Med Chem Res (2013) 22:1330–1338
DOI 10.1007/s00044-012-0104-0

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Aromatic sulfonyl hydrazides and sulfonyl hydrazones: antimicrobial activity and physical properties

H. Güzin Aslan · Nurcan Karacan

Received: 29 September 2011 / Accepted: 22 May 2012 / Published online: 14 June 2012

Abstract A series of novel aromatic sulfonamide derivatives (**1**–**7**) were synthesized and characterized by elemental analyses, FT-IR, ^{1}H NMR, ^{13}C NMR, and LC/MS techniques. The compounds' quantum mechanical descriptors, surface area, and molecular volume were calculated. All the synthesized compounds were evaluated in vitro as antimicrobial agents against representative strains of gram-positive and gram-negative bacteria and as antifungal agents for yeast by both the disc diffusion and minimal inhibition concentration methods for comparison. All the bacteria and fungi studied were screened against some commercial antibiotics to compare with our chemicals' zone diameters. Quantitative structure–activity relationship studies with multiple linear regression analysis were applied to find the correlation between the different calculated molecular descriptors of the synthesized compounds and biological activity.

Keywords Antimicrobial activity · Sulfonamides · Aromatic sulfonamide · MIC

Introduction

During the past two decades, the frequency and types of life-threatening infections has increased. In addition to the usual kinds of cases, there have been increasing numbers of immunocompromised patients (HIV infection, antitumoral treatments, organ transplant-associated immunosuppressive therapy) as well as patients undergoing more invasive medical procedures (extensive surgery, prosthetic implants), among others (Georgopapadakou and Walsh, 1996).

A major associated problem is that the incidence of drug-resistant isolated bacterial strains in the community has become quite alarming. For these reasons, the demand for new and better chemotherapic compounds has increased, and nowadays the search for new chemicals with antimicrobial activity has become an important field of research (Chohan *et al.*, 2006; Bruggraber *et al.*, 2004; Mills, 2006).

Sulfonamides, and their different derivatives are extensively used in medicine (Borrás *et al.*, 2004) due to their pharmacological antibacterial activity. They interfere with the use of *p*-aminobenzoic acid (PABA) in the biosynthesis of tetrahydrofolic acid, which is an essential growth factor for the bacteria's metabolism (Anand, 1996). In spite of the fact that the emergence of drug-resistant strains is one of the principal constraints of sulfonamide therapy, it has continued unabated. For instance, sulfonamides are considered as the drugs of choice for the treatment of nocardiosis and are mainly used for the treatment of urinary tract and methicillin-resistant bacteria infections (Anand,1996; Foye *et al.*, 1995).

Amsacrine ($C_{21}H_{19}N_3O_3S$) is used in cancer chemotherapy (Lomax and Narayanan, 1988; Jensen *et al.*, 1990; Finlay *et al.*, 1990; Rutner *et al.*, 1974). Some sulfonylhydrazines are known to have antineoplastic effects which prevent malignant cells from growing and spreading (Shyam *et al.*, 1990).

This prompted us to synthesize a series of novel symmetric aromatic sulfonamides. Therefore, we obtained a series of new sulfonamides (**1**–**7**) and characterized their

H. G. Aslan (✉)
Department of Chemistry, Science Faculty, Erciyes University, 38039 Melikgazi, Kayseri, Turkey
e-mail: guzina@erciyes.edu.tr

N. Karacan
Department of Chemistry, Science and Arts Faculty, Gazi University, 06500 Ankara, Turkey

structures by FT-IR, ^{1}H NMR, ^{13}C NMR, and LC/MS techniques. We investigated their antibacterial activities against gram-positive (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633, *Staphylococcus epidermidis* ATCC 12228, *Enterobacter aerogenes* ATCC 13048) and gram-negative bacteria (*Pseudomonas fluorescens* ATCC 49838, *Klebsiella pneumonia* ATCC 13883, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and as antifungal agents against *Candida albicans* (ATCC 90028), by both the disc diffusion and minimal inhibition concentration (MIC) methods for comparison. All the bacteria and fungi studied were screened against proper antibiotics to compare them with our chemicals' zone diameters.

Results and discussion

Figures 1 and 2 illustrate the general synthetic route used to prepare our compounds. The exothermic nucleophilic substitution reaction of benzene sulfonyl chloride with various amines was employed to form benzenesulfonicacid-1-methylhydrazide (**1**) (Dauban and Dodd, 2000; Newcombe, 1955, Kloes, 1959; Hrubiec *et al.*, 1986), *N*-(3-amino-2-hydroxypropylbenzenesulfonamide (**2**) (Karacan *et al.*, 2011) and *N*-(2-hydroxyethylbenzene sulfonamide (**3**).

