Journal of Molecular Structure 1100 (2015) 464–474

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Alkyl sulfonic acide hydrazides: Synthesis, characterization, computational studies and anticancer, antibacterial, anticarbonic anhydrase II (hCA II) activities

Ummuhan O. Ozdemir [a, *], Firdevs İlbiz [a], Ayla Balaban Gunduzalp [a], Neslihan Ozbek [b], Zuhal Karagoz Genç [c], Fatma Hamurcu [d], Suat Tekin [e]

[a] Department of Chemistry, Faculty of Science, Gazi University, Ankara, Turkey
[b] Department of Chemistry, Faculty of Education, Ahi Evran University, Kırşehir, Turkey
[c] Department of Materials Engineering, Faculty of Engineering, Adıyaman University, Adıyaman, Turkey
[d] Department of Molecular Biology and Genetics, Faculty of Science, Bartın University, Bartın, Turkey
[e] Department of Physiology, Faculty of Medicine, Inonu University, Malatya, Turkey

ARTICLE INFO

Article history:
Received 1 May 2015
Received in revised form
26 July 2015
Accepted 27 July 2015
Available online 30 July 2015

Keywords:
Alkyl sulfonic acide hydrazide
Sulfonamide
Anticancer
Antibacterial
Anti carbonic anhydrase II isoenzyme
Computational studies

ABSTRACT

Methane sulfonic acide hydrazide, $CH_3SO_2NHNH_2$ (**1**), ethane sulfonic acide hydrazide, $CH_3CH_2SO_2NHNH_2$ (**2**), propane sulfonic acide hydrazide, $CH_3CH_2CH_2SO_2NHNH_2$ (**3**) and butane sulfonic acide hydrazide, $CH_3CH_2CH_2CH_2SO_2NHNH_2$ (**4**) have been synthesized as homologous series and characterized by using elemental analysis, spectrophotometric methods ($^1H-^{13}C$ NMR, FT-IR, LC-MS). In order to gain insight into the structure of the compounds, we have performed computational studies by using 6−311G(d, p) functional in which B3LYP functional were implemented. The geometry of the sulfonic acide hydrazides were optimized at the DFT method with Gaussian 09 program package. A conformational analysis of compounds were performed by using NMR theoretical calculations with DFT/B3LYP/6−311++G(2d, 2p) level of theory by applying the (GIAO) approach. The anticancer activities of these compounds on MCF-7 *human breast cancer cell line* investigated by comparing IC_{50} values. The antibacterial activities of synthesized compounds were studied against Gram positive bacteria; *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* NRRL-B-3711, *Enterococcus faecalis* ATCC 29212 and Gram negative bacteria; *Escherichia coli* ATCC 11230, *Pseudomonas aeruginosa* ATCC 15442, *Klebsiella pneumonia* ATCC 70063 by using the disc diffusion method. The inhibition activities of these compounds on carbonic anhydrase II enzyme (hCA II) have been investigated by comparing IC_{50} and Ki values. The biological activity screening shows that butane sulfonic acide hydrazide (**4**) has more activity than the others against tested breast cancer cell lines MCF-7, Gram negative/Gram positive bacteria and carbonic anhydrase II (hCA II) isoenzyme.

1. Introduction

The molecules of sulfonic acide hydrazide involve two pharmacophoric fragments: sulfonamide group and hydrazine residue. Some of them exhibit strong cytostatic activity [1,2], antibacterial [3], anti-inflammatory and analgesic [4,5] activity, as well as carbonic anhydrase activating properties [6]. Special attention has been paid to the relationship between the cytotoxic effect of sulfonamides and their enzyme (carbonic anhydrases, cyclooxygenase-2 and dihydrofolate reductase) inhibitory activity [7–9]. Sulfonamides are of considerable interest and have been used as pharmaceutical agents for the treatment of different diseases such as infections [10], Alzheimer's disease [11] and HIV [12]. Sulfa drugs are still widely used for conditions such as acne and urinary tract infections, and are receiving renewed interest for the treatment of infections caused by bacteria resistant to other antibiotics. Also, a number of other activities, some of which have been recently observed, include endotelin antagonism, anti-inflammatory activity, tubular transport inhibition, insulin release and saluretic action, among others [13]. Due to Sulfonamides/sulfonylhydrazines/sulfonylhydrazones significant pharmacology applications and widespread use in medicine, these compounds have gained importance

* Corresponding author.
E-mail address: ummuhan@gazi.edu.tr (U. O. Ozdemir).

http://dx.doi.org/10.1016/j.molstruc.2015.07.054
0022-2860/

in bioinorganic and metal based drug chemistry.

