Sulfonamide-1,2,4-thiadiazole Derivatives as Antifungal and Antibacterial Agents: Synthesis, Biological Evaluation, Lipophilicity, and Conformational Studies

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A series of thirteen new thiadiazole compounds were synthesized and evaluated for *in vitro* antifungal and antibacterial activity. All compound 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 was shown for the synthetic analogue with methylpiperazine reactive group. Furthermore, it is apparent that different compounds reacted on different ways against bacteria. An effort was made to correlate the above mentioned differences in activity with lipophilicity studies. Furthermore, NMR and molecular modelling were used to obtain the main conformational features of a potent analogue, for future *in silico* studies.

Key words thiadiazole; antifungal; antibacterial; bifonazole; ketoconazole

Bacterial infections have increased dramatically in recent years. Bacteria have been the cause of some of the most deadly diseases and widespread epidemics in human civilization.[1)] Moreover, the widespread use and misuse of antibiotics has caused bacterial resistance. Some of these resistant strains, such as vancomycin-resistant enterococci (VRE) and multidrug resistant *Staphylococcus aureus* (MRSA), are capable of surviving the effects of most, if not all, antibiotics currently in use.[2—6)] With the increase in resistance of bacteria to antibiotic treatment, attention was given on developing novel approaches to antimicrobial therapy.[7—13)]

We have previously reported the significant antifungal activity of a series of sulfonamide-1,2,4-triazole derivatives against a series of micromycetes, compared to the commercial fungicide bifonazole. These compounds have also shown a comparable bactericidal effect to that of streptomycin but better activity than chlorampenicol respectively against various bacteria.[14)]

Thiadiazoles belong to the wider category of imidazole and triazole synthetic antifungal drugs which are designed to inhibit the enzyme cytochrome P450 14α-demethylase and inhibit the conversion of lanosterol to ergosterol, which is required in fungal cell membrane synthesis.

1,3,4-Thiadiazoles are known to possess antibacterial and antifungal properties similar to those of well known sulphonamide drugs.[15)] Thus, the 1,3,4-thiadiazoles exhibit a broad spectrum of biological activities possibly due to the presence of the toxophoric –N–C–S moiety.[16)] Prompted by these observations and in continuation of our search for bioactive molecules, we designed the synthesis of a series of novel sulfonamide-1,3,4-thiadiazoles, emphasizing, in particular, on the strategy of combining two chemically different but pharmacologically compatible molecules (the sulfonamide nucleus and the five member heterocycle) in one frame, in order to study their antibacterial and antifungal activities.

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

Starting from ethyl(2-chlorosulfonyl-4,5-dimethoxyphenyl)acetate (**1**)[17,18)] by reaction with secondary aliphatic amines in anhydrous benzene the corresponding sulfonamides (**2a**—**d**) were obtained. The latter were converted to the desired 2-(*N*-substituted sulfamoyl)-4,5-dimethoxyphenylacetylhydrazides (**3a**—**d**) by treatment with hydrazine hydrate in xylol. The hitherto unknown 1-[2-(*N*-substituted sulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-aryl-thiosemicarbazides (**4a**—**m**) were obtained upon the reaction of acid hydrazides (**3a**—**d**) with suitable aryl isothiocyanates.[4)] Cyclization of **4a**—**m** with concentrated sulfuric acid in cold resulted to the formation of *N*-{5-[2-(*N*-substituted sulfamoyl)-4,5-dimethoxy benzyl]-1,3,4-thiadiazole-2-yl}-*N*-arylamines (**5a**—**m**) respectively.

Results and Discussion

Biological Evaluation and Lipophilicity Studies The results of antibacterial and antifungal activity of compounds **5a**—**m** against a panel of selected Gram positive, Gram negative bacteria and fungi are presented in Tables 1 and 2 in comparison with those of the reference drugs ampicillin and streptomycin, bifonazole and ketoconazole respectively.

Results of antibacterial activity of compounds (Table 1) show that the minimum inhibitory concentration (MIC) of compounds varies in the range of 0.92—4.6×10^{-2} μmol/ml, while minimum bactericidal concentration (MBC) varies between 1.75—9.20×10^{-2} μmol/ml. Compounds **5a**, **5b**, **5c** and **5g** showed the lowest antibacterial activity among the tested compounds with **5a** being the worst, with MIC of 1.15—4.60×10^{-2} μmol/ml and MBC of 1.75—9.20×10^{-2} μmol/ml. Moderate activity was observed for compounds

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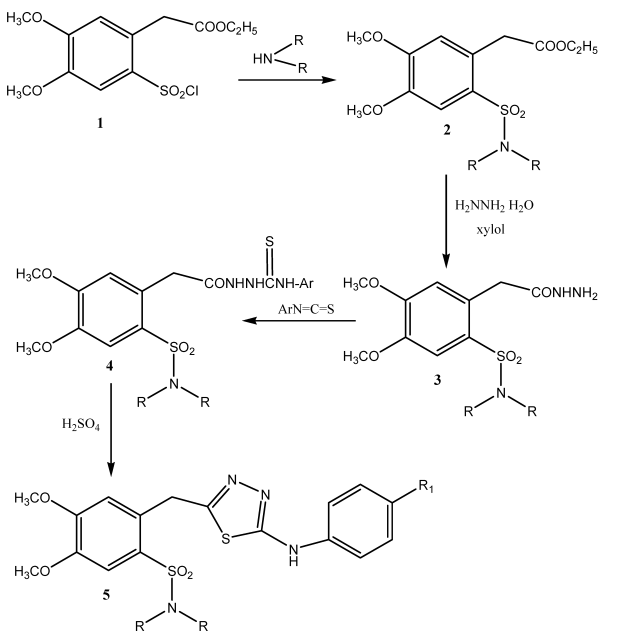


R_1 /	-H	-Cl	$-NO_2$	$-CF_3$
—N(CH3)2	**a**	**b**	**c**	**d**
—N(pyrrolidine)	**e**	**f**	**g**	**h**
—N(piperidine)		**i**	**j**	**k**
—N(N-CH3-piperazine)		**l**		**m**

Chart 1. Synthetic Pathway for the Preparation of the Thiadiazole Derivatives

5d—**f**, **5i**, **5j** and **5l**. Compounds **5h**, **5k** and **5m** showed the higher antibacterial activity with **5h** ranked as the best among them with MIC varying in the interval 0.95—1.89×10^{-2} μmol/ml and bactericidal effect between 1.89—3.78×10^{-2} μmol/ml. It should be noted that less active compounds have lower ClogP values which indicates that the increase in activity goes in parallel with their lipophilicity expressed as the calculated ClogP values.

The most sensitive bacterial species on these compounds is *Bacillus cereus*, while *Listeria monocytogenes* is the most resistant species. It can be seen that all the compounds tested for antibacterial activity showed much better effect than commercial antibiotics, streptomycin and ampicillin.

In general, compounds tested are more active against Gram positive bacteria than Gram negative while compound **5k** exhibited the best activity against Gram positive bacteria (*B. cereus*, *Micrococcus flavus*) with MIC 0.92—1.84×10^{-2} μmol/ml and the same good activity for Gram negative bacteria such as *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhimurium* (ATCC 13311). Almost the same behaviour was observed for compound **5h** which exhibited the best activity against Gram positive bacteria (*Bacillus cereus* and *Staphylococcus aureus* (ATCC 6538) as well as against Gram negative bacteria *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhimurium* (ATCC 13311).