Compound **1**, which was treated with various aldehydes and ketones, was synthesized to form salicylaldehyde benzenesulfonylhydrazone (**4**), 2-hydroxy-1-acetophenone benzenesulfonylhydrazone (**5**), 2-hydroxy-1-naphtaldehydebenzenesulfonylhydrazone (**6**), and thiophene-2-carbaldehyde

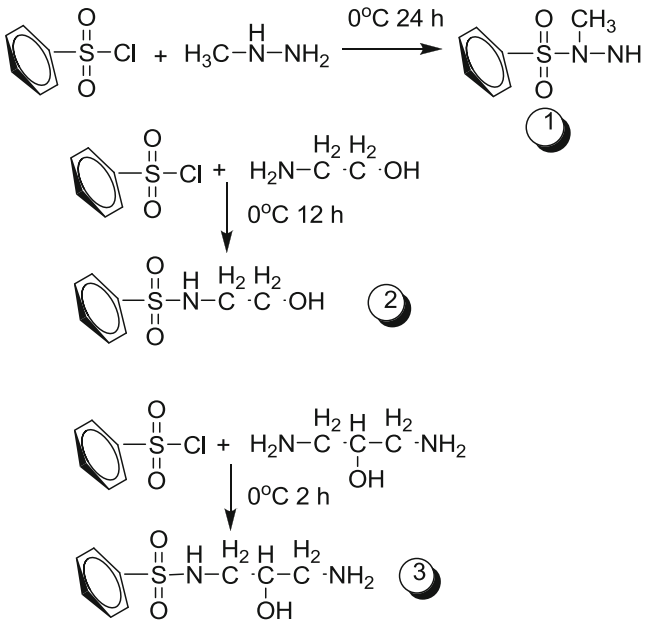


Fig. 1 Preparation of aromatic sulfonyl hydrazides

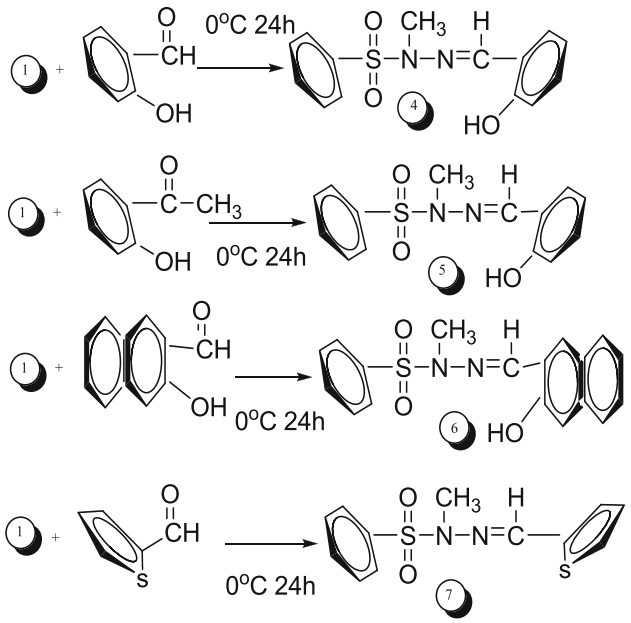


Fig. 2 Preparation of aromatic sulfonyl hydrazones

benzenesulfonyl hydrazone (**7**) (Aslan *et al.*, 2011; Aslan, 2008). Among the different solvents used, THF helped to facilitate a better nucleophilic substitution reaction for the series of compounds.

NMR spectra

The NMR spectra of compounds were recorded in DMSO-d_6, using TMS as an internal standard. The ^{1}H NMR and ^{13}C NMR data for half of the compounds are given in Tables 1, 2, 3, and 4, respectively. The ^{1}H NMR spectrum of compound **4** showed that the OH proton had a broad peak at 10.30 ppm, and the N=CH proton at 8.60 ppm. Based on these data, the structure of the phenol-imine compound of **4** was found in the DMSO solution. On the other hand, the IR spectrophotometric observation of (C–O) stretching vibration at 1,258 cm^{-1} and (C=N) stretching vibration at 1,659 cm^{-1} convinced us to propose that the structure of the phenol-imine compound was in the solid phase (Aslan *et al.*, 2011). The ^{1}H NMR spectrum of compound 6 showed that OH protons had a broad peak at 12.85 ppm, a singlet at 9.98 around the imine zone and a doublet at 8.66 ppm. Based on these data, the structures of the ketone-amine and phenol-imine compounds of **6** in DMSO solution indicate the presence of tautomeric forms. On the other hand, the IR spectrophotometric observation of (C–O) stretching vibration at 1,265 cm^{-1} and (C=N) stretching vibration at 1,636 cm^{-1} convinced us to propose that the structure of the phenol-imine was in the solid phase (Aslan, 2008). The ^{1}H NMR spectrum of the **1**, **4**, **5**, **6**, and **7** compounds showed that –N–CH_3 protons had peaks at

Table 1 The ^{1}H NMR data (ppm) of the sulfonyl hydrazide compounds

Assignment	Bsmh	Bsea	Bsdiap
N–NH_2	4.85 (s)	–	–
N–CH_3	2.6 (s)	–	–
Ar H	6.96–8.89 (s)	7.26–8.11	7.15 (w, 8H)
N*H*–CH_2	–	–	7.15 (w, 8H)
NH–C*H*$_2$	–	2.83 (t, 2H, 8Hz)	2.88 (d, 2H, 3.36 Hz)
NH–CH_2–C*H*$_2$	–	3.59 (t, 2H, 7.36 Hz)	–
CH_2–N*H*$_2$	–		7.15 (w, 8H)
NH–CH_2–C*H*	–		3.87 (s, 1H)
CHOH–C*H*$_2$	–		2.66 (d, 2H, 3.54 Hz.)
OH	–	5.18 (s, H)	5.28 (s, br)

Table 2 The ^{13}C NMR data (ppm) of the sulfonyl hydrazide compounds

Assignment	Bsmh	Bsea	Bsdiap
N–CH_3	40.46		
Ar C C1	125.95–148.13	125.77–134.0	129.07–128.04
NH–*CH*$_2$		45.42	42.47
NH–CH_2–*CH*$_2$		60.08	
NH–CH_2–*CH*			64.87
CHOH–*CH*$_2$–			40.59

Table 3 The ^{1}H NMR data (ppm) of the sulfonyl hydrazone compounds

Assignment	Hsalbsmh	Hafbsmh	Hnafbsmh	T2kbsmh
N–CH_3	2.94	2.69 (d, 3H, 8 Hz)	2.60	2.52
Ar H	6.85–7.65	7.67–6.94	8.05–7.31	7.18–8.75
C–CH_3		2.69 (d, 3H, 6 Hz)		
N=CH	8.70		9.98 (s, H)	8.84
			8.66 (d, H, 3.5 Hz)	
OH	10.25	12.93 (s, H)	12.85	

2.60, 2.94, 2.69, 2.60, and 2.52 ppm, respectively. It is known that rotation around a single bond brings together the mixture of conformers in the solution.