In our previous studies, we reported the antibacterial and cytotoxic effect of methane sulfonic acid hydrazide ($CH_3SO_2NHNH_2$) and its sulfonylhydrazone derivatives [14,15], as well as its metal complexes [16,17]. Methane, ethane and propane sulfonylhydrazone derivatives and their transition metal complexes were synthesized and screened for their antimicrobial activities [18–22]. Furthermore, ethanesulfonylhydrazone derivatives and their transition metal complexes, and different aromatic/heteroaromatic sulfonylhydrazone derivatives were investigated for inhibitory effects on carbonic anhydrase II (hCA II) enzyme [21,23].

As part of the ongoing studies, different alkyl sulfonyl hydrazides (compounds **1**–**4**) (Alkyl = methane, ethane, propane, butane) were synthesized. The structure of compound **1**was reported in previous work [14] and also its conformational analysis and vibrational spectroscopic investigation was reported before [24]. Compounds **2**–**4** were synthesized for the first time and characterized by using elemental analysis, $^1H/^{13}C$ NMR, FT-IR, LC–MS, spectroscopic methods. Gaussian 09 soft ware was used to obtain the most stable conformation of the compounds **1**–**4** based on DFT/B3LYP/6-311G (d,p) method. The geometrical parameters (bond lengths, bond angles and torsion angles) and electronic parameters (HOMO-LOMO energies) were calculated by this quantum set. NMR calculations were performed by 6-311++G (2d, 2p).

The anticancer activities of these compounds on MCF-7 *human breast cancer cell line* were investigated by comparing IC_{50} values. The antibacterial activities of synthesized compounds were studied against Gram positive bacteria; *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* NRRL-B-3711, *Enterococcus faecalis* ATCC 29212 and Gram negative bacteria; *Escherichia coli* ATCC 11230, *Pseudomonas aeruginosa* ATCC 15442, *Klebsiella pneumonia* ATCC 70063 by using the disc diffusion method. In addition, the inhibition activities of these compounds on carbonic anhydrase II enzyme (hCA II) have been investigated by comparing IC_{50} and Ki values.

2. Experimental

2.1. Physical measurements

The solvents used were purified and distilled according to routine procedures. Methane/ethane/propane and butane sulfonyl chloride, hydrazine hydrate were commercial products (purum). Elemental analyses were performed according to standard micro analytical procedures by Leco CHNS-932, ^1H and ^{13}C NMR spectra of dimethylsulfoxide-d_6 (DMSO-d_6) solutions of the compounds were registered on a Bruker WM-400 spectrometer (400 MHz) using tetra methyl silane as internal standard. The infrared spectra of the compounds as KBr-disks were recorded in the range of 4000–400 cm^{-1} with a Mattson 1000 FT-IR spectrometer. UV–Vis spectra were recorded on UNICAM-UV 2-100 spectrophotometer. Melting points of alkyl sulfonic acide hydrazides were determined with a Gallenkamp melting point apparatus. The anticancer activities of these compounds on MCF-7 *human breast cancer cell line* investigated by comparing IC_{50} values. The disc diffusion method were used to determine the antibacterial activity of compounds against Gram positive bacteria; *S. aureus* ATCC 6538, *B. subtilis* ATCC 6633, *B. cereus* NRRL-B-3711, *E. faecalis* ATCC 29212 and Gram negative bacteria; *E. coli* ATCC 11230, *P. aeruginosa* ATCC 15442, *K. pneumonia* ATCC 70063. The inhibition activities of synthesized compounds on carbonic anhydrase II (hCAII) were investigated by comparing K_i and IC_{50} values.

