1H, NH), 9.59 (s, 1H, NH).

Anal. Calcd for $C_{17}H_{26}N_4O_5S$: C, 51.25; H, 5.32; N, 14.07. Found: C, 51.20; H, 5.37; N, 14.11.

2.1.2.12. General procedure for the preparation of *N*-{5-[2-(*N*-dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-alkylamines (4). A mixture of the 1-[2-(*N*-sulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-thiosemicarbazide (1 mmol) in cold concentrated sulphuric acid (5 ml) was stirred for 15 min. The resulting solution was then allowed to reach ambient temperature, left stirring for 30 min and poured cautiously into ice cold water. The reaction mixture was alkalified to pH 8 with aqueous ammonia and the precipitated product was filtered, washed with water and recrystallized from the appropriate solvent.

The following compounds were prepared by an analogous procedure.

2.1.2.13. *N*-{5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-methylamine (4_a). Yield: 91%. Mp 190–191 °C (methanol–dichloromethane). IR cm^{-1}: 3280 (NH), 1600 (C=N), 1330 (S-O $_{antisym}$), 1140 (S-O_{sym}).

^{1}H NMR (DMSO-d_6) δ(ppm): 2.62 (s, 6H, $N(CH_3)_2$), 2.78 (d, J = 4.77 Hz, 3H, CH_3), 3.78 (s, 3H, CH_3O), 3.80 (s, 3H, CH_3O), 4.44 (s, 2H, CH_2), 7.04 (s, 1H, ArH), 7.21 (s, 1H, ArH), 7.43 (q, J = 4.77 Hz, 1H, NH).

Anal. Calcd for $C_{14}H_{20}N_4O_4S_2$: C, 45.16; H, 5.37; N, 15.05. Found: C, 45.12; H, 5.30; N, 15.09.

2.1.2.14. *N*-{5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-ethylamine (4_b). Yield: 82%. Mp 193–194 °C (methanol–chloroform). IR cm^{-1}: 3335 (NH), 1600 (C=N), 1330 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^{1}H NMR (DMSO-d_6) δ(ppm): 1.10 (t, J = 7.19 Hz, 3H, CH_3), 2.62 (s, 6H, $N(CH_3)_2$), 3.18 (m, 2H, CH_2), 3.78 (s, 3H, CH_3O), 3.80 (s, 3H, CH_3O), 3.98 (q, J = 7.05 Hz, 2H, NCH_2), 4.43 (s, 2H, CH_2), 7.04 (s, 1H, ArH), 7.21 (s, 1H, ArH), 7.49 (m, 1H, NH).

Anal. Calcd for $C_{15}H_{22}N_4O_4S_2$: C, 46.63; H, 5.70; N, 14.50. Found: C, 46.66; H, 5.66; N, 14.46.

2.1.2.15. *N*-{5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-propylamine (4_c). Yield: 70%. Mp 170–171 °C (methanol–chloroform). IR cm^{-1}: 3340 (NH), 1600 (C=N), 1325 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^{1}H NMR (DMSO-d_6) δ(ppm): 0.85 (t, J = 7.46 Hz, 3H, CH_3), 1.49 (hexaplet, J = 7.18 Hz, 2H, CH_2), 2.62 (s, 6H, $N(CH_3)_2$), 3.11 (q, J = 5.75 Hz, 2H, CH_2), 3.78 (s, 3H, CH_3O), 3.80 (s, 3H, CH_3O), 4.43 (s, 2H, CH_2), 7.05 (s, 1H, ArH), 7.21 (s, 1H, ArH), 7.52 (t, J = 5.37 Hz, 1H, NH).

Anal. Calcd for $C_{16}H_{24}N_4O_4S_2$: C, 48.00; H, 6.00; N, 14.00. Found: C, 47.97; H, 6.05; N, 14.05.

2.1.2.16. *N*-{5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-isopropylamine (4_d). Yield: 63%. Mp 192–193 °C (methanol). IR cm^{-1}: 3335 (NH), 1600 (C=N), 1325 (S-$O_{antisym}$), 1135 (S-O_{sym}).

^{1}H NMR (DMSO-d_6) δ(ppm): 1.11 (d, J = 6.43 Hz, 6H, $2CH_3$), 2.62 (s, 6H, $N(CH_3)_2$), 3.69 (m, 1H, CH), 3.79 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 4.43 (s, 2H, CH_2), 7.06 (s, 1H, ArH), 7.22 (s, 1H, ArH), 7.40 (d, J = 7.16 Hz, 1H, NH).

Anal. Calcd for $C_{16}H_{24}N_4O_4S_2$: C, 48.00; H, 6.00; N, 14.00. Found: C, 48.05; H, 5.94; N, 13.97.

2.1.2.17. *N*-{5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-butylamine (4_e). Yield: 53%. Mp 174–175 °C (methanol–chloroform). IR cm^{-1}: 3340 (NH), 1600 (C=N), 1330 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^{1}H NMR (DMSO-d_6) δ(ppm): 0.85 (t, J = 7.23 Hz, 3H, CH_3), 1.27 (hexaplet, J = 7.63 Hz, 2H, CH_2), 1.47 (pentaplet, J = 7.76 Hz, 2H, CH_2), 2.62 (s, 6H, $N(CH_3)_2$), 3.15 (tetraplet, J = 5.74 Hz, 2H, CH_2), 3.78 (s, 3H, CH_3O), 3.80 (s, 3H, CH_3O), 4.43 (s, 2H, CH_2), 7.05 (s, 1H, ArH), 7.21 (s, 1H, ArH), 7.50 (t, J = 5.47 Hz, 1H, NH).

Anal. Calcd for $C_{17}H_{26}N_4O_4S_2$: C, 49.27; H, 6.28; N, 13.52. Found: C, 49.22; H, 6.32; N, 13.49.

MS (HESI+). (m/z) 415.1473 (100%, $[M+H]^+$), 437.1292 (20%), $[M+Na]^+$).

2.1.3. General procedure for the preparation of *N*-{5-[2-(*N*-dimethylsulfa-moyl)-4,5-dimethoxy-benzyl]-1,3,4-oxadiazol-2-yl}-*N*-alkylamines (5)

To a stirred, cooled (0–5 °C) solution of the respective thiosemicarbazide derivative (1 mmol) in ethanol (6 ml) 2 N sodium hydroxide was added until the solution acquired pH 9. Iodine in potassium iodide solution (5%) was then added dropwise with stir-

ring at room temperature until the yellow colour of iodine persisted. The solvent was removed under reduced pressure and the residue was crystallized from the proper solvent to give the corresponding oxadiazole derivative.

The following compounds were prepared by an analogous procedure.

2.1.3.1. *N*-{5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-oxadiazol-2-yl}-*N*-methylamine (5_a). Yield: 60%. Mp 201–202 °C (ethanol–hexane). IR cm^{-1}: 3400 (NH), 1600 (C=N), 1330 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^1H NMR (DMSO-d_6) δ(ppm): 2.62 (s, 6H, $N(CH_3)_2$), 3.16 (s, 3H, CH_3), 3.67 (s, 3H, CH_3O), 3.79 (s, 3H, CH_3O), 4.20 (s, 2H, CH_2), 6.73 (s, 1H, ArH), 7.25 (s, 1H, ArH).

Anal. Calcd for $C_{14}H_{20}N_4O_4S_5$: C, 47.19; H, 3.61; N, 15.73. Found: C, 47.24; H, 5.64; N, 15.69.

2.1.3.2. *N*-{5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-oxadiazol-2-yl}-*N*-ethylamine (5_b). Yield: 81%. Mp 183–185 °C (ethanol). IR cm^{-1}: 3445 (NH), 1600 (C=N), 1330 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^1H NMR (DMSO-d_6) δ(ppm): 1.06 (t, J = 7.12 Hz, 3H, CH_3), 2.60 (s, 6H, $N(CH_3)_2$), 3.74 (s, 3H, CH_3O), 3.80 (s, 3H, CH_3O), 3.86 (q, J = 7.14 Hz, 2H, CH_2), 4.29 (s, 2H, CH_2), 6.96 (s, 1H, ArH), 7.23 (s, 1H, ArH).

Anal. Calcd for $C_{15}H_{22}N_4O_5S$: C, 48.64; H, 5.95; N, 15.13. Found: C, 48.60; H, 5.91; N, 15.17.