As regards the relationships between the structure and the detected antibacterial activity, it was observed that pyrrolidine derivatives are mostly endowed with higher activity with respect to piperidine, methylpiperazine and dimethylamino derivatives. Moreover the inhibitory effect appears to be dependent on the substitution at the benzene ring. Thus the introduction of CF_3 substituent as well as a chloro atom in the *para*-position of the benzene ring respectively, improved antibacterial activity in respect to compound **5a**.

Results of antifungal activity of the tested compounds are

Table 1. Antibacterial Activity of Compounds Tested by Microdilution Method (MIC and MBC in μmol$\times10^{-2}$)

Bacteria	**5a** mic / mbc	**5b** mic / mbc	**5c** mic / mbc	**5d** mic / mbc	**5e** mic / mbc	**5f** mic / mbc	**5g** mic / mbc	**5h** mic / mbc	**5i** mic / mbc	**5j** mic / mbc	**5k** mic / mbc	**5l** mic / mbc	**5m** mic / mbc	Str mic / mbc	Amp mic / mbc
Bacillus cereus	2.30 4.60	1.07 2.13	1.05 2.09	0.99 1.99	1.09 2.17	2.02 4.04	1.98 3.96	0.95 1.89	0.98 1.97	1.93 3.86	0.92 1.84	1.91 3.82	1.79 1.79	4.3 8.6	24.8 37.2
Micrococcus flavus	1.15 1.75	2.13 4.26	2.09 8.36	0.99 1.99	1.09 2.17	2.02 4.04	1.98 3.96	1.89 3.78	1.97 3.94	1.93 3.86	0.92 1.84	0.96 1.91	1.79 3.58	8.6 17.2	24.8 37.2
Staphylococcus aureus	4.60 9.20	2.13 4.26	4.18 8.36	1.99 3.98	2.17 4.34	2.02 4.04	1.98 3.96	0.95 1.89	1.97 3.94	3.86 7.72	1.84 3.68	3.82 7.64	1.79 3.58	17.2 34.4	24.8 37.2
Escherichia coli	4.60 4.60	4.26 8.52	2.09 8.36	3.98 7.96	2.17 4.34	1.52 4.04	1.98 3.96	1.89 3.78	1.97 3.94	1.93 3.86	1.84 3.68	1.91 7.64	1.79 1.79	17.2 34.4	37.2 49.2
Pseudomonas aeruginosa	4.60 4.60	4.26 8.52	1.57 4.18	1.99 3.98	2.17 4.34	2.02 4.04	1.98 3.96	0.95 1.89	0.98 1.97	0.97 1.93	0.92 1.84	1.91 7.64	1.79 3.58	17.2 34.4	74.4 124.0
Proteus mirabilis	4.60 4.60	2.13 4.26	4.18 8.36	1.99 7.96	2.17 4.34	2.02 4.04	3.96 7.92	1.89 3.78	1.97 7.87	3.86 7.72	3.68 7.36	3.82 7.64	1.79 3.58	17.2 34.4	37.2 49.2
Salmonella typhimurium	4.60 9.20	2.13 4.26	1.05 2.09	0.99 1.99	1.09 2.17	1.01 2.02	3.96 7.92	0.95 1.89	0.98 1.97	0.97 1.93	0.92 1.84	0.96 3.82	1.79 3.58	17.2 34.4	24.8 49.2
Listeria monocytogenes	4.60 9.20	8.52 8.52	4.18 8.36	3.98 7.96	2.17 4.34	4.04 4.04	1.98 3.96	1.89 1.89	3.94 7.88	1.93 7.72	1.84 7.36	1.91 7.64	1.79 3.58	25.8 51.6	37.2 74.4
ClogP	3.34	4.08	3.15	4.27	3.98	4.71	3.78	4.91	5.27	4.34	5.47	4.46	4.66		

Table 2. Antifungal Activity of Compounds Tested by Microdilution Method (MIC and MFC in μmol$\times10^{-2}$)

Fungi	**5a** mic mfc	**5b** mic mfc	**5c** mic mfc	**5d** mic mfc	**5e** mic mfc	**5f** mic mfc	**5g** mic mfc	**5h** mic mfc	**5i** mic mfc	**5j** mic mfc	**5k** mic mfc	**5l** mic mfc	**5m** mic mfc	Bif mic mfc	Ket mic mfc
Penicillium funiculosum	1.15 2.30	1.07 2.13	1.05 2.09	0.99 1.99	2.17 4.34	1.01 2.02	0.99 1.98	1.89 3.78	1.97 3.94	1.93 3.86	0.92 1.84	1.91 3.82	0.89 1.79	64.0 80.0	38.0 95.0
Penicillium ochrochloron	2.30 4.60	2.13 4.26	1.09 4.18	0.99 3.98	2.17 4.34	2.02 4.04	1.98 3.96	1.89 3.78	1.97 3.94	1.93 3.86	1.84 3.68	1.91 3.82	1.79 3.58	48.0 64.0	380.0 380.0
Trichoderma viride	1.15 2.30	1.07 2.13	1.05 2.09	0.99 1.99	1.09 2.17	1.01 2.02	0.99 1.98	0.95 1.89	0.98 1.97	0.97 1.93	0.92 1.84	0.96 1.91	0.89 1.79	64.0 80.0	475.0 570.0
Aspergillus fumigatus	1.15 4.60	1.07 4.26	2.09 4.18	1.99 3.98	2.17 4.34	2.02 4.04	0.99 1.98	1.89 3.78	1.97 3.94	1.93 3.86	0.92 3.68	0.96 3.82	0.89 1.79	48.0 64.0	38.0 95.0
Aspergillus niger	2.30 4.60	1.07 4.26	2.09 4.18	1.99 3.98	2.17 4.34	2.02 4.04	1.98 3.96	1.89 3.78	1.97 3.94	1.93 3.86	1.84 3.68	1.91 3.82	1.79 3.58	48.0 64.0	38.0 95.0
Aspergillus flavus	2.30 4.60	1.07 4.26	2.09 4.18	1.99 3.98	2.17 4.34	2.02 4.04	1.98 3.96	1.89 3.78	0.98 1.97	1.93 3.86	1.84 3.68	1.91 3.82	1.79 3.58	48.0 64.0	285.0 380.0
Aspergillus versicolor	1.15 2.30	1.07 4.26	2.09 4.18	0.99 3.98	2.17 4.34	2.02 4.04	1.98 3.96	1.89 3.78	0.98 1.97	1.93 3.86	0.92 1.84	3.82 3.82	1.79 3.58	32.0 64.0	38.0 95.0
Fulvia fulvum	1.15 2.30	2.13 8.52	1.05 2.09	0.99 1.99	1.09 2.17	1.01 2.02	0.99 1.98	1.89 7.56	0.98 1.97	0.97 1.93	0.92 1.84	0.96 1.91	0.89 1.79	32.0 64.0	38.0 95.0