2.2. General procedure for the synthesis

The nucleophilic substitution reactions of the hydrazine hydrate with various alkyl sulfonyl chloride (alkyl = methane, ethane, propane, butane) were carried out as follows [14,22]: An ethanol solution of the alkyl sulfonyl chloride, R-SO_2Cl (0.12 mol) was added dropwise to the ethanol solution of hydrazine hydrate (0.62 mol), maintaining the temperature between 10 and 12 °C. Then, the reaction mixture was stirred for 1 h at room temperature. After the completion of the reaction, the solvent was removed under vacuum and the viscose residue was taken to ether phase using a continuous extraction method in 5 days. Then the ether was removed with rotary evaporator. The resulting product recrystallized from ethyl acetate and then allowed to stand in the freeze and crystals were obtained after a few weeks.

2.3. Computational section

Molecular geometry optimizations, geometrical parameters such as bond lengths, bond angles and torsion angles and electronic parameters consist of HOMO-LUMO energies were examined with Becke's three-parameter exchange functional [25] in combination with the Lee–Yang–Parr correlation functional (B3LYP) [26] density functional theory (DFT)method with 6-311G (d, p) basis set by using the Gaussian 09 program package [27,28]. NMR chemical shifts were calculated by DFT/B3LYP/6–311++G(2d, 2p) method.

2.4. Biological activity

2.4.1. Cell culture and cytotoxicity determination

The cells were incubated under 5% CO_2/air at 37 °C conditions at Nuaire humidified carbon dioxide incubator (Playmouth, MN, USA). Cells' state was controlled by inverted microscope (Soif Optical Inc. China) and results are expressed as Mean ± STD. Statistical analysis and comparison between mean values for cytotoxicity were performed by Tukey variance analysis (SPSS 10.0 for Windows; Chicago, IL, USA).

A colorimetric cell viability assay under usage of the tetrazole 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) was used to evaluate the cytotoxic effects of the test compounds [29]. MCF-7 cancer cell line were grown as monolayer culture in a high glucose concentration (4.5 g/l) DMEM medium supplemented with 10% fetal calf serum (FCS), 1% L-glutamine (200 mM), 1% of mixture penicillin (100 IU/ml) and streptomycin (100 lg/ml) incubated at 37 °C in an atmosphere of 5% CO_2–95% air mixture. Briefly, 5×10^4 MCF-7 tumor cells were plated in triplicate in 96-well flat bottom tissue culture plates, and treated with different concentrations of drugs for the time indicated. MTT (0.005 g = mL in phosphate buffer saline) was added to the cell culture and incubated for 4 h at 37 °C in 5% CO_2 humidified incubator. The formazan crystals formed during the reaction of active mitochondria with MTT, were dissolved in 0.04 N (100 mL) in isopropanol and readings were taken in a microtiter plate reader using a 570 nm filter. Cytotoxic activity results was expressed as the IC_{50} (μM) and Docetaxel was used as positive control.

2.4.2. Procedure for antibacterial activity

The in vitro antibacterial activity of the alkyl sulfonic acide hydrazide were tested against the Gram positive bacteria; *S. aureus* ATCC 25923, *B. subtilis* RSKK 244, *Bacillus magaterium* RSKK 5117 and Gram negative bacteria, *Salmonella enteritidis* ATCC 13076, *E. coli* ATCC 11230 by using the disc diffusion method. The *Bacteria* cultures were obtained from Gazi University, Biology Department. Bacterial strains were cultured overnight at 310 K in Nutrient Broth. During the survey, these stock cultures were stored in the dark at

Table 1
Analytical and physical data for alkyl sulfonic acide hidrazides.

Compound	Empirical formula (formula weight)	Color	m.p. (°C)	Yield (%)	Found (calculated)			
					%C	%H	%N	%S
1	$CH_6N_2SO_2$ 110.05 g/mol	White	50–51	50	10.80 (10.91)	5.25 (5.45)	25.35 (25.45)	28.50 (29.09)
2	C_2H_8 N_2SO_2 124.14 g/mol	White	40–42	60	19.03 (19.36)	6.15 (6.45)	22.04 (22.58)	24.65 (25.81)
3	C_3H_{10} N_2SO_2 138.18 g/mol	White	40–41	65	25.92 (26.07)	7.10 (7.29)	19.85 (20.27)	22.80 (23.20)
4	C_4H_{12} N_2SO_2 152.31 g/mol	White	45–48	70	30.90 (31.58)	7.09 (7.89)	17.38 (18.42)	20.25 (21.05)