2.1.3.3. *N*-{5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-oxadiazol-2-yl}-*N*-propylamine (5_c). Yield: 78%. Mp 177–179 °C (ethanol). IR cm^{-1}: 3365 (NH), 1605 (C=N), 1330 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^1H NMR (DMSO-d_6) δ(ppm): 0. 37 (t, J = 7.41 Hz, 3H, CH_3), 1.38 (hexaplet, J = 7.57 Hz, 2H, CH_2), 2.62 (s, 6H, $N(CH_3)_2$), 3.64 (m, 2H, CH_2),3.67 (s, 3H, CH_3O), 3.79 (s, 3H, CH_3O), 4.24 (s, 2H, CH_2), 6.82 (s, 1H, ArH), 7.24 (s, 1H, ArH).

Anal. Calcd for $C_{16}H_{24}N_4O_5S$: C, 50.00; H, 6.25; N, 14.58. Found: C, 50.02; H, 6.21; N, 15.01.

2.1.3.4. *N*-{5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-oxadiazol-2-yl}-*N*-butylamine (5_d). Yield: 75%. Mp 128–129 °C (ethanol–*n*-hexane). IR cm^{-1}: 3450 (NH), 1600 (C=N), 1335 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^1H NMR (DMSO-d_6) δ(ppm): 0.78 (t, J = 7.20 Hz, 3H, CH_3), 1.17 (hexaplet, J = 7.31 Hz, 2H, CH_2), 1.33 (m, 2H, CH_2), 2.61 (s, 6H, $N(CH_3)_2$), 3.68 (s, 3H, CH_3O), 3.70 (m, 2H, CH_2), 3.79 (s, 3H, CH_3O), 4.24 (s, 2H, CH_2), 6.83 (s, 1H, ArH), 7.24 (s, 1H, ArH).

Anal. Calcd for $C_{17}H_{26}N_4O_5S$: C, 51.25; H, 6.53; N, 14.07. Found: C, 51.29; H, 6.49; N, 14.10.

2.1.3.5. *N*-{5-[2-(*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-oxadiazol-2-yl}-*N*-*tert*-butylamine (5_e). Yield: 95%. Mp 221–224 °C (ethanol–*n*-hexane). IR cm^{-1}: 3420 (NH), 1600 (C=N), 1330 (S-$O_{antisym}$), 1140 (S-O_{sym}).

^1H NMR (DMSO-d_6) δ(ppm): 1.84 (s, 9H, $3CH_3$), 2.59 (s, 6H, $N(CH_3)_2$), 3.77 (s, 3H, CH_3O), 3.82 (s, 3H, CH_3O), 4.48 (s, 2H, CH_2), 6.91 (s, 1H, ArH), 7.23 (s, 1H, ArH).

Anal. Calcd for $C_{17}H_{26}N_4O_5S$: C, 51.25; H, 6.53; N, 14.07. Found: C, 51.21; H, 6.56; N, 14.03.

2.2. Pharmacology

2.2.1. Antibacterial activity

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

The antibacterial activity of tested compounds against human pathogenic bacteria was determined using the microdilution method.[29]

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

2.2.2. Microdilution test

The minimum inhibitory and bactericidal concentrations (MICs and MBCs) were determined using 96-well microtitre plates. The bacterial suspension was adjusted with sterile saline to a concentration of 1.0×10^5 cfu/ml. Compounds to be investigated were dissolved in broth LB medium (100 μl) with bacterial inoculum (1.0×10^4 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 positive control. Streptomycin and ampicillin were used as a positive control (1 mg/ml DMSO). Two replicates were done for each compound.

2.2.3. Antifungal activity

For the antifungal bioassays, following fungi were used: *Aspergillus flavus* (ATCC 9643), *Aspergillus fumigatus* (human isolate), *Aspergillus niger* (ATCC 6275), *Aspergillus versicolor* (ATCC 11730), *Aspergillus ochraceus* (ATCC 12066), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112) and *Trichoderma viride* (IAM 5061).

The micromycetes were maintained on malt agar and the cultures stored at 4 °C and sub-cultured once a month.[1] In order to investigate the antifungal activity of the extracts, a modified microdilution technique was used.[29] The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 μl per well. The inocula were stored at 4 °C for further use. Dilutions of the inocula were cultured on solid malt agar to verify the absence of contamination and to check the validity of the inoculum.

Minimum inhibitory concentration (MIC) determinations were performed by a serial dilution technique using 96-well microtiter plates. The compounds investigated were dissolved in 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.

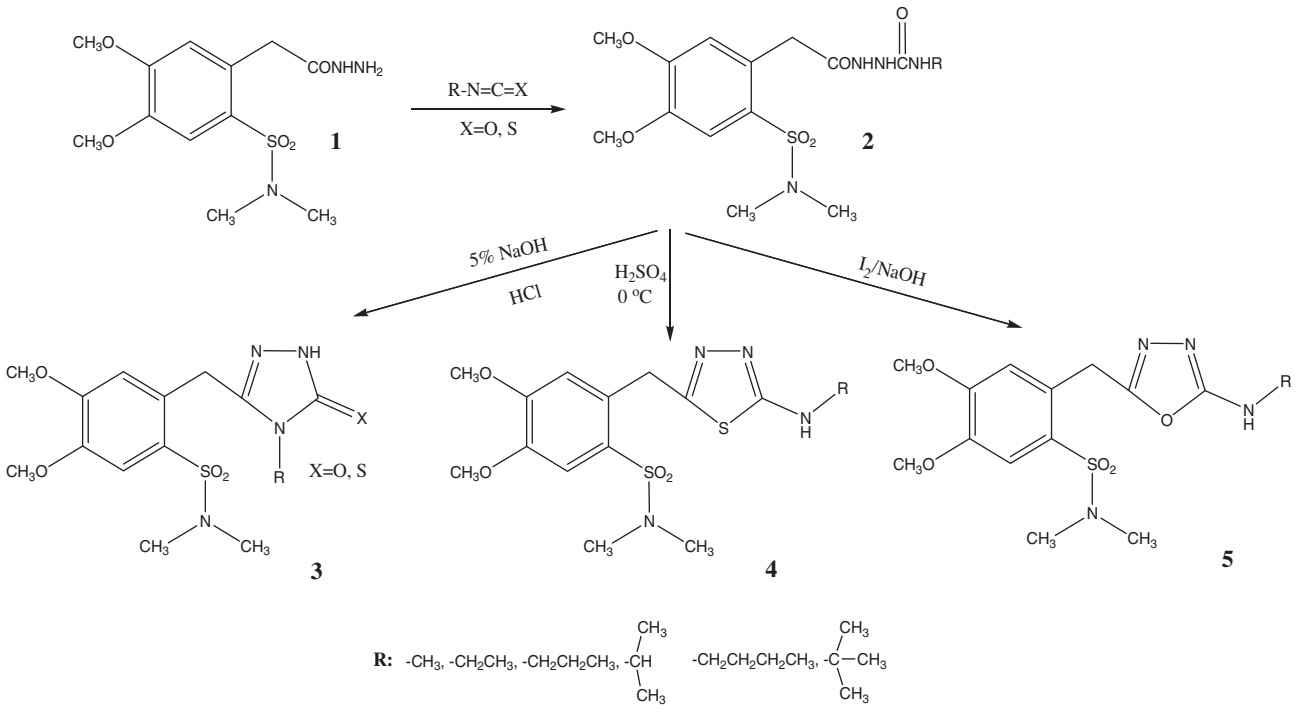
The fungicidal concentrations (MFCs) were determined by serial subcultivation of a 2 μl into microtitre plates containing 100 μl of broth per well and further incubation 5 days at 28 °C. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. DMSO was used as a negative control, commercial fungicides, bifonazole and ketoconazole, were used as positive controls (1–5000 μg/ml).

2.3. NMR spectroscopy

DMSO-d_6 and ultra precision NMR tubes (Norell 509-UP-7, 5 mm) were used for the NMR experiments. Compounds were dissolved in DMSO-d_6 to a final concentration of 10 mM and a series of experiments were performed using Varian 600 MHz spectrometer at 300 K temperature. All data are collected using pulse sequences and phase-cycling routines provided in the Varian libraries. The ^{1}H spectral width was set to 8500 Hz at 600 MHz. Typically the 2D NOESY spectrum was acquired with 4096 data points in t_2 dimension, 64 scans, 256 increments in t_1 dimension, 150 ms mixing time and a relaxation delay of 1 s. Data processing including apodization with cosine square Bell function, Fourier transformation and phasing, were performed using Varian VNMR software. Intramolecular distances were calculated from cross-peak volumes in NOESY while a tolerance of ±10% of the calculated value was applied to produce the upper and the lower limit distance constraints.