NMR chemical shift calculations for the most stable conformers were performed using the GIAO approach in order to correctly assign proton and carbon peaks, which are now widely used in efficient assignment.

Table 4 The ^{13}C NMR data (ppm) of the sulfonyl hydrazone compounds

Assignment	Hsalbsmh	Hafbsmh	Hnafbsmh	T2kbsmh
N–CH_3	35.22	Covered	37	37
Ar C	158.22–117.13	130.45–119.81	164–112	122–144
C–CH_3		15.55		
N=CH	141.61	133.60	161	156

Table 5 Wave number (cm^{-1}) of selected vibration of the compounds

Comp.	νNH_{as}	νNH_s	$\nu(SO_2)_{as}$	$\nu(SO_2)_s$	νC=*N*
Bsmh	3,141	3,100	1,249 (w)	1,157 (m)	–
Bsea	3,290 (sh)	–	1,361 (w)	1,151 (m)	–
Bsdiap	3,331 (sh)	3,360	1,334 (w)	1,156 (m)	–
Hsalbsmh	–	–	1,295 (w)	1,180 (m)	1,612
Hafbsmh	–	–	1,329	1,150	1,606
Hnafbsmh	–	–	1,344	1,162	1,636
T2kbsmh	–	–	1,316	1,166	1,617

v Stretching vibration, *sh* sharp, *m* medium, *w* weak

FT-IR spectra

The selected vibration wave numbers of the IR spectra compounds are listed in Table 5. The presence of NH_2 vibrations in the prepared compounds **1** and **3**, which are observed between 3,100 and 3,250 cm^{-1} as double bonds and the presence of a single and strong NH band observed at ~3,275–3,293 cm^{-1} confirms the presence of secondary amine groups in our compounds (**2**–**3**). $\nu_{as}(SO_2)$ and $\nu_s(SO_2)$ vibrations are observed between 1,249–1,361 and 1,150–1,180 cm^{-1}.

LC/MS spectra

LC/MS shows that compounds **1** ($[M]^+$), **2** ($[M+H^+]$), **3** ($[M-H^+]$), **4** ($[M+Na]^+$), **5** ($[M-H]^+$), **6** ($[M+H]^+$), and **7** ($[M-H]^+$) give ions at 186.1, 231.2, 200.3, 313.1, 303.25, 341.2, and 279.80 *m*/*z*, respectively.

All compounds of the C_6H_5 group fragment separately and this was observed at 79.2 *m*/*z*.

Biological activity

In this study, the antimicrobial activities of the synthesized compounds were screened against various pathogens in vitro by the disc diffusion and microdilution methods. Tetracycline, trimethoprim, miconazole, and nystatin were used as positive controls against both bacteria and yeast using the disc diffusion method. The results of the anti-bacterial and antifungal studies of all the synthesized

Table 6 The MICs of the tested compounds and reference antibiotics

	MIC (μg/mL)										
Tested compound number:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
S. aureus ATCC 25923	125	>2,000	>2,000	250	500	500	250	0.000	1.000	0.000	0.000
B. cereus ATCC 11778	125	>2,000	>2,000	500	500	250	250	0.000	0.000	0.000	0.000
E. faecalis ATCC 29212	250	>2,000	>2,000	500	1,000	500	1,000	0.625	0.500	0.000	0.000
B. subtilis ATCC 6633	125	>2,000	1,000	500	500	250	125	0.075	1.000	0.000	0.000
S. epidermidis ATCC 12228	125	>2,000	>2,000	250	500	500	125	0.125	0.000	0.000	0.000
E. aerogenes ATCC 13048	125	1,000	1,000	1,000	1,000	500	250	0.150	1.000	0.000	0.000
P. fluorescens ATCC 49838	62.5	1,000	1,000	500	1,000	250	125	0.125	1.000	0.000	0.000
K. pneumonia ATCC 13883	125	>2,000	1,000	1,000	1,000	250	125	0.150	1.000	0.000	0.000
E. coli ATCC 25922	125	1,000	1,000	500	1,000	500	125	0.075	0.000	0.000	0.000
P. aeruginosa ATCC 27853	62.5	1,000	1,000	1,000	1,000	250	125	0.125	1.000	0.000	0.000
C. albicans ATCC 90028	>2,000	1,000	1,000	500	500	500	1,000	0.000	0.000	0.150	0.125

8 Tetracycline (μg/mL), **9** trimethoprim(μg/mL), **10** miconazole (μg/mL), **11** nystatin (μg/mL)

compounds are listed in Tables 6 and 7. As seen in Table 6, compound **1** is the most potent sulfonyl hydrazide of this series. It showed good antibacterial activity against both gram-negative and gram-positive bacteria, and has also shown antifungal activity against *C. albicans* at >2,000 μg/mL. Similarly, sulfonylhydrazone (**7**) showed observable activity against gram-negative bacteria and gram-positive bacteria. As shown in Table 6, the sulfonyl hydrazones which are part of the –OH group show antifungal activity against *C. albicans*. Using the disc diffusion method we found that compound **6** displays good activity against all tested microorganisms except *E. coli*.