277 K. The inocula of microorganisms were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity.

The alkyl sulfonic acide hydrazides were dissolved in dimethylsulfoxide (20% DMSO) to a final concentration of 5.0 mg/mL and sterilized by filtration by 0.45 μm millipore filters. Antimicrobial tests were then carried out by the disc diffusion method using 100 μL of suspension containing 10^8 CFU mL^{-1} bacteria spread on a nutrient agar (NA) medium. The discs (6 mm in diameter) were impregnated with 20 μL of each compound (100 μg/disc) at the concentration of 5.0 mg/mL and placed on the inoculated agar. DMSO impregnated discs were used as negative control. Sulfamethoxazole (300 μg/disk) and sulfisoxazole (300 μg/disk) were used as positive reference standards to determine the sensitivity of one strain/isolate in each microbial species tested. The inoculated plates were incubated at 37 °C for 24 h for bacterial strains isolates. Antimicrobial activity in the disc diffusion assay was evaluated by measuring the zone of inhibition against the test organisms. Each assay in this experiment was repeated twice. Percentage of inhibition by comparing distance of the compounds to the positive control using (Sulfamethoxazole) the equation below [30].

$$\%\ Inhibition = \left[\frac{diameter\ of\ the\ sample}{diameter\ of\ the\ positive\ control}\right]\cdot 100$$

2.4.3. Procedure for hCA II enzyme inhibitor activity

Carbonic anhydrase activity was assayed by the hydrolysis of p-nitrophenylacetate [31]. IC_{50} and Ki values of compounds were determined on hCA II enzyme. Acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide) **AAZ**, a clinically used in hCA II inhibition has also been investigated as standard inhibitor.

In order to determine IC_{50} values, 100 μL of 3.0 mM p-nitrophenylacetate as substrate and four different concentrations (3×10^{-2}; 3×10^{-3}; 5×10^{-4}; 3×10^{-4} M) of inhibitors were used. Reaction was started by adding of 170 μL of 0.05 M tris-SO_4 buffer (pH: 7.6) and 0.1 μL enzyme solution for total volume of 300 μL. The absorbance was determined at 348 nm after 6 min [32]. This study was repeated three times for each inhibitor. In order to determine IC_{50} values, Graphs were drawn by using inhibition % values by a statistical packing program on a computer. The IC_{50} concentrations of the compounds were determined from graphs [33]. This method was applied to determine K_i values. In the media with or without inhibitor, the substrate concentrations were 0.3, 0.6, 1.0, 3.0 mM. For this aim, inhibitor solutions were used for the reaction medium in four different concentrations (3×10^{-2}; 3×10^{-3}; 5×10^{-4}; 3×10^{-4} M). The Linewear-Burk graphs were obtained and K_i values were calculated according to *Cheng Prusoff equation*.

3. Results and discussion

Analytical data and some physical properties of the alkyl sulfonic acide hydrazides are summarized in Table 1. The general synthetic route used to prepare the compounds are illustrated in Figs. 1–2. The exothermic nucleophilic substitution reaction of corresponding alkyl sulfonyl chloride with hydrazine hydrate were employed to form alkyl sulfonic acide hydrazides (methane sulfonic acide hydrazide (**1**), ethane sulfonic acide hydrazide (**2**), propane sulfonic acide hydrazide (**3**) and butane sulfonic acide hydrazide (**4**)).

The geometry optimization was performed on DFT/B3LYP/6-311G(d, p) methods with Gaussian 09 program. Optimized structure of the sulfonic acide hydrazides are given in Fig. 3. The calculated geometrical parameters of these possible stable conformers for compounds **1**–**4** are presented in Tables 2–5 with respect to the bond lengths, bond angles and torsion angles.