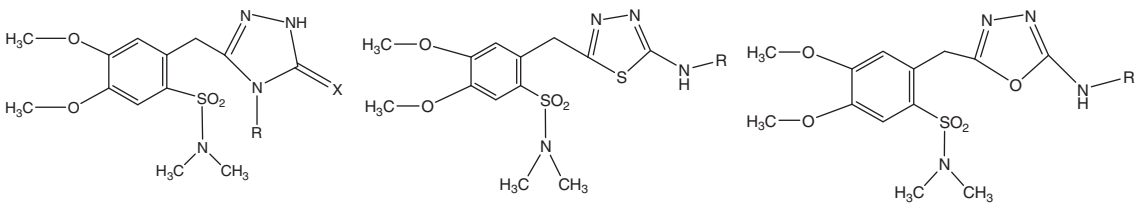
2.4. Molecular modeling

Computer calculations were performed using Schrodinger Suite 2011 molecular modeling package. More specifically, Molecular



Scheme 1. Synthetic pathway for the preparation of the title compounds.

Table 1
Chemical structures of synthesized molecules



	X = S	X = O		
–CH_3	**3$_a$**	–	**4$_a$**	**5$_a$**
–CH_2CH_3	**3$_b$**	**3$_g$**	**4$_b$**	**5$_b$**
–$CH_2CH_2CH_3$	**3$_c$**	**3$_h$**	**4$_c$**	**5$_c$**
–CH(CH$_3$)$_2$	**3$_d$**	**3$_i$**	**4$_d$**	–
–$CH_2CH_2CH_2CH_3$	**3$_e$**	**3$_j$**	**4$_e$**	**5$_d$**
–C(CH$_3$)$_3$	**3$_f$**	**3$_k$**	–	**5$_e$**

Table 2
Antibacterial activity of compounds $\mathbf{3_{a-k}}$ tested by microdilution method (MIC and MBC in μmol)

Bacteria	Activity	$\mathbf{3_a}$	$\mathbf{3_b}$	$\mathbf{3_c}$	$\mathbf{3_d}$	$\mathbf{3_e}$	$\mathbf{3_f}$	$\mathbf{3_g}$	$\mathbf{3_h}$	$\mathbf{3_i}$	$\mathbf{3_j}$	$\mathbf{3_k}$	Str	Amp
Staphylococcus aureus	MIC	0.27	0.26	0.50	0.38	0.24	0.24	0.27	0.26	0.39	0.25	0.39	0.04	0.25
	MBC	0.54	0.52	0.50	0.38	0.48	0.42	0.54	0.39	0.39	0.50	0.39	0.09	0.37
Bacillus cereus	MIC	0.27	0.52	0.25	0.25	0.24	0.24	0.27	0.26	0.26	0.25	0.25	0.09	0.25
	MBC	0.54	0.52	0.50	0.50	0.48	0.48	0.81	0.65	0.78	0.76	0.78	0.17	0.37
Micrococcus flavus	MIC	0.27	0.26	0.25	0.63	0.24	0.24	0.40	0.65	0.39	0.63	0.39	0.17	0.25
	MBC	0.54	0.52	0.50	0.63	0.48	0.42	0.40	0.65	0.39	0.63	0.39	0.34	0.37
Listeria monocytogenes	MIC	0.27	0.26	0.25	0.63	0.24	0.24	0.40	0.65	0.39	0.38	0.39	0.17	0.37
	MBC	0.27	0.52	0.25	0.63	0.24	0.42	0.54	0.78	0.39	0.38	0.39	0.34	0.49
Pseudomonas aeruginosa	MIC	0.54	0.26	0.50	0.63	0.48	0.24	0.40	0.65	0.39	0.38	0.39	0.17	0.74
	MBC	0.54	0.26	0.50	0.63	0.48	0.42	0.54	0.78	0.39	0.38	0.39	0.34	1.24
Enterobacter cloacae	MIC	0.27	0.26	0.25	0.63	0.24	0.24	0.54	0.65	0.39	0.50	0.25	0.17	0.37
	MBC	0.27	0.52	0.25	0.63	0.24	0.42	0.54	0.78	0.39	0.76	0.25	0.34	0.49
Salmonella typhimurium	MIC	0.27	0.26	0.50	0.63	0.24	0.24	0.40	0.65	0.39	0.50	0.25	0.17	0.25
	MBC	0.27	0.26	0.50	0.63	0.24	0.42	0.54	0.65	0.39	0.76	0.25	0.34	0.49
Escherichia coli	MIC	0.54	0.26	0.50	0.63	0.48	0.24	0.27	0.65	0.39	0.13	0.13	0.26	0.37
	MBC	0.54	0.52	0.50	0.63	0.48	0.42	0.27	0.65	0.39	0.25	0.25	0.52	0.74

Table 3
Antifungal activity of compounds $\mathbf{3_{a-k}}$ tested by microdilution method (MIC and MFC in μmol)

Fungi	Activity	$\mathbf{3_a}$	$\mathbf{3_b}$	$\mathbf{3_c}$	$\mathbf{3_d}$	$\mathbf{3_e}$	$\mathbf{3_f}$	$\mathbf{3_g}$	$\mathbf{3_h}$	$\mathbf{3_i}$	$\mathbf{3_j}$	$\mathbf{3_k}$	Bif	Keto
Penicillium funiculosum	MIC	0.14	0.13	0.13	0.13	0.12	0.12	0.14	0.13	0.26	0.13	0.13	0.64	0.38
	MFC	0.27	0.26	0.25	0.25	0.24	0.24	0.27	0.26	0.26	0.25	0.25	0.8	0.95
Penicillium ochrochloron	MIC	0.14	0.13	0.13	0.13	0.12	0.12	0.14	0.13	0.13	0.13	0.25	0.48	3.8
	MFC	0.27	0.26	0.25	0.38	0.48	0.36	0.27	0.39	0.39	0.38	0.38	0.64	3.8
Trichoderma viride	MIC	0.07	0.07	0.06	0.01	0.06	0.24	0.14	0.07	0.03	0.03	0.03	0.64	4.75
	MFC	0.14	0.13	0.13	0.03	0.12	0.24	0.27	0.13	0.07	0.06	0.25	0.8	5.7
Aspergillus fumigatus	MIC	0.14	0.13	0.13	0.13	0.12	0.02	0.14	0.13	0.03	0.25	0.25	0.48	0.38
	MFC	0.27	0.26	0.25	0.25	0.24	0.12	0.27	0.26	0.13	0.25	0.38	0.64	0.95
Aspergillus niger	MIC	0.07	0.07	0.06	0.13	0.06	0.24	0.27	0.13	0.26	0.13	0.25	0.48	0.38
	MFC	0.14	0.13	0.13	0.38	0.12	0.36	0.45	0.26	0.39	0.38	0.38	0.64	0.95
Aspergillus flavus	MIC	0.07	0.07	0.06	0.13	0.06	0.24	0.27	0.13	0.26	0.13	0.25	0.48	2.85
	MFC	0.14	0.26	0.13	0.38	0.24	0.36	0.45	0.26	0.39	0.38	0.38	0.64	3.8
Aspergillus versicolor	MIC	0.14	0.13	0.25	0.13	0.24	0.24	0.14	0.13	0.13	0.13	0.25	0.32	0.38
	MFC	0.27	0.26	0.5	0.38	0.48	0.36	0.45	0.39	0.39	0.38	0.38	0.64	0.95
Fulvia fyulvum	MIC	0.07	0.07	0.06	0.25	0.12	0.24	0.27	0.26	0.26	0.25	0.25	0.32	0.38
	MFC	0.14	0.07	0.13	0.25	0.24	0.24	0.27	0.26	0.26	0.25	0.25	0.64	0.95

Table 4
Minimal inhibitory and bactericidal concentration (MIC and MBC (μmol/ml)) of tested compounds, $\mathbf{4_{a-e}}$

Bacteria	Activity	$\mathbf{4_a}$	$\mathbf{4_b}$	$\mathbf{4_c}$	$\mathbf{4_d}$	$\mathbf{4_e}$	Streptomycin	Ampicillin
Staphylococcus aureus	MIC	0.27	0.52	0.5	0.38	0.48	0.04	0.25
	MBC	0.54	0.52	0.5	0.46	0.48	0.09	0.37
Bacillus cereus	MIC	0.27	0.26	0.25	0.25	0.24	0.09	0.25
	MBC	0.27	0.26	0.5	0.5	0.24	0.17	0.37
Micrococcus flavus	MIC	0.27	0.26	0.25	0.38	0.24	0.17	0.25
	MBC	0.54	0.52	0.5	0.38	0.48	0.34	0.37
Listeria monocytogenes	MIC	0.27	0.52	0.5	0.38	0.24	0.17	0.37
	MBC	0.54	0.52	0.5	0.46	0.48	0.34	0.49
Pseudomonas aeruginosa	MIC	0.27	0.26	0.5	0.25	0.48	0.17	0.74
	MBC	0.27	0.26	0.5	0.38	0.48	0.34	1.24
Enterobacter cloacae	MIC	0.27	0.52	0.5	0.46	0.24	0.17	0.37
	MBC	0.27	0.52	0.5	0.46	0.24	0.34	0.49
Salmonella typhimurium	MIC	0.27	0.26	0.25	0.25	0.24	0.17	0.25
	MBC	0.27	0.26	0.5	0.5	0.24	0.34	0.49
Escherichia coli	MIC	0.27	0.26	0.25	0.38	0.48	0.26	0.37
	MBC	0.27	0.26	0.25	0.46	0.48	0.52	0.74

Mechanics calculations were performed under Macromodel[30] module of Schrodinger Suite 2011, using the OPLS 2005 force field. Compounds were first minimized with TNCG (Truncated Newton Conjugate Gradient) algorithm using 1000 iterations and an energy tolerance of 0.01 kcal/mol^{-1} Å^{-1}, to reach a local minimum. The dielectric constant (ε) was set to 47 during minimization, simulating the DMSO environment of the NMR solvent.