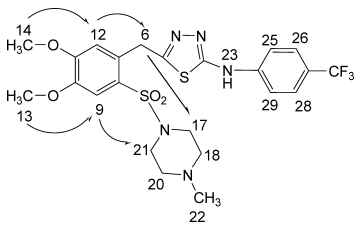
presented in Table 2. As in the case of the antibacterial activity, compounds showed strong antifungal potential. MIC varies between 0.89—2.30$\times10^{-2}$ μmol/ml and minimal fungicidal concentration (MFC) in the interval 1.79—8.52$\times10^{-2}$ μmol/ml. The lowest antifungal activity was observed for compounds **5e**, **5f**, **5h**, **5j** and **5l**. Among them, compound **5e** possessed the lowest antifungal potential, by inhibiting fungal growth at 1.09—2.17$\times10^{-2}$ μmol/ml and showing fungicidal effect at 2.17—4.34$\times10^{-2}$ μmol/ml. Compounds **5a**—**d**, **5g** and **5i** were ranked in the middle of the activities score, according to their antifungal potential. Compounds **5k** and **5m**, showed the best activity against all the fungi, with 5m exhibiting the highest antifungal potential (MIC 0.89—1.79$\times10^{-2}$ μmol/ml and MFC 1.79—3.58$\times10^{-2}$ μmol/ml). The majority of compounds showed the best activity against *Trichoderma viride* while *Fulvia fulvum* is the most resistant species. Fungi were in general more sensitive than bacterial species.

In conclusion, according to obtained results, compound **5h** showed the best antibacterial activity while compounds **5k** and **5m** showed the best antifungal activity.

Conformational Analysis Studies As a first step for future *in silico* docking and pharmacophore alignment studies, we performed a conformational study using NMR and molecular modelling. Here we demonstrate the conformational properties of the most active compounds **5m**, **5h** and of the lowest activity compound **5a**. 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 double quantum filtered correlation spectroscopy (DQF-COSY) and rotating frame Overhauser enhancement spectroscopy (ROESY) spectra. It was observed that all the tested compounds had common ROEs which reflect similar conformational properties (Table 3). This result indicates that the conformation of the molecules' scaffold is not influenced by the performed substitutions.

Table 3. Observed ROE Constraints, Common in All Studied Compounds

Distance constraints	**5m** structure
12–14 9–13 9–17/21 6–17/21 6–12	

In Fig. 1, we present the ROESY spectrum for the **5m** analogue indicating the critical ROE correlations. Resonance peaks assignment is indicated on the 1D projection. The observed ROE between H6 and the aromatic proton at 7.15 ppm clearly attributed this singlet peak to H12. Furthermore, the aromatic protons H9 and H12 showed ROE correlations with the methoxy protons H13 and H14 respectively leading to the unequivocal assignment of those proton resonances. ROE between H23 and the doublet at 7.73 ppm (not shown) led to the assignment of the more deshielded doublet to the H25/H29 while the peak attributed to H26/28 was confirmed by COSY correlation with the neighbouring nuclei. The observed ROE signals between both H6 and H9 with H17, H21 are indicative of the spatial proximity between the methylene group and the dimethoxy benzyl ring with the methylpiperazine moiety. Interestingly, no ROE signal was observed between the benzene protons (H25—29) and the rest of the

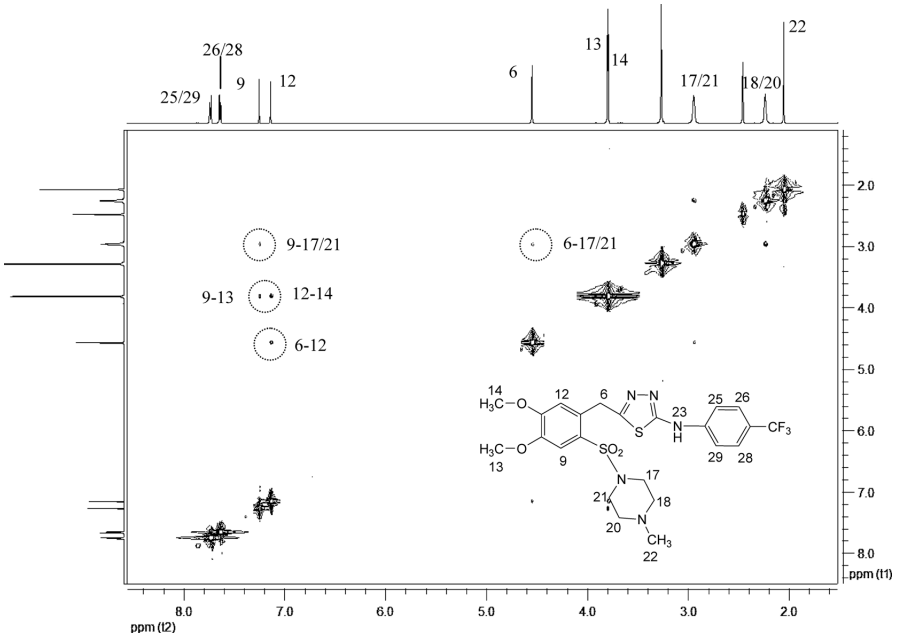


Fig. 1. ROESY Spectrum of **5m** Acquired at 600 MHz
Distance constraints are indicated in red circles.

molecule and this was valid for all of the tested compounds.

In order to identify low energy conformations consistent with the observed ROE constrains, we performed molecular modelling studies for the **5a**, **5h** and **5m**. The most important conformational features for all the analogues are related to the dihedral angles (τ_1—τ_8) which are presented in Fig. 2. The 3D models of the studied molecules following their optimization were subjected to Monte Carlo conformational search. The produced low energy conformations for each analogue were clustered according to the dihedral angles values and the lowest energy members of the families were further investigated for their consistency with the ROE data. Here we present the results for the **5m** analogue stating that similar configurations were observed for the rest of the compounds. Three representative favourable conformations of **5m** namely **5m_a**, **5m_b** and **5m_c** are displayed in Fig. 3. Conformer **5m_a** forms a cluster between the thiadiazole system and methylpiperazine group while **5m_b** adopts an "open" conformation, differing in the orientation of methylpiperazine which is now closer to the aromatic ring. Both **5m_a** and **5m_b** conformers support the experimentally observed proximity of the methylpiperazine (H17, H21) with the methylene group H6 and the H9 proton of the aromatic ring. On the other hand, **5m_c** conformer brings in spatial proximity the methylpiperazine group and the benzene ring which is inconsistent with the ROE data. Moreover, the absence of any ROE signal between the benzene ring (H25—29) and the rest of the molecule features indicates its distal positioning with its conformational flexibility depending on τ_7 and τ_8 dihedrals. Finally, the thiadiazole system may adopt different orientations bringing the sulphur atom or the diazole moiety in geometrically opposite directions.

In order to further examine the conformational relationship between the thiadiazole and the benzene rings, we performed systematic search through τ_7 and τ_8 dihedrals. The isoenergetic map (Fig. 4) confirmed a coplanar position of

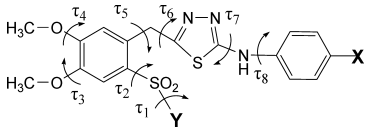


Fig. 2. Critical Torsion Angles of the Thiadiazole Analogues for Their Conformational Properties
X and Y indicate the substitution groups for all compounds.