Different quantum mechanical descriptors including dipole moment, energies of the frontier orbitals (HOMO, LUMO), refractivity, and dipole moment were calculated by HyperChem 7.5 software.

The log *P* value definition is an important method used to determine antimicrobial activity. The octanol–water partition coefficient (log *P* value) of a drug substance is an indicator of compound lipophilicity and solubility. A higher log *P* value increases the lipophilic property of compounds. According to the Lipinski (Lipinski *et al.*, 2001) and Ghose (Ghose *et al.*,1999; Brendan *et al.*, 2007) rules, the compound's log *P* value must be between −0.4 and +5.6. In our study, the activity in the sulfonamides (**1**–**3**) increased with increasing log *P* value. Similarly, the antimicrobial activity in the sulfonyl hydrazones (**5**–**6**) increased with increasing log *P* value. However, compound **7** showed the highest activity and lowest log *P* value because this compound was located in the thiophene ring, instead of in the phenol group.

Electronic potential maps enable us to visualize the charge distributions of molecules; charge is related to the properties of molecules. In Figs. 3 and 4, the blue areas on the maps represent the positive potential values, and the red regions correspond to the negative potential values.

Table 7 Inhibition zones (diameter) in mm of compound and reference antibiotic discs against tested microorganisms by the disc diffusion method

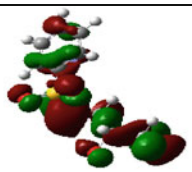
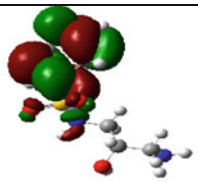
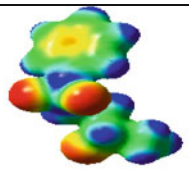
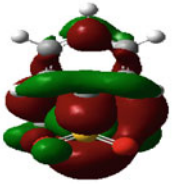
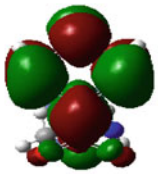
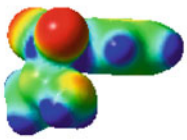
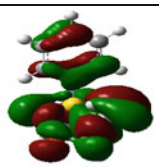
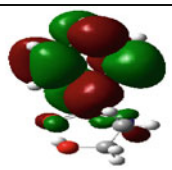
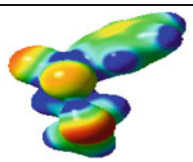
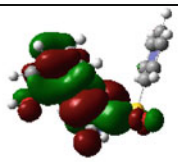
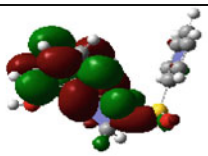
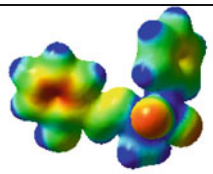
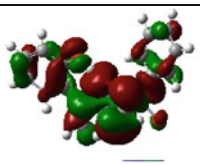
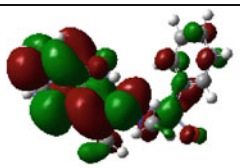
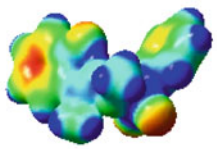
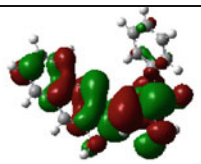
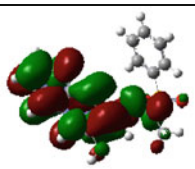
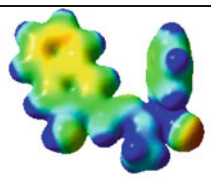
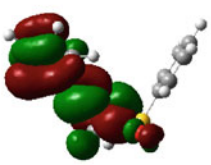
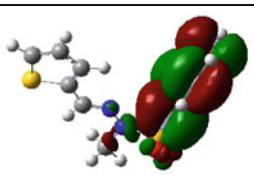
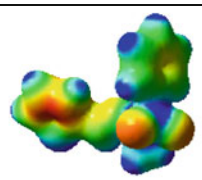
Bacteria and fungi	İnhibition zone (mm, 140 μg/disk)										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
S. aureus ATCC 25923	19	0	0	13	10	19	12	0	37	0	0
B. cereus ATCC 11778	15	0	0	11	10	26	25	19	0	0	0
E. faecalis ATCC 29212	15	0	0	11	9	17	0	0	27	0	0
B. subtilis ATCC 6633	16	0	0	9	9	28	24	26	20	0	0
S. epidermidis ATCC 12228	14	0	0	14	12	16	15	19	29	0	0
E. aerogenes ATCC 13048	16	0	0	6	5	18	17	0	28	0	0
P. fluorescens ATCC 49838	22	0	0	12	11	26	25	19	0	0	0
K. pneumonia ATCC 13883	17	0	0	6	6	23	21	12	0	0	0
E. coli ATCC 25922	18	0	0	13	11	17	17	23	0	0	0
P. aeruginosa ATCC 27853	19	0	0	6	5	25	24	23	31	0	0
C. albicans ATCC 90028	0	0	0	14	12	16	0	0	0	26	18

8 Tetracycline (μg/mL), **9** trimethoprim(μg/mL), **10** miconazole (μg/mL), **11** nystatin (μg/mL)

In this study, QSAR analysis was performed by the stepwise multiple linear regression method. The statiscially significant QSAR models are given below.