To generate random conformers, the 3D models of the studied molecules following their optimization were subjected to Conformational Search (Macromodel) using the Mixed torsional/Low-mode sampling. This method uses a combination of the random changes in torsion angles and/or molecular position from the torsional sampling (MCMM) method, together with the low-mode steps from the LMOD method, which is highly efficient and has the advantage that ring structures and variable torsion angles do not need to be specified.

Epik was used for the tautomers generation of compound **3$_f$** by employing protonation and tautomerization state adjustment consistent with a specified pH range. The tautomerization facility of Epik relies on a database of tautomeric templates. Tautomers in the database are assigned probabilities to assist in focusing on the most highly populated tautomeric forms.[31–33]

QikProp predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules. It rapidly analyses atom types and charges, rotor counts, and the sample molecule's volume and surface area. QikProp then uses this information, along with the physical descriptors calculated using algorithms, which mimic the full Monte Carlo simulations and produce comparable results with experimentally determined properties, in regression equations. This procedure results in an accurate prediction of a molecule's pharmacologically relevant properties.[34]

The calculations of the hydrophilic and hygrophobic surfaces were performed by the Hydrophobic/philic Surfaces panel of Maestro.[35]

3. Results and discussion

3.1. Chemistry

The synthetic pathway followed for the preparation of the title compounds was accomplished as shown in Scheme 1. All synthesized molecules are presented in Table 1.

Table 5
Minimal inhibitory and fungicidal concentration (MIC and MFC μmol/ml) of tested compounds **4$_{a–e}$**

Fungi	Activity	**4$_a$**	**4$_b$**	**4$_c$**	**4$_d$**	**4$_e$**	Bifonazole	Ketoconazole
Penicillium funiculosum	MIC	0.14	0.13	0.13	0.25	0.12	0.64	0.38
	MFC	0.27	0.26	0.25	0.5	0.24	0.8	0.95
Penicillium ochrochloron	MIC	0.14	0.13	0.13	0.5	0.12	0.48	3.8
	MFC	0.27	0.26	0.25	0.75	0.24	0.64	3.8
Trichoderma viride	MIC	0.07	0.07	0.06	0.13	0.06	0.64	4.75
	MFC	0.13	0.13	0.13	0.25	0.12	0.8	5.7
Aspergillus fumigatus	MIC	0.14	0.13	0.13	0.13	0.12	0.48	0.38
	MFC	0.27	0.26	0.25	0.25	0.24	0.64	0.95
Aspergillus niger	MIC	0.07	0.13	0.06	0.25	0.06	0.48	0.38
	MFC	0.14	0.26	0.13	0.75	0.12	0.64	0.95
Aspergillus flavus	MIC	0.14	0.13	0.13	0.25	0.12	0.48	2.85
	MFC	0.27	0.26	0.25	0.75	0.24	0.64	3.8
Aspergillus versicolor	MIC	0.27	0.26	0.25	0.25	0.12	0.32	0.38
	MFC	0.54	0.52	0.5	0.75	0.24	0.64	0.95
Fulvia fyulvum	MIC	0.07	0.07	0.06	0.13	0.06	0.32	0.38
	MFC	0.07	0.07	0.13	0.25	0.12	0.64	0.95

Table 6
Antibacterial activity of compounds **5$_{a–e}$** tested by microdilution method (MIC and MBC in μmol)

Bacteria	Activity	**5$_a$**	**5$_b$**	**5$_c$**	**5$_d$**	**5$_e$**	Str	Amp
Staphylococcus aureus	MIC	0.28	0.14	0.26	0.25	0.25	0.04	0.25
	MBC	0.28	0.27	0.26	0.38	0.5	0.09	0.37
Bacillus cereus	MIC	0.28	0.14	0.26	0.25	0.39	0.09	0.25
	MBC	0.28	0.27	0.26	0.25	0.39	0.17	0.37
Micrococcus flavus	MIC	0.56	0.27	0.13	0.25	0.25	0.17	0.25
	MBC	0.84	0.54	0.26	0.5	0.5	0.34	0.37
Listeria monocytogenes	MIC	0.42	0.27	0.13	0.25	0.25	0.17	0.37
	MBC	0.42	0.54	0.26	0.75	0.39	0.34	0.49
Pseudomonas aeruginosa	MIC	0.42	0.27	0.26	0.13	0.39	0.17	0.74
	MBC	0.56	0.54	0.26	0.25	0.39	0.34	1.24
Enterobacter cloacae	MIC	0.7	0.67	0.63	0.63	0.63	0.17	0.37
	MBC	0.84	0.67	0.75	0.75	0.63	0.34	0.49
Salmonella typhimurium	MIC	0.42	0.14	0.13	0.13	0.13	0.17	0.25
	MBC	0.42	0.27	0.26	0.25	0.25	0.34	0.49
Escherichia coli	MIC	0.28	0.14	0.63	0.63	0.39	0.26	0.37
	MBC	0.42	0.27	0.63	0.63	0.39	0.52	0.74

3.2. Pharmacology

3.2.1. Triazoles group

Results of antibacterial activity of the triazole compounds **3a–k** are presented in Table 2. All the compounds showed antibacterial activity with MIC in range 0.13–0.65 μmol/ml and MBC of 0.24–0.76 μmol/ml. The best antibacterial activity was obtained for compound **3f** with MIC and MBC of 0.24–0.48 μmol/ml while the lowest antibacterial activity was achieved for compound **3g** with MIC and MBC of 0.27–0.81 μmol/ml. It can be seen that differences between MIC and MBC of these compounds are very small. Streptomycin showed MIC in range of 0.04–0.26 μmol/ml and MBC of 0.09–0.52 μmol/ml. Ampicillin showed inhibitory effect at 0.25–0.74 μmol/ml and bactericidal at 0.37–1.24 μmol/ml. Compounds **3a**, **3c** and **3e** showed better antibacterial activity than streptomycin against *Listeria monocytogenes*. Compound **3b** also possessed better activity than streptomycin but only against *Pseudomonas aeruginosa*. *Enterobacter cloacae* and *Salmonella typhimurium* were more sensitive to compounds **3a**, **3e** and **3k** than to streptomycin. *Escherichia coli* was the most sensitive bacteria to the tested compounds, especially to **3f**, **3i** and **3k**, where the MIC and MBC were lower than for streptomycin.

Compounds **3a**, **3b**, **3c**, **3e**, **3f**, **3i** and **3k** possessed better antibacterial activity than ampicillin against *Enterobacter cloacae* and *Salmonella typhimurium*. All compounds tested showed stronger antibacterial potential than ampicillin against *Escherichia coli* and *Pseudomonas aeruginosa*. Compounds **3a**, **3c**, **3e**, **3f**, **3i**, **3j** and **3k** reacted with higher antibacterial activity than ampicillin against *Listeria monocytogenes*.

From the obtained results it can be noticed that several compounds possessed better antibacterial activity than streptomycin and ampicillin.

Results of antifungal activity are presented in Table 3. It can be seen that all the compounds showed very good antifungal activity with MIC of 0.01–0.27 μmol/ml and MFC 0.06–0.50 μmol/ml better than the commercial antifungal agents, bifonazole (MIC 0.32–0.64 μmol/ml, MFC 0.64–0.80 μmol/ml) and ketoconazole (MIC 0.38–4.75 μmol/ml, MFC 0.95–5.70 μmol/ml). Triazoles, exhibited much better antifungal activity than these mycotics, in some cases (*A. niger, A. flavus* and *T. viride*) this activity was 10–70 times higher. Compound **3d** showed the best antifungal activity among all the others with lowest MIC (0.01–0.25 μmol/ml) and MFC (0.03–0.38 μmol/ml). The most sensitive fungi to all tested compounds were *Trichoderma viride*, while *Aspergillus versicolor* was the most resistant.