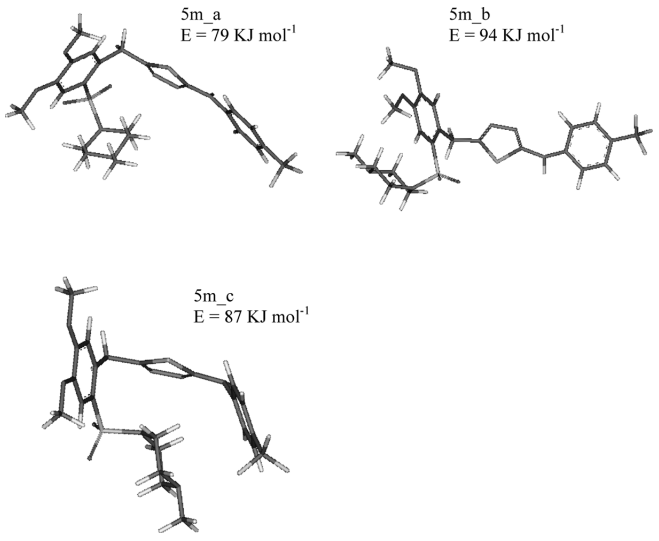


Fig. 3. Representative Low Energy Conformers of **5m**

the H23 with the thiadiazole plane (τ_7=0° or 180°). Furthermore, H23 seems to be preferably directed towards the sulphur instead of the nitrogen atom.

Concerning the benzene ring flexibility, no specific limitations are observed since τ_8 dihedral energetically favours several values around ±35°, ±145°.

Systematic search was also performed for τ_3 and τ_4 dihe-

dral angles to examine the relative orientation of the methoxy groups. Results showed that energetically low conformations are achieved when adopting opposite directions, $\tau_3 = 0°$ and $\tau_4 = 180°$ and *vice versa* with a margin of $\pm 60°$. All these orientations are accepted as they are in accordance with ROE data.

As already stated, detailed conformational search of the studied compounds, revealed similar conformational features for **5m** (most active antifungal and good antibacterial), **5h** (most active antibacterial and least active antifungal) and **5a** (least active antibacterial). In an effort to explain the different activity profile, we calculated the lipophilicity maps for the derived favorable conformations of the three compounds (Fig. 5). All molecules showed distinct lipophilic and hydrophilic areas giving a more amphiphilic character. This characteristic has been proved crucial for lateral diffusion through lipid bilayers.[19,20] Interestingly, **5a** has significantly lower lipophilic area (*ca.* 20 Å^2) compared to **5h** (*ca.* 66 Å^2) which may partially explain their difference in antibacterial activity.

These results will be used for further *in silico* studies, providing information on their pharmacophore models. It has to be noticed that fruitful discussion of quantitative structure–activity relationship (QSAR) is difficult, based on such narrow-range activity data.

Conclusion

Prompted by the well established antibacterial and antifungal properties of 1,3,4-thiadiazoles, similar to those of well known sulphonamide drugs, we presented a series of novel sulfonamide-1,3,4-thiadiazoles emphasizing, in particular, on the strategy of combining two chemically different but pharmacologically compatible molecules (the sulfonamide nucleus and the five member heterocycle) in one frame. All the compounds showed very strong antibacterial and antifungal activity against all the species tested. Compounds **5h**, **5k** and **5m** showed better antibacterial potential with **5h** ranking as the more active while compounds **5k** and **5m**, showed better activity against all the fungi, with **5m** showing the highest antifungal potential.

As regards the relationships between the structure and the detected antibacterial activity, it was observed that in general pyrrolidine derivatives are mostly endowed with higher activity with respect to piperidine, methylpiperazine and dimethylamino derivatives. Moreover the inhibitory effect appears to be dependent on the substitution at the benzene ring. Thus the introduction of CF_3 substituent as well as a chloro atom in the *para*-position of the benzene ring respectively, improved antibacterial activity in respect to compound **5a**.

Conformational studies of more and less potent analogues with NMR and molecular modelling techniques provided data for further pharmacophore generation and *in silico* docking studies on CYP450. All studied analogues provided similar low energy conformations consistent with ROE experimental data which indicates that the conformation of the molecules' scaffold is not influenced by the performed substitutions. The lipoplilic surface of the resulting conformers show an amphoteric character, important for the interaction of the molecules with membrane bilayers while supports that greater lipophilic surface is related to antibacterial activity increase.

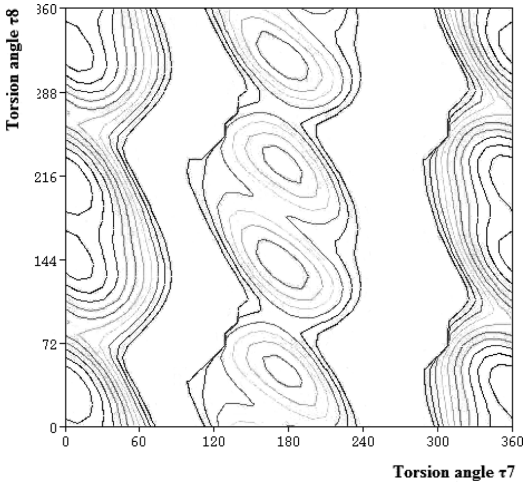


Fig. 4. Isoenergetic Map from Simultaneous Grid Scan of τ_7 and τ_8 Dihedrals

Energy minimum values are achieved when τ_7 is at 0, 180, 360°. This indicates a coplanar orientation of NH and thiadiazole group.

Experimental

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 Varian 300 MHz spectrometer using deuterated dimethylsulfoxide (DMSO-d_6) and are reported in δ (ppm) units. Thin layer chromatography (TLC) was performed in E. Merck precoated silica gel plates. 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 ($\pm 0.4\%$ of the calculated values).

General Procedure for the Preparation of the Ethyl-[2-(*N*-substituted sulfamoyl)-4,5-dimethoxy-phenyl]acetate (2) To a flask containing 0.01 mol of ethyl-(2-chloro-sulfonyl-4,5-dimethoxy-phenyl) acetate (**1**) 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 sulfon-

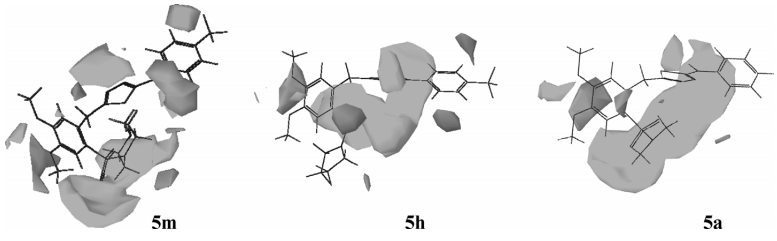


Fig. 5. Selected Low Energy Conformers of **5m**, **5h**, and **5a** in Accordance with ROE Experimental Data and Their Lipophilicity Maps Showing Amphiphilic Character for the Selected Molecules

Light gray represents hydrophilic and dark gray represents hydrophobic areas.

amides. The title compounds prepared are reported below.