QSAR model for antimicrobial activity against *Candida albicans*

Fig. 3 Frontier molecular orbitals of sulfonyl hydrazides and sulfonyl hydrazones (Color figure online)

	HOMO	LUMO	EP Map
Bsdiap			
Bsmh			
Bsea			
Salbsmh			
Afbsmh			
Nafbsmh			
T2kbsmh			

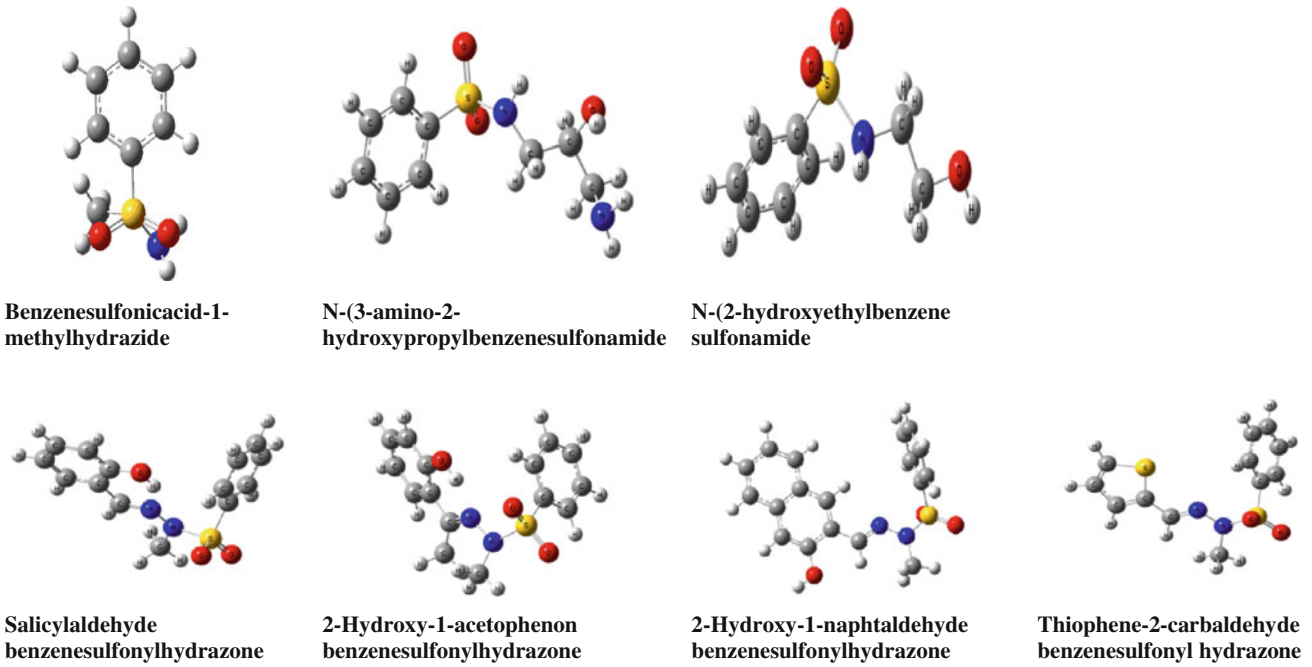


Fig. 4 Optimized geometries of aromatic sulfonyl hydrazides and aromatic sulfonyl hydrazones (Color figure online)

$$-\log \mathrm{MIC} = \{-2.676(\pm 0.08)\} + \{\ \mathrm{V9}\ 3.586(\pm 0.639) - \mathrm{V10}\ 0.150(\pm 0.007)\ \}$$
$$n = 7, r^2 = 0.955, s = 0.002, F = 42.548,$$
$$\mathrm{V9} = \mathrm{HOMO},\ \text{and}\ \ \mathrm{V10} = \mathrm{LUMO}\ \ \mathrm{SPRESS} = 0.307.$$

HOMO and LUMO are responsible for the formation of many charge transfer complexes. According to the frontier molecular orbital theory (FMO) of chemical reactivity, the formation of a transition state is due to an interaction between the frontier orbitals (HOMO and LUMO) of the reacting species. Thus, the treatment of the frontier molecular orbitals separately from the other orbitals is based on the general principles governing the nature of chemical reactions (Fukui, 1975).

The statistical analyses were performed with SPSS software (version 13, 2002). The abbreviations for the statistical parameters given for each equation were: n (number of data points), r^2 (squared correlation coefficient), and s (standard error of estimate), SPRESS (sum of square standard error), and F (Fischer statistics).

Statistically, a significant relationship was observed between the antimicrobial activity of sulfonyl hydrazides and sulfonyl hydrazones against *Candida albicans* (ATCC 90028) and HOMO–LUMO.

Experimental methods

Elemental analyses (C, H, N, and S) were performed on a LECO-CHSNO-9320 type elemental analyzer. The IR spectra were recorded on a Mattson-1000 FT-IR spectrophotometer with samples prepared as KBr pellets. The NMR spectra were obtained on a Bruker-Spectrospin Avance DPX-400 Ultra-Shield (400 MHz) using DMSO-d_6 and $CDCl_3$ as solvents and TMS as an internal standard. LC/MS-APC1 was acquired with an AGILENT 1100. Melting point, were recorded on an Opti MELT 3 hot stage apparatus. TLC was conducted on 0.25 mm silica gel plates (60F254, Merck). The synthesized compounds (except **4**) were purified by column chromatography.