3.2.2. Thiadiazoles group

The results of antibacterial activity of thiadiazole compounds **4a–e** are presented in Table 4. The range of antibacterial activity was in 0.24–0.54 μmol/ml (MIC and MBC). All compounds tested possessed higher antibacterial activity than streptomycin against *Escherichia coli*. *Pseudomonas aeruginosa* and *Escherichia coli* were more sensitive to all the compounds than to ampicillin. Compound **4e** showed the best antibacterial activity with MIC and MBC of 0.24–0.48 μmol/ml. Compounds **4a**, **4b** and **4e** showed better bactericidal activity than streptomycin against *Escherichia coli* and *Salmonella typhimurium* and better bactericidal activity than ampicillin against *Bacillus cereus* and *Salmonella typhimurium*. Better bactericidal potential than ampicillin against *Enterobacter cloacae* was achieved also for compounds **4a**, **4d** and **4e**, while **4d** and **4e** showed better activity than ampicillin only against *Listeria monocytogenes*.

The results of antifungal activity are presented in Table 5. The tested compounds showed very good antifungal activity with MIC of 0.06–0.50 μmol/ml and MFC 0.07–0.75 μmol/ml. All the compounds showed higher antifungal potential than ketoconazol and almost all showed higher potential than bifonazole (MIC, MFC), except compound **4d** which exhibited slightly lower potential than bifonazole against *Penicillium ochrochloron*, *Aspegillus niger*, *Aspergillus versicolor* and *A. flavus*.

The best antifungal potential could be seen for compound **4e** MIC (0.06–0.12 μmol/ml) and MFC (0.12–0.24 μmol/ml). The most sensitive fungus on all tested compounds was *Fulvia fulvum*, while *Aspergillus versicolor* was the most resistant species. In conclusion, thiadiazoles exhibited much better antifungal activity than used mycotics, in some cases (*A. niger, T. viride* and *F. fulvum*) this activity was 8–10 times higher.

3.2.3. Oxadiazoles group

The results of antibacterial activity of the oxadiazoles **5a–e** are presented in Table 6. Antibacterial activity was achieved at 0.13–0.70 μmol/ml (MIC) and 0.25–0.84 μmol/ml (MBC). All compounds showed almost the same activity with small differences. The highest inhibition and bactericidal potential was observed for compound **5b** with MIC of 0.14–0.67 μmol/ml and MBC of 0.27–0.67 μmol/ml. Compounds **5d** and **5c** showed better antibacterial activity than streptomycin against *Salmonella typhimurium*, while compound **5c** was also more effective than streptomycin against *Listeria moonocytognes* and *Micrococcus flavus*. Compounds **5b**, **5c** and **5d** possessed higher antibacterial activity than streptomycin against *Salmonella typhimurium*. Ampicillin was less effective than

Table 7
Antifungal activity of compounds **5a–e** tested by microdilution method (MIC and MFC in μmol)

Fungi	Activity	5a	5b	5c	5d	5e	Bif	Keto
Penicillium funiculosum	MIC	0.14	0.14	0.26	0.25	0.13	0.64	0.38
	MFC	0.28	0.27	0.78	0.5	0.25	0.8	0.95
Penicillium ochrochloron	MIC	0.14	0.14	0.13	0.25	0.13	0.48	3.8
	MFC	0.45	0.27	0.26	0.75	0.38	0.64	3.8
Trichoderma viride	MIC	0.03	0.03	0.07	0.13	0.06	0.64	4.75
	MFC	0.14	0.03	0.13	0.13	0.13	0.8	5.7
Aspergillus fumigatus	MIC	0.14	0.27	0.13	0.13	0.13	0.48	0.38
	MFC	0.28	0.27	0.26	0.25	0.25	0.64	0.95
Aspergillus niger	MIC	0.14	0.14	0.13	0.25	0.25	0.48	0.38
	MFC	0.45	0.14	0.39	0.75	0.38	0.64	0.95
Aspergillus flavus	MIC	0.14	0.14	0.13	0.25	0.13	0.48	2.85
	MFC	0.45	0.4	0.39	0.75	0.25	0.64	3.8
Aspergillus versicolor	MIC	0.14	0.14	0.26	0.25	0.13	0.32	0.38
	MFC	0.45	0.4	0.39	0.75	0.38	0.64	0.95
Fulvia fyulvum	MIC	0.28	0.27	0.13	0.13	0.25	0.32	0.38
	MFC	0.45	0.27	0.26	0.25	0.25	0.64	0.95

Table 8
Predicted properties for the synthesized analogues

Compd	Dipole	FOSA	SASA	FISA	PISA	WPSA	Volume	HBd	Hba	Dip^2/V	QPlogPC16	QPlogPoct	QPlogPw	QPlogPo/w	QPlogS	ClQPlogS	IP (eV)	EA (eV)	PSA	3*HBd − QPlogPo/w
3a	8.85	347.19	566.06	100.93	43.33	74.61	1053.98	1	9.5	0.074	9.90	18.20	11.59	1.63	−2.59	−3.66	8.43	1.02	90.50	1.37
3b	10.94	398.13	620.73	115.93	36.94	69.72	1130.80	1	9.5	0.106	10.53	19.37	11.56	1.93	−3.30	−3.93	8.31	1.01	92.44	1.07
3c	13.07	431.90	655.81	115.89	38.41	69.61	1193.70	1	9.5	0.143	11.11	20.47	11.43	2.31	−3.72	−4.21	8.29	1.01	92.33	0.69
3d	11.51	428.21	603.51	89.23	24.70	61.37	1163.64	1	9.5	0.114	10.58	19.86	11.25	2.30	−2.98	−4.21	8.13	1.05	89.97	0.7
3e	9.85	424.29	608.36	91.69	30.35	62.03	1185.88	1	9.5	0.082	10.85	19.28	10.87	2.44	−2.74	−4.49	8.24	1.09	90.47	0.56
3f	10.29	449.34	614.25	81.26	25.94	57.72	1192.99	1	9.5	0.089	10.76	19.88	11.23	2.53	−3.14	−4.49	8.03	0.99	83.76	0.47
3g	3.59	382.77	574.58	147.66	44.14	0.00	1074.05	1	9	0.012	9.88	16.97	11.19	1.34	−2.42	−3.82	9.25	0.77	109.32	1.66
3h	7.73	438.51	624.23	152.05	33.28	0.38	1146.92	1	9	0.052	10.42	18.16	11.05	1.72	−3.07	−4.09	9.15	0.95	113.38	1.28
3i	9.46	434.23	587.91	128.48	24.91	0.29	1131.22	1	9	0.079	10.16	18.56	10.96	1.81	−2.64	−4.09	8.82	0.88	108.27	1.19
3j	6.46	460.10	639.31	137.77	40.62	0.82	1190.30	1	9	0.035	10.77	18.26	10.82	2.10	−3.16	−4.37	9.13	0.83	106.98	0.9
3k	8.65	478.64	620.48	114.35	27.49	0.00	1177.02	1	9	0.064	10.42	18.87	10.96	2.18	−3.17	−4.37	8.81	0.76	103.63	0.82
4a	5.85	425.45	617.69	120.17	49.08	22.99	1103.10	1	8.5	0.031	10.04	17.22	10.66	1.98	−3.41	−4.05	8.66	0.63	90.19	1.02
4b	7.61	410.68	595.64	123.31	50.28	11.37	1112.64	1	8.5	0.052	10.15	17.48	10.41	2.00	−2.83	−4.33	8.99	0.77	89.41	1
4c	2.38	462.68	648.03	117.45	46.31	21.59	1186.42	1	8.5	0.005	10.77	17.50	10.25	2.50	−3.59	−4.61	8.90	0.66	89.75	0.5
4d	5.23	502.38	670.46	117.15	43.31	7.62	1210.41	1	8.5	0.023	10.88	18.20	10.50	2.58	−4.07	−4.61	8.63	0.77	90.69	0.42
4e	6.15	512.77	705.50	120.00	47.61	25.13	1272.23	1	8.5	0.030	11.65	18.73	10.20	3.01	−4.42	−4.89	8.68	0.66	90.67	−0.01
5a	9.77	407.30	589.95	123.96	58.19	0.50	1062.84	1	9	0.090	9.63	17.73	11.16	1.44	−2.67	−3.54	8.98	0.75	100.12	1.56
5b	10.04	425.53	600.75	116.33	58.32	0.57	1097.01	1	9	0.092	9.90	17.96	10.92	1.70	−2.69	−3.82	8.94	0.77	96.89	1.3
5c	1.37	460.04	633.77	124.56	47.65	1.52	1163.24	1	9	0.002	10.48	17.37	10.76	2.03	−3.07	−4.09	8.87	0.73	100.51	0.97
5d	3.39	488.34	665.08	124.43	51.80	0.51	1222.44	1	9	0.009	11.05	18.03	10.64	2.39	−3.43	−4.37	8.97	0.67	99.71	0.61
5e	1.99	466.32	626.54	112.13	47.36	0.73	1183.71	1	9	0.003	10.60	17.95	10.88	2.25	−3.11	−4.37	8.83	0.74	97.22	0.75

all the compounds tested against *Pseudomonas aeruginosa* and *Salmonella typhimurium*. More specifically, compound **5c** showed higher antibacterial activity than ampicillin against *Micrococcus flavus*, *Bacillus cereus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*, while **5d** suppressed *Bacillus cereus*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* better than ampicillin. *Listeria monocytogenes* was more sensitive to compounds **5c** and **5e** than to ampicillin. Compound **5b** showed better activity than ampicillin against *Listeria monocytogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli* and *Bacillus cereus*.