Ethyl-[2-(*N*,*N*-dimethyl sulfamoyl)-4,5-dimethoxy-phenyl]acetate (**2a**): mp 108—110 °C (methanol) (ref. 14; 109—110 °C).

Ethyl-[2-(1-pyrrolidine sulfamoyl)-4,5-dimethoxy-phenyl]acetate (**2b**): mp 107—108 °C (ethylacetate) (ref. 14; 107—108 °C).

Ethyl-[2-(1-piperidine sulfamoyl)-4,5-dimethoxy-phenyl]acetate (**2c**): mp 100—101 °C (methanol) (ref. 17; 100—101 °C).

Ethyl-[2-(4-methyl-piperazine sulfamoyl)-4,5-dimethoxy-phenyl]acetate (**2d**): Yield: 64%. mp 119—120 °C (ethylacetate). IR cm^{-1}: 1734 (COO), 1329 (S–$O_{antisym}$), 1142 (S–O_{sym}). *Anal.* Calcd for $C_{17}H_{26}N_2O_6S$: C, 52.85; H, 6.73; N,7.25. Found: C, 52.91; H, 6.68; N, 7.31.

General Procedure for the Preparation of the 2-(*N*-Substituted sulfamoyl)-4,5-dimethoxy-phenylacetylhydrazides (3) 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 hydrate. 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.

2-(*N*,*N*-Dimethyl sulfamoyl)-4,5-dimethoxy-phenylacetylhydrazide (**3a**): mp 147—149 °C (ethanol) (ref. 14; 147—148 °C).

2-(1-Pyrrolidine sulfamoyl)-4,5-dimethoxy-phenylacetylhydrazide (**3b**): mp 139—141 °C (ethanol) (ref. 14; 140—141 °C).

2-(1-Piperidine sulfamoyl)-4,5-dimethoxy-phenylacetylhydrazide (**3c**): mp 134—136 °C (ethanol) (ref. 14; 136—137 °C).

2-(4-Methyl-piperazine sulfamoyl)-4,5-dimethoxy-phenylacetylhydrazide (**3d**): Yield: 90%, mp 127—129 °C (powder ethanol–*n*-hexane), IR cm^{-1}: 3275, 3090 (NH, NH_2), 1672 (CONH), 1320 (S–$O_{antisym}$), 1135 (S–O_{sym}). *Anal.* Calcd for $C_{15}H_{24}N_4O_5S$: C, 48.38; H, 6.45; N,15.05. Found: C, 48.44; H, 6.50; N, 15.09.

General Procedrure for the Preparation of the 1-[2-(*N*-Substituted sulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-aryl-thiosemicarbazides (4) Equimolar quantities of hydrazide (1 mmol) and aryl isothiocyanate (1 mmol) in 3 ml of absolute ethanol were refluxed on a stream bath for 1 h. The resulting solid was filtered and recrystallized from the appropriate solvent.

The following compounds were prepared by an analogous procedure.

1-[2-(*N*,*N*-Dimethyl sulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-phenyl-thiosemicarbazide (**4a**): mp 218—220 °C (methanol–dichloromethane) (ref. 14; 219—221 °C).

1-[2-(*N*,*N*-Dimethyl sulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-(*p*-chlorophenyl)-thiosemicarbazide (**4b**): mp 197—198 °C (methanol–dichloromethane) (ref. 14; 196—198 °C).

1-[2-(*N*,*N*-Dimethyl sulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-(*p*-nitrophenyl)-thiosemicarbazide (**4c**): Yield: 76%. mp 207—208 °C (methanol–chloroform). IR cm^{-1}: 3350, 3312, 3177 (3NH), 1685 (CONH), 1516 (C=S), 1338 (S–$O_{antisym}$), 1142 (S–O_{sym}). ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.66 (s, 6H, $N(CH_3)_2$, 3.80 (s, 3H, CH_3O), 3.82 (s, 3H, CH_3O), 3.91 (s, 2H, CH_2), 7.09 (s, 1H, ArH), 7.20 (s, 1H, ArH), 7.92 (d, 2H, ArH, J=8.75 Hz), 8.20 (d, 2H, ArH, J=8.95 Hz), 9.61 (s, 1H, NH), 10.15 (s, 1H, NH), 10.22 (s, 1H, NH). *Anal.* Calcd for $C_{19}H_{23}N_5O_7S_2$: C, 45.87; H, 4.62; N, 14.08. Found: C, 45.83; H, 4.67; N, 14.01.

1-[2-(*N*,*N*-Dimethyl sulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-(*p*-trifluoromethylphenyl)-thiosemicarbazide (**4d**): Yield: 69%. mp 196—197 °C (methanol). IR cm^{-1}: 3377, 3329, 3188 (3NH), 1684 (CONH), 1525 (C=S), 1323 (S–$O_{antisym}$), 1126 (S–O_{sym}). ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.66 (s, 6H, $N(CH_3)_2$), 3.80 (s, 3H, CH_3O), 3.82 (s, 3H, CH_3O), 3.90 (s, 2H, CH_2), 7.09 (s, 1H, C_6), 7.20 (s, 1H, C_3), 7.68 (d, 2H, ArH, J=8.4 Hz), 7.79 (d, 2H, ArH, J=8.7 Hz), 9.47 (bs, 1H, NH), 9.98 (s, 1H, NH), 10.18 (s, 1H, NH). *Anal.* Calcd for $C_{20}H_{23}F_3N_4O_5S_2$: C, 46.15; H, 4.42; N, 10.77. Found: C, 46.09; H, 4.47; N, 10.81.

1-[2-(1-Pyrrolidinesulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-phenyl-thiosemicarbazide (**4e**): mp 188—190 °C (ethanol–dichloromethane) (ref. 14; 189—191 °C).

1-[2-(1-Pyrrolidinesulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-(*p*-chlorophenyl)-thiosemicarbazide (**4f**): mp 212—213 °C (ethanol) (ref. 14; 212—214 °C).

1-[2-(1-Pyrrolidinesulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-(*p*-nitrophenyl)-thiosemicarbazide (**4g**): Yield: 74%. mp 200—201 °C (methanol). IR cm^{-1}: 3333, 3196, 3090 (3NH), 1670 (CONH), 1514 (C=S), 1325 (S–$O_{antisym}$), 1145 (S–O_{sym}). ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.72—1.77 (m, 4H, pyrrolidine), 3.13—3.18 (m, 4H, pyrrolidine), 3.79 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 3.93 (s, 2H, CH_2), 7.08 (s, 1H, ArH), 7.24 (s, 1H, ArH), 7.92 (d, 2H, ArH, J=8.7 Hz), 8.20 (d, 2H, ArH, J=9 Hz), 9.60 (s, 1H, NH), 10.16 (s, 1H, NH), 10.23 (s, 1H, NH). *Anal.* Calcd for $C_{21}H_{25}N_5O_7S_2$: C, 48.18; H, 4.78; N, 13.38. Found: C, 48.26; H, 4.68; N, 13.25.