3.1. Characterization of compounds

3.1.1. NMR spectra

^1H–^{13}C NMR spectra of compounds **1**–**4** were obtained in

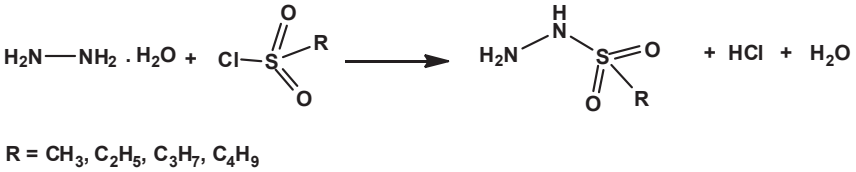

Fig. 1. Preparation of different alkyl sulfonic acid hydrazide (**1**–**4**).

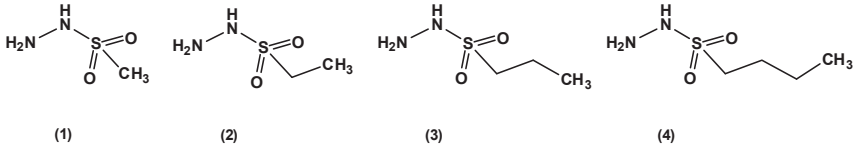

Fig. 2. Structures of alkyl sulfonic acid hydrazides (**1**–**4**) as homologous series.

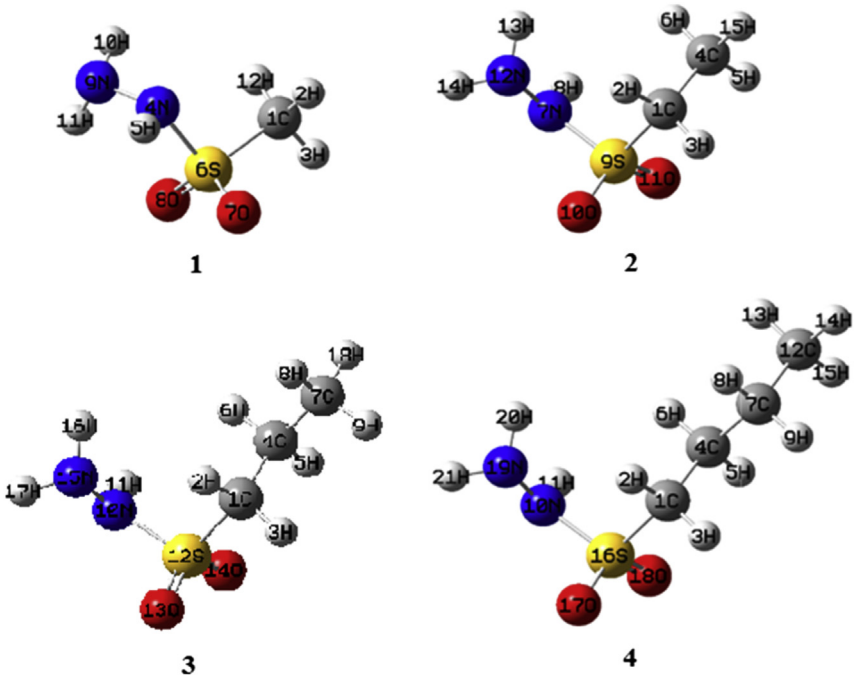

Fig. 3. Optimized geometries of the alkyl sulfonic acid hydrazides (**1**–**4**).

Table 2
Optimized geometric parameters of compound **1** by using B3LYP-6-311G(d,p) basic set.

Bond lengths (Å)		Bond angles (°)		Torsion angles (°)	
9N–10H	1.0140	10H–9N–11H	110.3739	10H–9N–4N–15H	−25.3404
9N–11H	1.0126	10H–9N–4N	112.0571	11H–9N–4N–15H	97.4290
9N–4N	1.4045	11H–9N–4N	109.4309	10H–9N–4N–6S	111.5934
4N–5H	1.0147	9N–4N–5H	118.5500	11H–9N–4N–6S	−125.6372
4N–6S	1.6987	9N–4N–6S	116.0504	9N–4N–6S–7O	62.2019
6S–7O	1.4538	4N–6S–7O	108.0207	9N–4N–6S–8O	−167.2273
6S–8O	1.4617	4N–6S–8O	103.4225	9N–4N–6S–1C	−52.0349
6S–1C	1.8177	7O–6S–8O	103.4225	5H–4N–6S–7O	−157.9417
1C–2H	1.0918	7O–6S–1C	106.0981	5H–4N–6S–8O	153.1209
1C–12H	1.0700	8O–6S–1C	109.4772	5H–4N–6S–1C	−98.7782
1C–3H	1.0923	6S–1C–2H	104.9729	4N–6S–1C–2H	56.9062
		6S–1C–3H	103.7590	4N–6S–1C–3H	170.6086
		6S–1C–12H	113.8230	4N–6S–1C–12H	−67.3870