Results of antifungal activity are presented in Table 7. Inhibitory activity of compounds was in range of 0.03–0.28 μmol/ml and fungicidal in range of 0.03–0.75 μmol/ml. The tested compounds showed higher antifungal potential than bifonazole, except compound **5d** which exhibited slightly lower potential against *Penicillium ochrochloron*, *Aspegillus niger*, *A. versicolor* and *A. flavus*. Moreover, oxadiazoles possessed much better antifungal potential than ketokonazol. The best antifungal potential was achieved for compound **5b** with MIC 0.03–0.27 μmol/ml and MFC 0.03–0.40 μmol/ml. Compounds **5a**, **5b** and **5e** showed almost the same activity with small differences in MIC, while compounds **5c** and **5d** possessed similar antifungal potential. The most sensitive fungus on all tested compounds was *Trichoderma viride*, while *Aspergillus versicolor* and *Penicillium* species were the most resistant.

3.3. SAR—structure activity relationship

From the biological results, it becomes clear that different substituents on triazole, thiadiazole and oxadiazole scaffolds have a noticeable effect on antibacterial and antifungal activities. In general, studied molecules exhibited much better antifungal than antibacterial activity.

3.3.1. Antibacterial activity

The antimicrobial activity of all compounds is promising. It seems that the presence of the aliphatic chain increases lipophilicity which is mandatory for the increased activity. More specifically, the activity is strongly depended on the number of carbon atoms of the chain length. In general oxadiazole analogues with propyl (**5c**) and butyl (**5d**) chains exhibited the best MIC activity over most of the studied bacteria.

3.3.2. Antifungal activity

All the tested compounds exhibited much better antifungal activity than ketokonazol and bifonazole against all fungi species being 8 to 10-fold higher in cases of *A. niger, T. viride, A. flavus* and *F. fulvum*. Among all groups of tested compounds, triazole-3-thiones, in general, exhibited the best antifungal potency over all fungal species.

3.3.2.1. *Penicillium funiculosum* and *Penicillium ochrochloron*. Triazole-3-thiones analogues exhibited the best MIC activity against these species especially those with butyl (**3e**) and *tert* butyl (**3f**) substitutions. The thiadiazole analogue with butyl chain substitution (**4e**) also exhibited equipotent activity.

3.3.2.2. *Trichoderma viride*. The best MIC activity was exhibited by triazole-3-thione with isopropyl substitution (**3d**). In general, triazole-3-ones with longer chains (isopropyl, butyl, *tert* butyl) and oxadiazoles with methyl or ethyl substitutions showed remarkable inhibition.

3.3.2.3. *Aspergillus fumigates*. Triazole-3-thione with *tert* butyl substitution (**3f**) and triazole-3-one with isopropyl chain (**3i**) exhibited the best activity against this specie.

3.3.2.4. *Aspergillus niger*. The best MIC activity show triazole-3-thiones and thiadiazoles with propyl and butyl chain substitutions (**3c**, **3e**, **4c**, **4e**).

3.3.2.5. *Aspergillus flavus*. All triazole-3-thiones substituted with linear alkyl chains (methyl, ethyl, propyl, butyl) exhibited very good activity.

3.3.2.6. *Aspergillus versicolor*. Almost all triazole-3-thiones and triazole-3-ones showed very good activity. From the thiadiazole analogues, **4e** exhibited the best activity.

3.3.2.7. *Fulvia fyulvum*. Thiadiazoles and triazole-3-thiones exhibited the best activity against this specie.

3.4. Prediction of molecular properties

In order to proceed with prediction of physically significant descriptors and pharmaceutically relevant properties of the synthesized molecules, QikProp (Maestro) as a fast and accurate prediction software was used. The produced values can be used as descriptors for QSAR and in silico screening techniques. The following properties[34] were selected, calculated and presented in Table 8.

(a) Computed dipole moment of the molecule (Dipole); (b) total solvent accessible surface area (SASA) in square angstroms using a probe with a 1.4 Å radius, (c) hydrophobic component of the SASA (FOSA); (d) hydrophilic component of the SASA (FISA); (e) π component of the SASA (PISA); (f) weakly polar component of the SASA (halogens, P, and S) (WPSA); (g) total solvent-accessible volume in cubic angstroms using a probe with a 1.4 Å radius (volume); (h) estimated average number of hydrogen bonds (taken over a number of configurations) that would be donated by the solute to water molecules in an aqueous solution (HBd); (i) estimated average number of hydrogen bonds (taken over a number of configurations) that would be accepted by the solute from water molecules in an aqueous solution (HBa); (j) square of the dipole moment divided by the molecular volume (dip^2/V), a relevant parameter for

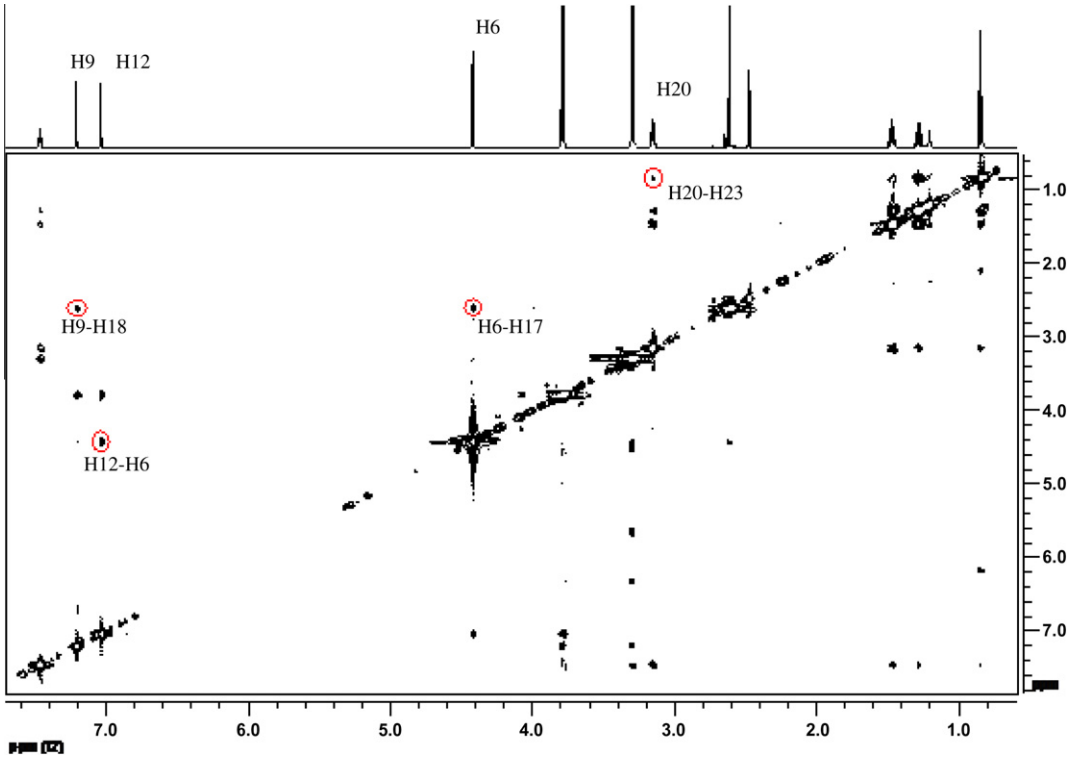
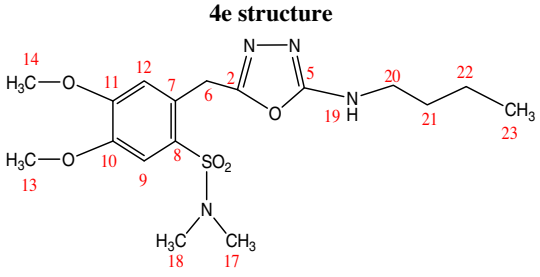


Figure 1. NOESY NMR spectrum for compound **4e** indicating the critical NOE signals.