1-[2-(1-Pyrrolidinesulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-(*p*-trifluoromethyl-phenyl)-thiosemicarbazide (**4h**): Yield: 78%. mp 199—200 °C (methanol). IR cm^{-1}: 3396, 3327, 3219 (3NH), 1684 (CONH), 1518 (C=S), 1325 (S–$O_{antisym}$), 1124 (S–O_{sym}). ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.72—1.76 (m, 4H, pyrrolidine), 3.13—3.17 (m, 4H, pyrrolidine), 3.79 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 3.92 (s, 2H, CH_2), 7.08 (s, 1H, ArH), 7.24 (s, 1H, ArH), 7.69 (d, 2H, ArH, J=8.7 Hz), 7.80 (d, 2H, ArH, J=8.4 Hz), 9.46 (bs, 1H, NH), 9.98 (s, 1H, NH), 10.20 (s, 1H, NH). *Anal.* Calcd for $C_{22}H_{25}F_3N_4O_5S_2$: C, 48.35; H, 4.57; N, 10.25. Found: C, 48.22; H, 4.66; N, 10.33.

1-[2-(1-Piperidinesulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-(*p*-chlorophenyl)-thiosemicarbazide (**4i**): mp 211—213 °C (methanol–dichloromethane) (ref. 14; 212—213 °C).

1-[2-(1-Piperidinesulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-(*p*-nitrophenyl)-thiosemicarbazide (**4j**): Yield: 56%. mp 197—198 °C (methanol). IR cm^{-1}: 3333, 3298, 3086 (3NH), 1670 (CONH), 1514 (C=S), 1332 (S–$O_{antisym}$), 1145 (S–O_{sym}). ^{1}H-NMR (DMSO-d_6) δ (ppm): −1.40—1.49 (m, 6H, piperidine), 2.99 (s, 4H, piperidine), 3.80 (s, 6H, $2CH_3O$), 3.90 (s, 2H, CH_2), 7.08 (s, 1H, ArH), 7.20 (s, 1H, ArH), 7.93 (d, 2H, ArH, J=6.97 Hz), 8.20 (d, 2H, ArH, J=7.92 Hz), 9.58 (s, 1H, NH), 10.17 (s, 1H, NH), 10.23 (s, 1H, NH). *Anal.* Calcd for $C_{22}H_{27}N_5O_7S_2$: C, 49.16; H, 5.02; N, 13.03. Found: C, 49.23; H, 5.15; N, 12.97.

1-[2-(1-Piperidinesulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-(*p*-trifluorophenyl)-thiosemicarbazide (**4k**): Yield: 60%. mp 202—203 °C (methanol). IR cm^{-1}: 3306, 3078 (3NH), 1687 (CONH), 1514 (C=S), 1325 (S–$O_{antisym}$), 1145 (S–O_{sym}). ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.40—1.49 (m, 6H, piperidine), 2.99 (s, 4H, piperidine), 3.79 (s, 3H, CH_3O), 3.80 (s, 3H, CH_3O), 3.88 (s, 2H, CH_2), 7.08 (s, 1H, ArH), 7.19 (s, 1H, ArH), 7.69 (d, 2H, ArH, J=7.56 Hz), 7.79 (d, 2H, ArH, J=7.58 Hz), 9.42 (bs, 1H, NH), 9.98 (s, 1H, NH), 10.20 (s, 1H, NH). *Anal.* Calcd for $C_{23}H_{27}F_3N_4O_5S_2$: C, 49.28; H, 4.82; N, 10.00. Found: C, 49.39; H, 4.72; N, 10.12.

1-[2-(4-Methylpiperazine sulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-(*p*-chlorophenyl)-thiosemicarbazide (**4l**): Yield: 84%. mp 177—179 °C (ethanol powder). IR cm^{-1}: 3306, 3180 (3NH), 1697 (CONH), 1518 (C=S), 1325 (S–$O_{antisym}$), 1141 (S–O_{sym}). ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.15 (s, 3H, N–CH_3), 2.34 (s, 4H, piperazine), 3.01 (s, 4H, piperazine), 3.80 (s, 3H, CH_3O), 3.82 (s, 3H, CH_3O), 3.88 (s, 2H, CH_2), 7.09 (s, 1H, ArH), 7.20 (s, 1H, ArH), 7.38 (d, 2H, ArH, J=8.9 Hz), 7.53 (d, 2H, ArH, J=8.9 Hz), 9.30 (bs, 1H, NH), 9.85 (s, 1H, NH), 10.16 (s, 1H, NH). *Anal.* Calcd for $C_{22}H_{28}ClN_5O_5S_2$: C, 48.75; H, 5.17; N, 12.92. Found: C, 48.82; H, 5.09; N, 12.85.

1-[2-(4-Methylpiperazine sulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-(*p*-trifluoromethylphenyl)-thiosemicarbazide (**4m**): Yield: 87%. mp 148—151 °C (methanol powder). IR cm^{-1}: 3304, 3190 (3NH), 1697 (CONH), 1518 (C=S), 1325 (S–$O_{antisym}$), 1141 (S–O_{sym}). ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.15 (s, 3H, N–CH_3), 2.36 (s, 4H, piperazine), 3.02 (s, 4H, piperazine), 3.80 (s, 3H, CH_3O), 3.82 (s, 3H, CH_3O), 3.91 (s, 2H, CH_2), 7.10 (s, 1H, ArH), 7.21 (s, 1H, ArH), 7.70 (d, 2H, ArH, J=8.4 Hz), 7.81 (d, 2H, ArH, J=8.5 Hz), 9.49 (s, 1H, NH), 10.02 (bs, 1H, NH), 10.22 (s, 1H, NH). *Anal.* Calcd for $C_{23}H_{28}F_3N_5O_5S_2$: C, 48.00; H, 4.87; N, 12.17. Found: C, 48.11; H, 4.81; N, 12.25.

General Procedure for the Preparation of *N*-{5-[2-(*N*-Substituted sulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-arylamines (5) A mixture of the 1-[2-(*N*-substituted sulfamoyl)-4,5-dimethoxy-phenylacetyl]-4-aryl-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 made alkaline 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.

N-{5-[2-(*N*,*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-phenylamine (**5a**): Yield: 69%. mp 227—228 °C (methanol–chloroform). IR cm^{-1}: 3285 (NH), 1329 (S–$O_{antisym}$), 1138 (S–O_{sym}). ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.67 (s, 6H, $2NCH_3$), 3.83 (s, 3H, CH_3O), 3.84 (s, 3H, CH_3O), 4.56 (s, 2H, CH_2), 6.97 (t, 1H, ArH, J= 7.32—7.34 Hz), 7.14 (s, 1H, ArH), 7.26 (s, 1H, ArH), 7.32 (t, 2H, ArH, J=7.55–8.22 Hz), 7.56 (d, 2H, ArH, J=7.88 Hz), 10.17 (s, 1H, NH). *Anal.* Calcd for $C_{19}H_{22}N_4O_4S_2$: C, 52.53; H, 5.07; N, 12.90. Found: C, 52.45; H, 5.15; N, 12.81.