Table 3
Optimized geometric parameters of compound **2** by using B3LYP-6-311G(d,p) basic set.

Bond lengths (Å)		Bond angles (°)		Torsion angles (°)	
12N–13H	0.9985	13H–12N–14H	108.8825	13H–12N–7N–8H	47.7103
12N–14H	0.9993	13H–12N–7N	106.1289	14H–12N–7N–8H	163.4455
12N–7N	1.4499	14H–12N–7N	106.1143	12N–7N–9S–10O	57.1721
7N–8H	1.0027	12N–7N–8H	107.8193	12N–7N–9S–11O	−173.9170
7N–9S	1.7726	12N–7N–9S	119.4341	12N–7N–9S–1C	−58.1511
9S–10O	1.4586	7N–9S–10O	107.4512	7N–9S–1C–2H	66.6720
9S–11O	1.4589	7N–9S–11O	104.9825	7N–9S–1C–3H	179.8091
9S–1C	1.8163	8H–7N–9S	114.7419	9S–1C–4C–15H	−177.0512
1C–2H	1.1097	10O–9S–1C	108.4132	9S–1C–4C–5H	−55.2836
1C–3H	1.1105	11O–9S–1C	109.7618	9S–1C–4C–6H	−126.7633
1C–4C	1.5183	9S–1C–2H	110.3219	2H–1C–4C–15H	57.6746
4C–15H	1.0700	9S–1C–3H	108.4143	2H–1C–4C–5H	179.4422
4C–6H	1.1091	9S–1C–4C	116.1186	2H–1C–4C–6H	−63.9219
4C–5H	1.1095	2H–1C–14C	108.9658	3H–1C–4C–15H	−54.7707
4C–15H	1.0700	3H–1C–14C	108.4169	3H–1C–4C–5H	66.9969
		1C–4C–15H	111.2333	3H–1C–4C–6H	−176.3672
		1C–4C–5H	110.1261		
		1C–4C–6H	110.4466		

Table 4
Optimized geometric parameters of compound **3** by using B3LYP-6-311G(d,p) basic set.

Bond lengths (Å)		Bond angles (°)		Torsion angles (°)	
15N–16H	0.9983	16H–15N–17H	108.8946	17H–15N–10N–11H	158.8473
15N–17H	0.9993	10N–15N–16H	106.2943	16H–15N–10N–12S	176.2359
15N–10N	1.4490	10N–15N–17H	105.9997	17H–15N–10N–12S	−67.9912
10N–11H	1.0026	15N–10N–11H	108.0264	15N–10N–12S–13O	−121.5073
10N–12S	1.7735	15N–10N–12S	119.2989	15N–10N–12S–14O	−168.1788
12S–13O	1.4582	11H–10N–12S	114.5713	11H–10N–12S–14O	−37.8816
12S–14O	1.4595	16H–15N–10N	106.2943	11H–10N–12S–13O	−166.6337
12S–1C	1.8151	10N–12S–13O	107.4813	10N–12S–1C–2H	54.0249
1C–2H	1.1097	10N–12S–14O	104.8960	10N–12S–1C–13H	167.3003
1C–3H	1.1106	130-12S–14O	119.9841	10N–12S–1C–4C	−70.5446
1C–4C	1.5189	10N–12S–1C	104.6616	13O–12S–1C–2H	−60.4432
4C–5H	1.1091	12S–1C–4C	115.7496	13O–12S–1C–3H	52.8322
4C–6H	1.1087	12S–1C–2H	110.6630	13O–12S–1C–4C	174.9874
4C–7C	1.5130	12S–1C–3H	108.6220	14O–12S–1C–4C	−19.7980
7C–8H	1.0983	2H–1C–4C	108.9587	12S–1C–4C–7C	−177.3669
7C–9H	1.0983	14O–12S–1C	110.2971	12S–1C–4C–6H	60.8825
7C–18H	1.0976	13O–12S–14O	108.3884	12S–1C–4C–5H	−129.3138
		3H–1C–4C	108.3935	2H–1C–4C–7C	57.1870
		1C–4C–7C	111.3058	2H–1C–4C–6H	−64.5636
		1C–4C–5H	110.0246	2H–1C–4C–5H	179.1553
		1C–4C–6H	110.0904	3H–1C–4C–7C	−55.0904
		6H–4C–5H	105.8470	3H–1C–4C–5H	66.8779
		6H–4C–7C	109.6100	3H–1C–4C–6H	−176.8410
		5H–4C–7C	109.8175	1C–4C–7C–18H	−179.7049
		4C–7C–8H	111.7956	1C–4C–7C–8H	−59.7845
		4C–7C–9H	111.7314	1C–4C–7C–9H	60.4768
		4C–7C–18H	111.0259	6H–4C–7C–18H	127.7377
		8H–7C–18H	107.4038	6H–4C–7C–8H	62.2449
		9H–7C–18H	107.3560	6H–4C–7C–9H	−177.4939
				5H–4C–7C–18H	58.2069
				5H–4C–7C–9H	−61.6115
				5H–4C–7C–8H	178.1273