All calculations reported here in were carried out with the GAUSSIAN 03 software (Frisch *et al.*, 2003). Quantum chemical descriptors (HOMO, LUMO, dipole moment, heat of formation) were calculated by using the B3LYP/6-31** method with the same software. The surface areas (approx) and molecular volumes of the compounds were calculated by using the HyperChem Release 7.5 package program (HyperChem 7.5 program, 2002).

General procedure for the synthesis

The nucleophilic substitution reaction of the different aliphatic diamines with benzene sulfonyl chloride was carried out as follows (only compounds **1**–**3**): A THF solution of aromatic sulfonyl chlorides (Ar-SO_2Cl) was added dropwise to the THF solution of methyl hydrazide (1:2 equiv), maintaining the temperature between −5 and −10 °C. Then, the reaction mixture was stirred for 24 h at room temperature (completion of the reaction was monitored by TLC). After the completion of the reaction, the solvent was

removed under vacuum and the solid residue was purified by column chromatography. Compounds (**4**–**7**) were synthesized according to the following general procedure: A THF solution of various aldehydes and ketones was added to the THF solution of compound **1** maintaining the temperature at −5 and −10 °C. Then the reaction mixture was stirred for 48 h at room temperature (completion of the reaction was monitored by TLC). After completion of the reaction, the solvent was removed under vacuum. The solid residue was purified by column chromatography (except for compound **4**).

*Synthesis of benzenesulfonicacid-1-methylhydrazide (**1**)*

The general synthetic method described above afforded a white solid from methylhydrazide (4.4 mL, 0.08 mol) and benzenesulfonyl chloride (5.05 mL, 0.04 mol). The product was purified by column chromatography. The results were as follows: yield 62 %; mp 67–68 °C; analytical calculation for $C_7H_{10}N_2O_2S$: C, 45.090; H, 5.430; N, 15.090; S, 17.190. Found: C, 45.140; H, 5.410; N, 15.040; S, 17.210.

*Synthesis of N-(3-amino-2-hydroxypropylbenzenesulfonamide (**2**)*

The general synthetic method described above afforded a white solid from benzenesulfonyl chloride (5.05 mL, 0.04 mol) and 1.3 diaminopropan-2-ol (7.21 g, 0.08 mol). The product was purified by column chromatography. The results were as follows: yield 51 %; mp 155 °C; analytical calculation for $C_9H_{14}N_2O_3S$: C, 47.000; H, 6.130; N, 12.104; S, 13.910. Found: C, 46.940; H, 6.127; N, 12.164; S, 13.922.

*Synthesis of N-(2-hydroxyethylbenzene sulfonamide (**3**)*

The general synthetic method described above afforded a white solid from benzenesulfonyl chloride (5.05 mL, 0.04 mmol) and ethanolamine (4.83 mL, 0.08 mol). The product was purified by column chromatography. The results were as follows: yield 65 %; mp 79–80 °C; analytical calculation for $C_8H_{11}NO_2S$: C, 47.700; H, 5.610; N, 7.000; S, 15.833. Found: C, 47.740; H, 5.519; N, 6.960; S, 15.931.

*Synthesis of salicylaldehyde benzenesulfonylhydrazone (**4**)*

The general synthetic method described above afforded a yellow solid from bsmh (3.1 mL, 46.0 mmol) and salicylaldehyde (3 mL, 23.0 mmol). The product was crystallized from acetonitrile. The results were as follows: yield 59 %; mp 167–168 °C; analytical calculation for $C_{14}H_{14}N_2SO_3$: C, 57.931; H, 4.880; N, 9.663; S, 11.022. Found: C, 57.916; H, 4.860; N, 9.648; S, 11.042.

*Synthesis of 2-hydroxy-1-acetophenone benzenesulfonylhydrazone (**5**)*

The general synthetic method described above afforded a yellow solid from bsmh (3.95 mL, 47.5 mmol) and 1-hydroxy acetophenone (3.0 mL, 23.7 mmol). The product was purified by column chromatography. The results were as follows: yield 68 %; mp 194–195 °C; analytical calculation for $C_{15}H_{16}N_2SO_3$: C, 59.201; H, 5.289; N, 9.212; S, 10.525. Found: C, 59.193; H, 5.298; N, 9.203; S, 10.533.

*Synthesis of 2-hydroxy-1-naphtaldehyde benzenesulfonylhydrazone (**6**)*

The general synthetic method described above afforded a yellow solid from bsmh (4.7 mL, 47.4 mmol) and 2-hydroxy-1-naphthaldehyde (3.0 mL, 23.7 mmol). The product was purified by column chromatography. The results were as follows: yield 70 %; mp 259–262 °C; analytical calculation for $C_{18}H_{16}N_2SO_3$: C, 63.523; H, 4.732; N, 8.234; S, 9.408. Found: C, 63.512; H, 4.737; N, 8.229; S, 9.419.

*Synthesis of thiophene-2-carbaldehyde benzenesulfonyl hydrazone (**7**)*

The general synthetic method described above afforded a yellow solid from bsmh (2.0 mL, 16.0 mmol) and thionyl chloride (1.06 mL, 8.1 mmol). The product was purified by column chromatography. The results were as follows: yield 35 %; mp 83.7 °C; analytical calculation for $C_{12}H_{12}N_2S_2O_2$: C, 41.428; H, 4.326; N, 9.979; S, 22.851. Found: C, 41.409; H, 4.314; N, 9.991; S, 22.870.