N-{5-[2-(*N*,*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-(*p*-chlorophenyl)amine (**5b**): Yield: 85%. mp 264—265 °C (methanol–chloroform). IR cm^{-1}: 3262 (NH), 1329 (S–$O_{antisym}$), 1136

($S–O_{sym}$). ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.61 (s, 6H, $2NCH_3$), 3.80 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 4.54 (s, 2H, CH_2), 7.12 (s, 1H, ArH), 7.24 (s, 1H, ArH), 7.34 (d, 2H, ArH, J=8.50 Hz), 7.58 (d, 2H, ArH, J=8.43 Hz), 10.28 (s, 1H, NH). *Anal.* Calcd for $C_{19}H_{21}ClN_4O_4S_2$: C, 48.66; H, 4.48; N, 11.95. Found: C, 48.75; H, 4.43; N, 12.03.

N-{5-[2-(*N*,*N*-Dimethylsulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-(*p*-nitrophenyl)amine (**5c**): Yield: 89%. mp 268—269 °C (methanol–dichloromethane). IR cm^{-1}: 3263 (NH), 1329 ($S–O_{antisym}$), 1136 ($S–O_{sym}$). ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.64 (s, 6H, $2NCH_3$), 3.81 (s, 6H, $2CH_3O$), 4.59 (s, 2H, CH_2), 7.14 (s, 1H, ArH), 7.24 (s, 1H, ArH), 7.77 (d, 2H, ArH, J=8.70 Hz), 8.21 (d, 2H, ArH, J=8.70 Hz), 10.90 (s, 1H, NH). *Anal.* Calcd for $C_{19}H_{21}N_5O_6S_2$: C, 47.60; H, 4.38; N, 14.61. Found: C, 47.68; H, 4.32; N, 14.55.

N-{5-[2-(*N*,*N*-Dimethyl sulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-(*p*-trifluoromethylphenyl)amine (**5d**): Yield: 73%. mp 253—254 °C (methanol–dichloromethane). IR cm^{-1}: 3263 (NH), 1329 ($S–O_{antisym}$), 1138 ($S–O_{sym}$). ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.65 (s, 6H, $2NCH_3$), 3.81 (s, 3H, CH_3O), 3.82 (s, 3H, CH_3O), 4.57 (s, 2H, CH_2), 7.13 (s, 1H, ArH), 7.24 (s, 1H, ArH), 7.65 (d, 2H, ArH, J=8.58 Hz), 7.75 (d, 2H, ArH, J=8.57 Hz), 10.61 (s, 1H, NH). *Anal.* Calcd for $C_{20}H_{21}F_3N_4O_4S_2$: C, 47.80; H, 4.18; N, 11.15. Found: C, 47.75; H, 4.27; N, 11.07.

N-{5-[2-(1-Pyrrolidinesulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-phenylamine (**5e**): Yield: 57%. mp 242—243 °C (methanol–chloroform). IR cm^{-1}: 3292 (NH), 1333 ($S–O_{antisym}$), 1142 ($S–O_{sym}$). ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.68—1.73 (m, 4H, pyrrolidine), 3.09—3.14 (m, 4H, pyrrolidine), 3.80 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 4.56 (s, 2H, CH_2), 6.94 (t, 1H, ArH, J=7.27 Hz), 7.12 (s, 1H, ArH), 7.29 (s, 1H, ArH), 7.29 (t, 2H, ArH, J=7.14—8.71 Hz), 7.53 (d, 2H, ArH, J=7.83 Hz), 10.14 (s, 1H, NH). *Anal.* Calcd for $C_{21}H_{24}N_4O_4S_2$: C, 54.78; H, 5.21; N, 12.17. Found: C, 54.87; H, 5.28; N, 12.20.

N-{5-[2-(1-Pyrrolidinesulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-(*p*-chlorophenyl)amine (**5f**): Yield: 75%. mp 275—277 °C (methanol–chloroform). IR cm^{-1}: 3267 (NH), 1331 ($S–O_{antisym}$), 1141 ($S–O_{sym}$). ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.68—1.73 (m, 4H, pyrrolidine), 3.09—3.13 (m, 4H, pyrrolidine), 3.80 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 4.56 (s, 2H, CH_2), 7.12 (s, 1H, ArH), 7.29 (s, 1H, ArH), 7.34 (d, 2H, ArH, J=9Hz), 7.58 (d, 2H, ArH, J=9 Hz), 10.29 (s, 1H, NH). *Anal.* Calcd for $C_{21}H_{23}ClN_4O_4S_2$: C, 50.96; H, 4.65; N, 11.32. Found: C, 50.91; H, 4.71; N, 11.37.

N-{5-[2-(1-Pyrrolidinesulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-(*p*-nitrophenyl)amine (**5g**): Yield: 89%. mp 285—286 °C (methanol–chloroform). IR cm^{-1}: 3262 (NH), 1332 ($S–O_{antisym}$), 1140 ($S–O_{sym}$). ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.66—1.73 (m, 4H, pyrrolidine), 3.09—3.14 (m, 4H, pyrrolidine), 3.80 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 4.61 (s, 2H, CH_2), 7.15 (s, 1H, ArH), 7.29 (s, 1H, ArH), 7.76 (d, 2H, ArH, J=9.3Hz), 8.22 (d, 2H, ArH, J=8.22 Hz), 10.94 (s, 1H, NH). *Anal.* Calcd for $C_{21}H_{23}N_5O_6S_2$: C, 49.90; H, 4.55; N, 13.86. Found: C, 49.93; H, 4.61; N, 13.89.

N-{5-[2-(1-Pyrrolidinesulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-(*p*-trifluoromethylphenyl)amine (**5h**): Yield: 90%. mp 260—261 °C (methanol–chloroform). IR cm^{-1}: 3265 (NH), 1329 ($S–O_{antisym}$), 1142 ($S–O_{sym}$). ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.68—1.73 (m, 4H, pyrrolidine), 3.09—3.14 (m, 4H, pyrrolidine), 3.80 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 4.59 (s, 2H, CH_2), 7.14 (s, 1H, ArH), 7.29 (s, 1H, ArH), 7.65 (d, 2H, ArH, J=8.7 Hz), 7.74 (d, 2H, ArH, J=8.4 Hz), 10.56 (s, 1H, NH). *Anal.* Calcd for $C_{22}H_{23}F_3N_4O_4S_2$: C, 50.00; H, 4.35; N, 10.60. Found: C, 50.04; H, 4.29; N, 10.64.

N-{5-[2-(1-Piperidinesulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-(*p*-chlorolphenyl)amine (**5i**): Yield: 86%. mp 258—259 °C (methanol–chloroform). IR cm^{-1}: 3262 (NH), 1333 ($S–O_{antisym}$), 1142 ($S–O_{sym}$). ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.37—1.39 (m, 2H, piperidine), 1.45—1.48 (m, 4H, piperidine), 2.95—2.97 (m, 4H, piperidine), 3.80 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 4.53 (s, 2H, CH_2), 7.10 (s, 1H, ArH), 7.24 (s, 1H, ArH), 7.35 (d, 2H, ArH, J=4.5 Hz), 7.57 (d, 2H, ArH, J=4.5 Hz), 10.29 (s, 1H, NH). *Anal.* Calcd for $C_{22}H_{25}ClN_4O_4S_2$: C, 51.91; H, 4.91; N, 11.01. Found: C, 51.96; H, 4.85; N, 10.97.