Table 9
Critical interproton distances, calculated from ROE signals

Distance constrains ±10% (Å)		**4e** Structure
9–18	2.55–3.11	
9–13	2.03–2.49	
12–14	2.05–2.50	
12–6	2.04–2.49	
6–17	2.65–3.24	

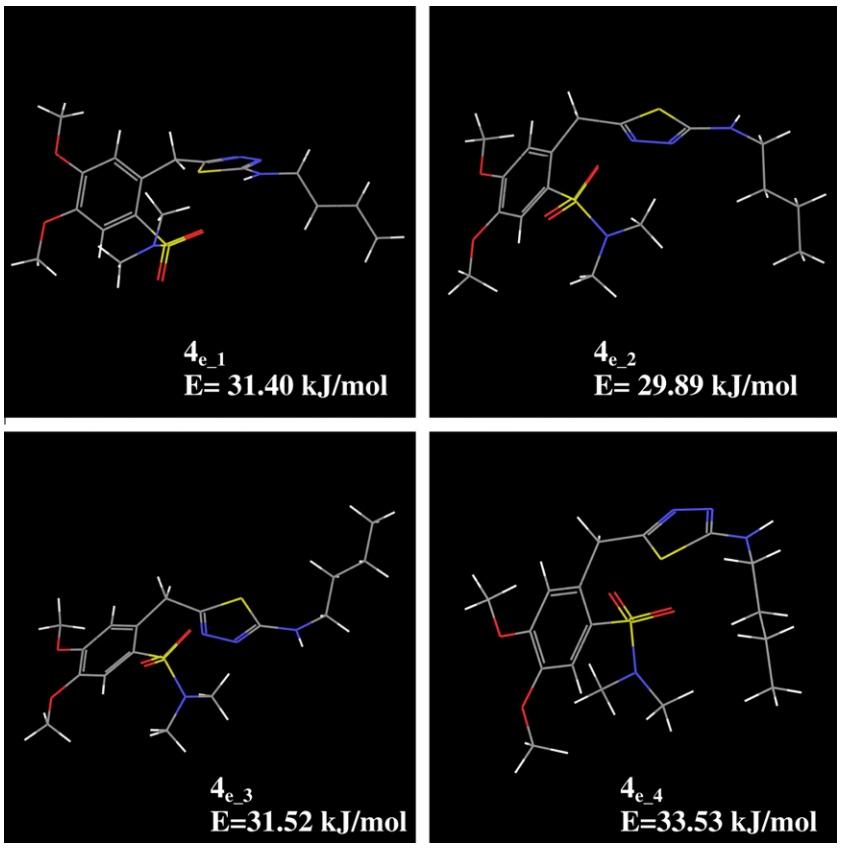


Figure 2. Representative low energy conformers of $\mathbf{4_e}$.

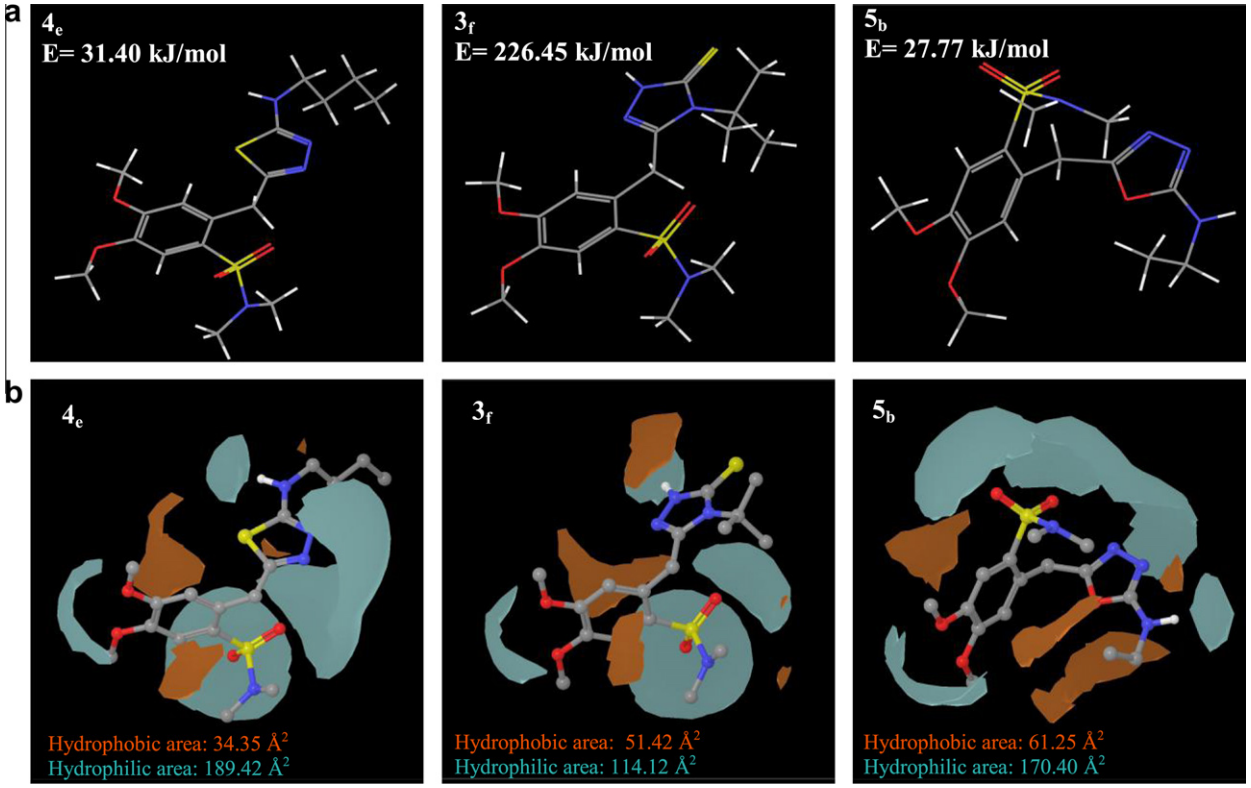


Figure 3. (a) Low energy conformers of compounds $\mathbf{4_e}$, $\mathbf{3_f}$, $\mathbf{5_b}$, consistent with NOE data; (b) hydrophobic (brown) and hydrophilic (blue) surface area distribution.

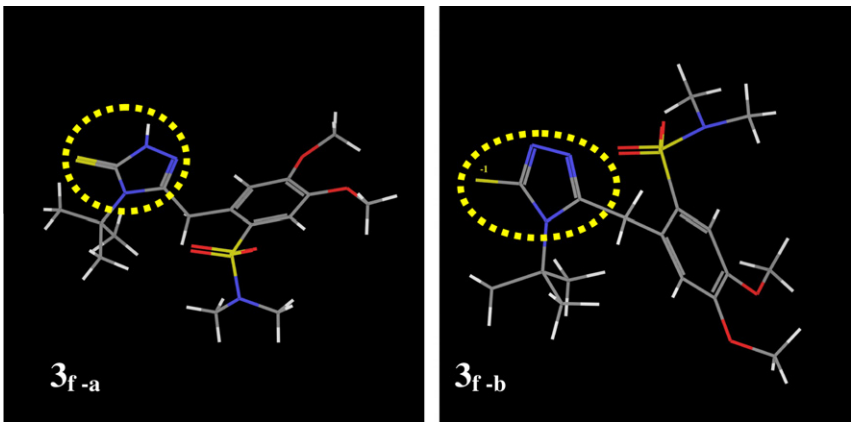


Figure 4. Tautomeric forms of compound $\mathbf{3_f}$ ($\mathbf{3_{f-a}}$ and $\mathbf{3_{f-b}}$) at pH 7 ± 2.

the energy of solvation of a dipole of volume V; (k) hexadecane/gas partition coefficient (QPlogPC16); (l) octanol/gas partition coefficient (QPlogPoct); (m) water/gas partition coefficient (QPlogPw); (n) octanol/water partition coefficient (QPlogPo/w); (o) aqueous solubility in mol dm^{-3} (QPlogS); (p) conformation-independent predicted aqueous solubility (CIQPlogS); (q) PM3 calculated ionization potential (IP(ev)); (r) PM3 calculated electron affinity (EA(eV)); (s) van der Waals surface area of polar nitrogen and oxygen atoms (PSA).