Biological activity: in vitro evaluation

The biological activity of the synthesized sulfonamide derivatives was individually screened against a panel of microorganisms, including gram-positive (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633, *Staphylococcus epidermidis* ATCC 12228, *Enterobacter aerogenes* ATCC 13048), and gram-negative bacteria (*Pseudomonas fluorescens* ATCC 49838, *Klebsiella pneumonia* ATCC 13883, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and yeast (*Candida albicans* ATCC 90028). The cultures were obtained from Erciyes University's Department of Biology. Bacterial strains were cultured overnight at 35 °C in Triptic Soy Broth (TSB), and the yeast was cultured overnight at 30 °C in Yeast Peptone Dextrose Broth (YPDB) in a rotary shaker at

200 rpm. Overnight cultures were then transferred to fresh media and the turbidity of all broth cultures was adjusted to 0.1 absorbance at 640 nm on a spectrophotometer. These stock cultures were stored in the dark at 4 °C during the survey.

Minimal inhibitory concentration

MIC were determined by the micro dilution broth method following the procedures recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2000). Briefly, synthesized sulfonamide derivatives dissolved in dimethylsulfoxide (DMSO) were first diluted to the highest concentration, and then serial twofold dilutions were made in a concentration range from 31.25 to 2,000 μg/mL in a sterile 2.4 mL 96-well Masterblock dish containing either MHB for the bacterial strains or YPDB for the yeast. For the test, the 96-well plates were prepared by dispensing into each well 195 μL of the serial dilutions of the synthesized sulfonamide derivatives which were then transferred into seven consecutive wells in each column. The last well, containing only 195 μL of MHB or YPDB without compound, was used as a negative control. For each column of the plate, 5 μL of the strains to be tested was inoculated into each well individually. The final volume in each well was brought up to 200 μL. For the bacterial cells, ampicillin, trimethoprim, and tetracycline in MHB and for the yeast miconazole and nystatin in YPDB at the concentration range of 1,000–7.8 μg/mL were used as standard drugs for positive control. The contents of each well were mixed on a plate shaker at 300 rpm for 20 s and then incubated at 35 °C for 18–24 h. Microbial growth was determined by measuring absorbance at 600 nm using the Tecan Sunrise microplate reader and with the presence of a white "pellet" on the well bottom for visual examination. Concentrations resulting in no growth were confirmed by plating 5 μL samples from the clear wells on MHA or YPDA. The compounds tested in this work were screened twice against each organism. The MIC was defined as the lowest concentrations of the antimicrobial agents that inhibited visible growth of the microorganism.

Determination of inhibition zones

The disk diffusion antibacterial screening (Shyam *et al.*, 1987; Kretov *et al.*, 1973; Bolli *et al.*, 2003; Bauer *et al.*, 1966; Committee for Clinical Laboratory Standards (NCCLS) (2000); National Committee for Clinical Laboratory Standards (NCCLS) (2000)) of the synthesized sulfonamide derivatives dissolved in DMSO was performed using Miller Hinton Agar (MHA) for the bacterial strains and Yeast Peptone Dextrose Agar (YPDA) for the yeast cells. One hundred microliters of the culture suspensions adjusted to the OD_{640} 0.1 (equivalent to 0.5 Mc Farland Turbidity Tubes) was swabbed over the entire surface of the medium. Sterile 6-mm-diameter disks (1.2 mm thickness Whatman filter paper) were impregnated with 50 μL dissolved extracts (including 1.2 mg total extract per disk) and placed onto MHA or YPDA. Negative controls were prepared by just using DMSO. For the bacterial cells, ampicillin (50 μg/disk), trimethoprim (30 μg/disk), and tetracycline (30 μg/disk) on MHA and for the yeast miconazole (40 μg/disk) and nystatin (30 μg/disk) on YPDB were used as reference standards to determine the sensitivity of each strain. The inoculated plates were incubated at 35 °C for 18–24 h. The antibacterial activity was measured as the diameter (mm) of the clear zone of growth inhibition. Four disks per plate were used and each test was run in duplicate.

Conclusion

In conclusion, a series of aromatic sulfonyl hydrazides and sulfonyl hydrazones (**1**–**7**) were synthesized, and their antimicrobial activities were evaluated. All compounds demonstrated potent inhibition against all the strains tested. Further research in this area is in progress in our laboratory. Consequently, these compounds may be recommended for industrial application.

Acknowledgments The authors are grateful to the TUBITAK Research Foundation for financial support under project number 104 T 390.