N-{5-[2-(1-Piperidinesulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-(*p*-nitrolphenyl)amine (**5j**): Yield: 74%. mp 273—274 °C (methanol–dichloromethane). IR cm^{-1}: 3262 (NH), 1337 ($S–O_{antisym}$), 1142 ($S–O_{sym}$). ^{1}H-NMR (DMSO-d_6) δ (ppm): 1,42 (m, 2H, piperidine), 1,51 (m, 4H, piperidine), 3,01 (m, 4H, piperidine), 3.84 (s, 6H, $2CH_3O$), 4.61 (s, 2H, CH_2), 7.16 (s, 1H, ArH), 7.27 (s, 1H, ArH), 7.79 (d, 2H, ArH, J=8,89 Hz), 8,25 (d, 1H, ArH, J=8,86 Hz), 10.96 (s, 1H, NH). *Anal.* Calcd for $C_{22}H_{25}N_5O_6S_2$: C, 50.86; H, 4.81; N, 13.48. Found: C, 50.91; H, 4.85; N, 13.45.

N-{5-[2-(1-Piperidinesulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-(*p*-trifluoromethylphenyl)amine (**5k**): Yield: 80%. mp 253—254 °C (methanol–dichloromethane). IR cm^{-1}: 3268 (NH), 1331 ($S–O_{antisym}$), 1142 ($S–O_{sym}$). ^{1}H-NMR (DMSO-d_6) δ (ppm): 1,41 (m, 2H, piperidine), 1,51 (m, 4H, piperidine), 3,00 (m, 4H, piperidine), 3.83 (s, 3H, CH_3O), 3.84 (s, 3H, CH_3O), 4.59 (s, 2H, CH_2), 7.15 (s, 1H, ArH), 7.27 (s, 1H, ArH), 7.68 (d, 2H, ArH, J=8,77 Hz), 7,78 (d, 2H, ArH, J=8,66 Hz), 10.62 (s, 1H, NH). *Anal.* Calcd for $C_{23}H_{25}F_3N_4O_4S_2$: C, 50.92; H, 4.61; N, 10.33. Found: C, 50.98; H, 4.70; N, 10.37.

N-{5-[2-(4-Methylpiperazinesulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-(*p*-chlorophenyl)amine (**5l**): Yield: 67%. mp 263—264 °C (methanol–chloroform). IR cm^{-1}: 3262 (NH), 1342 ($S–O_{antisym}$), 1138 ($S–O_{sym}$). ^{1}H-NMR (DMSO-d_6) δ (ppm): 2,08 (s, 3H, $N–CH_3$), 2.26 (m, 4H, piperazine), 2.96 (m, 4H, piperazine), 3.81 (s, 3H, CH_3O), 3.82 (s, 3H, CH_3O), 4.54 (s, 2H, CH_2), 7.14 (s, 1H, ArH), 7.26 (s, 1H, ArH), 7.34 (d, 2H, ArH, J=8,78 Hz), 7,59 (d, 2H, ArH, J=8,77 Hz), 10.31 (s, 1H, NH). *Anal.* Calcd for $C_{22}H_{26}ClN_5O_4S_2$: C, 50.43; H, 4.96; N, 13.37. Found: C, 50.48; H, 4.91; N, 13.41.

N-{5-[2-(4-Methylpiperazinesulfamoyl)-4,5-dimethoxy-benzyl]-1,3,4-thiadiazol-2-yl}-*N*-(*p*-trifluoromethylphenyl)amine (**5m**): Yield: 67%. mp 248—249 °C (methanol–dichloromethane). IR cm^{-1}: 3271 (NH), 1329 ($S–O_{antisym}$), 1136 ($S–O_{sym}$). ^{1}H-NMR (DMSO-d_6) δ (ppm): 2,08 (s, 3H, $N–CH_3$), 2.27 (m, 4H, piperazine), 2.97 (m, 4H, piperazine), 3.83 (s, 6H, $2CH_3O$), 4.58 (s, 2H, CH_2), 7.17 (s, 1H, ArH), 7.28 (s, 1H, ArH), 7.67 (d, 2H, ArH, J=8,56 Hz), 7,77 (d, 2H, ArH, J=8,54 Hz), 10.62 (s, 1H, NH). *Anal.* Calcd for $C_{23}H_{26}F_3N_5O_4S_2$: C, 49.55; H, 4.66; N, 12.56. Found: C, 49.61; H, 4.61; N, 12.50.

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

The antibacterial assay was carried out by microdilution method [21—23] in order to determine the antibacterial activity of compounds tested against the human pathogenic bacteria.

The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^5 colony forming unit (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.

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 subcultivation 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.

Test for Antifungal Activity For the antifungal bioassays, *Aspergillus flavus* (ATCC 9643), *Aspergillus fumigatus* (plant isolate), *Aspergillus niger* (ATCC 6275), *Aspergillus versicolor* (ATCC 11730)), *Fulvia fulvum* (TK 5318), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112) and *Trichoderma viride* (IAM 5061) were used.

The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković," Belgrade, Serbia.

The micromycetes were maintained on malt agar and the cultures stored at 4 °C and sub-cultured once a month[24] in order to investigate the antifungal activity of the extracts, a modified microdilution technique was used.[21—23] 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 microtiter plates containing 100 μl of broth per well and further incubation 72 h at 28 °C. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. DMSO was used as a negative control, commercial fungicides, bifonazole and ketoconazole, were used as positive controls (1—3000 μg/ml).

NMR Spectroscopy DMSO-d_6 and ultra precision NMR tubes Wilmad 535—5 mm (SPINTEC ROTOTEC) were used for the NMR experiments. The compounds were dissolved in DMSO-d_6 to a final concentration of around 10 mM and a series of experiments were performed using Varian 600MHz spectrometer at 300 K. 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. The offset compensated ROESY experiments were performed using a mixing time of 150 ms in the phase-sensitive mode, a relaxation delay of 1 s and 4 kHz spin-locking field strength. The performance of a series of nuclear Overhauser effect spectroscopy (NOESY) experiments using mixing times of 100 ms, 150 ms, 300 ms, 500 ms and 1 s revealed that the mixing time of 150 ms could ensure the operation at the initial linear part of the NOE buildup curve. Experimental data were processed using VNMR routines. Chemical shifts (δ) are reported in ppm while spectra were referenced by the standard experimental setup.

Molecular Modeling Conformational analyses studies and lipophilicity maps calculations were performed using MacroModel 9.5 (Schrödinger Inc.) s/w. The OPLS5 force field was applied for the potential energy calculations and all studies were run using a dielectric constant ε=45 to simulate DMSO environment. Monte Carlo search was performed using MCMM/LMOD routine. PRCG algorithm was used for energy optimization with 0.005 convergence threshold and 5000 steps. Consequently, the minimized conformers are representatives of the local minima of the potential energy surface. The derived conformers were clustered by torsional RMSD in 5 families, the lowest energy members of which were chosen for further analysis. Grid scan search was performed using Maestro Dihedral Scan. The initial structures were subjected to a systematic variation around the specific torsion angles from 0° to 360° applying a torsion step of 10°. The derived conformations were optimized by applying PRCG algorithm with an energy gradient tolerance of 0.001 Kcal/mol Å as convergence criterion. Visualization of the 3D structures has been enabled by the use of Accelrys DS Visualizer s/w.

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