The empirical equation 3∗HBd − QPlogPo/w, was also calculated, which combines descriptors for both polarity and lipophilicity, and could serve as a predictor for compounds' bioavailability.[36] A value above 6 is correlated to very poor bioavailability. All of the tested compounds showed considerable lower values (Table 8).

The obtained results show that molecules which exhibited good antifungal activities against all tested species, are characterized by high dipole moments, increased number of hydrogen bond acceptors, high dip^2/V values leading to high energy of solvation, increased polar contributions to the total solvent accessible surface area, high water/gas partition coefficients, as well as increased electron affinity and low ionization potential values. Interestingly, the class of triazole-3-thiones is following the trend described above.

3.5. Conformational analysis

Conformational analysis was performed using NMR and molecular modeling techniques to one representative compound from each category, exhibiting different activities. From the triazoles, $\mathbf{3_f}$ was chosen among the best antifungal and antibacterial agents. Compound $\mathbf{4_e}$ from the thiadiazole group was selected showing good activity against tested bacteria and fungi but less compared to $\mathbf{3_f}$. Finally, for comparison reasons, $\mathbf{5_b}$ was selected from the oxadiazole group, because it exhibited lower activity relatively to the $\mathbf{3_f}$ and $\mathbf{4_e}$.

Here we demonstrate the conformational analysis of the thiadiazole $\mathbf{4_e}$. The solvent used in NMR analysis is DMSO-d_6 and not D_2O because of little solubility of tested compounds in aqueous media. Selection of the solvent though is not inadequate since drug's bioactive conformation is finally determined from the environment of the active site of the target protein.

Structure elucidation for each of the above mentioned compounds was performed following standard procedures using homonuclear gCOSY and NOESY spectra.

In Figure 1, we present the NOESY spectrum for the $\mathbf{4_e}$ analogue indicating the NOE correlations. Resonance peaks assignment is indicated on the 1D projection. The triplet peak at 0.85 ppm is attributed to H23. From COSY correlation of the neighbouring nuclei, peaks at 1.28, 1.47, 3.16 and 7.47 ppm are attributed to H22, H21, H20 and H19 correspondingly. The single peak at 4.43 ppm, integrated for two protons is attributed to H6. The observed NOE between H6 and the aromatic proton at 7.05 ppm clearly attributed this singlet peak to H12. Furthermore, NOE correlation of H12 with the single peak at 3.78 ppm, leads to the unequivocal assignment of those proton resonances to the methoxy protons H14. Thus, aromatic H9 and the second methoxy group H13 are assigned to 7.21 and 3.80 ppm correspondingly. Finally, the single peak at 2.62 ppm is integrated for 6 protons and is assigned for H17 and H18.

Critical interproton distances for the conformation of $\mathbf{4_e}$ in solution are calculated from NOE signal volumes and are presented in Table 9. Signals between H12 with H14 and H9 with H13 define the orientation of the methoxy groups towards the aromatic ring. Signal between H9 with H18 and H6 with H17 define the orientation of the dimethyl sulfamoyl group. Finally, signals between protons of the alkyl chain were not taken into consideration due its high mobility in solution.

In order to identify low energy conformations, consistent with the experimentally observed constrains, we performed molecular modeling studies for the $\mathbf{4_e}$. The 3D models of the studied molecules following their optimization were subjected conformational search after examining the existence of possible tautomers. The produced low energy conformations for each analogue were clustered according to their heavy atoms and the lowest energy members of the families were further investigated for their consistency with the NOE data.

Four representative favourable conformations of $\mathbf{4_e}$ namely $\mathbf{4_{e\_1}}$, $\mathbf{4_{e\_2}}$, $\mathbf{4_{e\_3}}$ and $\mathbf{4_{e\_4}}$, are displayed in Figure 2. $\mathbf{4_{e\_1}}$ and $\mathbf{4_{e\_3}}$ adopt a more extended conformation without forming any kind of clusters between functional groups as in the case of $\mathbf{4_{e\_2}}$ and $\mathbf{4_{e\_4}}$. From the presented conformers, $\mathbf{4_{e\_1}}$ is in accordance with the critical NOE data of Table 9.

The same procedure was followed for compounds $\mathbf{3_f}$ and $\mathbf{5_b}$. Low energy conformers, consistent with NOE data are shown in Figure 3a together with $\mathbf{4_{e\_1}}$. Compound $\mathbf{3_f}$ is found in two tautomeric forms ($\mathbf{3_{f-a}}$ and $\mathbf{3_{f-b}}$) at pH 7 ± 2 (Fig. 4) as calculated using Epik module of Schrodinger software. Nevertheless, the presence of a broad single peak at ~13 ppm in the NMR spectrum proves the existence of $\mathbf{3_f}$ in the 'a' form. Interestingly, the compound $\mathbf{3_f}$ exhibits much higher potential energy relative to $\mathbf{4_e}$ and $\mathbf{5_b}$, which is attributed to the increased bend energy of the triazole-3-thione ring due to the steric interactions of the *tert*-butyl group.

Proposed conformations consistent with NOE data were used to generate the hydrophobic (brown) and hydrophilic surfaces (blue)

Table 10
Predicted properties for $\mathbf{3_f}$ (including its two tautomers a and b), $\mathbf{4_e}$ and $\mathbf{5_b}$ analogues

Compd	Dipole	FOSA	SASA	FISA	PISA	WPSA	Volume	HBd	HBa	Dip^2/V	QPlogPC16	QPlogPoct	QPlogPw	QPlogPo/w	QPlogS	ClQPlogS	IP (eV)	EA (eV)
$\mathbf{3_{f\_a}}$	11.74	424.82	620.92	102.86	30.89	62.354	1181.45	1	9.5	0.1167	10.844	20.202	11.423	2.317	−3.269	−4.486	8.21	0.87
$\mathbf{3_{f\_b}}$	10.29	425.64	621.48	97.389	34.95	63.51	1181.6	0.8	8	0.0897	10.797	18.471	9.424	2.988	−3.61	−5.084	8.65	0.60
$\mathbf{4_e}$	6.896	529.03	709.05	113.48	36.54	29.995	1273.12	1	8.5	0.0374	11.58	18.764	10.088	3.065	−4.503	−4.89	8.69	0.72
$\mathbf{5_b}$	9.926	397.84	569.25	131.95	39.46	0	1073.34	1	9	0.0918	9.762	17.718	10.82	1.449	−2.168	−3.815	8.98	0.75

of the selected compounds (Fig. 3b). Results show that all molecules have an amphoteric character, important for antifungal activity, with higher hydrophilic and lower hydrophobic areas.

As a next step, we recalculated the molecular properties of compounds $\mathbf{3_f}$ (both tautomers), $\mathbf{4_e}$ and $\mathbf{5_b}$, this time using their low energy conformations satisfying the experimental NOE data. Differences are expected to occur compared to Table 8, only to properties which depend on the molecules' conformation. Results are presented in Table 10 and indicate that higher WPSA, Qplog-Poct and lower FISA, PISA and IP values may be related to higher antifungal activity to most of the tested species.

4. Conclusions

The synthesis of a series of 21 novel sulfonamide-1,2,4-triazoles, sulfonamide-1,3,4-thiadiazoles and sulfonamide-1,3,4-oxadiazoles is presented emphasizing, on the strategy of combining two chemically different but pharmacologically compatible molecules (the sulfomamide nucleus and the five member) heterocycles in one frame. Synthesized compounds were tested in vitro for antibacterial and antifungal activity. Results indicate that increase of the length of the aliphatic chain, increases lipophilicity which is mandatory for antibacterial activity. More specifically, oxadiazole analogues with propyl ($\mathbf{5_c}$) and butyl ($\mathbf{5_d}$) chains exhibited the best MIC activity over most of the studied bacteria. Although the title compounds did not exhibit significantly higher antifungal activity than previous synthesized by our group triazole and thiadiazole analogues, all of them exhibited much better antifungal activity than commercial ketokonazol and bifonazol. In some cases of fungi (i.e., *A. niger*, *T. viride*, *A. flavus* and *F. fulvum*) this activity was 8–10 times higher. More specifically, triazole-3-thiones exhibited the best activities over all fungal species. Prediction of the molecular properties of synthesized molecules showed that triazole-3-thiones share by high dipole moment, increased number of hydrogen bond acceptors, high energy of solvation, increased polar contributions to the total solvent accessible surface area, high water/gas partition coefficients, as well as increased electron affinity and low ionization potential values.

In general, larger alkyl groups (butyl, *tert*-butyl, isopropyl) gave better results against fungi. Nevertheless, main differences in the antifungal activity seem to depend more on the triazol-3-thione core rather than the different length of the alkyl chain substitutions.

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