References

Anand N (1996) Burger's medicinal chemistry and drug discovery. In: Wollf ME (ed) Therapeutic agents, 5th edn. J. Wiley & Sons, New York, pp 527–544

Aslan G (2008) Çeşitli Sülfonamit Türevleri Ve Bunların Bazı Geçiş Metal Komplekslerinin Sentezi, Antimikrobiyal Etkilerinin İncelenmesi. Ph.D. Thesis, Gazi University, Ankara

Aslan G, Özcan S, Karacan N (2011) Synthesis, characterization and antimicrobial activity of salicylaldehyde benzenesulfonylhydrazone (Hsalbsmh) and its nickel(II), palladium(II), platinum(II), copper(II), cobalt(II) complexes. Inorg Chem Commun 14(9):1550–1553

Bauer AW, Kirby WM, Sheris JV, Truck M (1966) Antibiotic susceptibility testing by standardized single disc method. Am J Clin Pathol 45:493–496

Bolli MH, Boss C, Clozel M, Fischli W, Hess P, Weller T (2003) The use of sulfonylamido pyrimidines incorporating an unsaturated side chain as endothelin receptor antagonists. Bioorg Med Chem Lett 13(5):955–959

Borras J, Alzuet G, Ferrer S, Supuran CT (2004) Metal complexes of heterocyclic sulfonamides as carbonic anhydrase inhibitors. In: Supuran CT, Scozzafava A, Conway J (eds) Carbonic anhydrase: its inhibitors and activators, vol 36. CRC Press, Boca Raton, FL, pp 183–207

Brendan L, Wilkinson LF, Bornaghi AD, Wright A, Todd A, Poulsen H (2007) Anti-mycobacterial activity of a bis-sulfonamide. Bioorg Med Chem Lett 17:1355–1357

Bruggraber SFA, French G, Thompson RPH, Powell JJ (2004) Selective and effective bactericidal activity of the cobalt(II) cation against. Helicobacter 9:422–428

Chohan ZH, Pervez H, Rauf A, Khan KM, Supuran CT (2006) Antibacterial cobalt(II), copper(II), nickel(II) and zinc(II) complexes of mercaptothiadiazole-derived furanyl, thienyl, pyrrolyl, salicylyl and pyridinyl schiff bases. J Enzym Inhib Med Chem 21:193–201

Committee for Clinical Laboratory Standards (NCCLS) (2000) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, fifth ed. Approved standards, M7-A5. National Clinical and Laboratory Standards Institute, Wayne, PA

Dauban P, Dodd RH (2000) Synthesis and GABA(A) receptor activity of 6-oxa-analogs of neurosteroids. Org Lett 2:2327–2329

Finlay GJ, Baguley BC, Snow K, Judd W (1990) Multiple patterns of resistance of human leukemia cell sublines to amsacrine analogues. J Natl Cancer Inst 82:82–662

Foye WO, Lemke TL, Williams DA (1995) Principles of medicinal chemistry, 4th edn. Williams & Williams, Philadelphia

Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA (2003) Gaussian, Inc., Pittsburg PA, Gaussian 03 (Revision B.04)

Fukui K (1975) Theory of orientation and stereoselection. Springer, New York, pp 34–39

Georgopapadakou NH, Walsh TJ (1996) Antifungal agents: chemotherapeutic targets and immunologic strategies. Antimicrob Agents Chemother 40:279–291

Ghose A, Viswanadhan K, Vellarkad N, Wendoloski J (1999) A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. J Comb Chem 1:55–68

Hrubiec R, Shyam K, Cosby L, Sortorelli A (1986) Synthesis and evaluation of 2-substituted 1-methyl-1-(4-tolylsulfonyl) hydrazines as antineoplastic agents. J Med Chem 29:1777–1779

HyperChem 7.5 program (2002) Hypercube Inc.,Toronto, Canada

Jensen PB, Soerensen BS, Demant JFE, Sehested M, Jensen PS, Vindeloey L et al (1990) Antagonistic effect of aclarubicin on the cytotoxicity of etoposide and 4′-(9-acridinylamino)methanesulfon-*m*-anisidide in human small cell lung cancer cell lines and on topoisomerase II-mediated DNA cleavage. Cancer Res 50:3311

Karacan MS, Aslantaş N, Aslan HG (2011) Enrichment and removal of iron(II), copper(II) and strontium(II) ions with sulfonamides on activated carbon in environmental samples and determination by ICP-OES analysis. Presenius Environ Bull 20(5):1167–1173

Kloes H (1959) Hydrazides of aliphatic sulfonic acids. Farbenfabriken Bayer Akt Ges 45(7):456–458

Kretov AE, Momsenko AP, Bespalyi AS (1973) Synthesis of dihydro-1,3-thiazine derivatives. III. Zh Obshch Khim 5:595–597

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 46:3–26

Lomax NR, Narayanan VL (1988) Chemical structures of interest to the division of cancer treatment, developmental therapeutics program, vol VI. National Cancer Institute, Bethesda

Mills SD (2006) Mercaptothiadiazole-derived furanyl, thienyl, pyrrolyl, salicyl and pyridinyl. When will the genomics investment pay off for antibacterial discovery? Biochem Pharmacol 71:1096–1102

National Committee for Clinical Laboratory Standards (NCCLS) (2000) Performance standards for antimicrobial disk susceptibility tests, seventh ed. Approved standards, M2-A7. National Clinical and Laboratory Standards Instıtute, Wayne, PA

Newcombe AG (1955) Mesyl derivatives of hydrazine. Can J Chem 33:1250–1255

Rutner H, Lewin N, Woodburry EC, McBride TJ, Rao KV (1974) 4-Chloro-2-(morpholinomethyl) phenol. Cancer Chemother Rep 58:803–811

Shyam K, Hrubiec RT, Furubayashi R, Cosby LA, Sartorelli AC (1987) 1,2-Bis(sulfonyl)hydrazines. 3. Effects of structural modification on antineoplastic activity. J Med Chem 30:2157–2161

Shyam K, Penketh PG, Divo AA, Loomis RH, Patton CL, Sorterelli AC (1990) ChemInform Abstract: synthesis and evaluation of 1,2,2-tris(sulfonyl)hydrazines as antineoplastic and trypanocidal agents. Med Chem 33:2259–2264