Table 5
Optimized geometric parameters of compound **4** by using B3LYP-6-311G(d,p) basic set.

Bond lengths (Å)		Bond angles (°)		Torsion angles (°)	
20H–19N	0.9985	20H–19N–21H	108.8792	20H–19N–10N–11H	47.6042
21H–19N	0.9993	20H–19N–10N	106.1350	20H–19N–10N–16S	−179.2285
19N–10N	1.4498	21H–19N–10N	44.8232	21H–19N–10N–11H	−22.8373
10N–11H	1.0026	19N–10N–11H	109.8331	21H–19N–10N–16S	−63.4982
10N–16S	1.7729	19N–10N–16S	119.3379	19N–10N–16S–17O	56.3370
16S–17O	1.4587	11H–10N–16S	114.6885	19N–10N–16S–18O	−174.7531
16S–18O	1.4588	10N–16S–17O	107.5211	19N–10N–16S–1C	−58.7766
16S–1C	1.8158	10N–16S–18O	105.1382	11H–10N–16S–17O	−173.4959
1C–2H	1.1098	10N–16S–1C	104.3330	11H–10N–16S–18O	−44.5859
1C–3H	1.1106	17O–16S–1C	108.5176	10N–16S–1C–2H	55.2414
1C–4C	1.5183	18O–16S–1C	39.2610	10N–16S–1C–3H	168.4877
4C–5H	1.1096	16S–1C–2H	110.5685	10N–16S–1C–4C	−69.3232
4C–6H	1.1093	16S–1C–3H	108.6187	17O–16S–1C–2H	−59.1693
4C–7C	1.5218	16S–1C–4C	115.6789	17O–16S–1C–3H	54.0770
7C–8H	1.1084	2H–1C–3H	103.7696	17O–16S–1C–4C	176.2660
7C–9H	1.1084	1C–4C–5H	110.1663	18O–16S–1C–2H	167.6423
7C–12C	1.5121	1C–4C–6H	110.2458	18O–16S–1C–3H	−79.1114
12C–13H	1.0980	1C–4C–7C	111.2749	16S–1C–4C–5C	−56.2041
12C–14H	1.0974	5H–4C–7C	109.7269	16S–1C–4C–6C	60.2030
12C–15H	1.0983	6H–4C–7C	109.4732	16S–1C–4C–7C	−178.1340
		4C–7C–12C	111.2523	2H–1C–4C–6H	−65.1418
		6H–4C–5H	105.8065	2H–1C–4C–5H	178.4511
		8H–7C–9H	105.5463	2H–1C–4C–7C	56.5212
		8H–7C–12C	109.9950	3H–1C–4C–5H	66.0699
		9H–7C–12C	109.9527	3H–1C–4C–6H	−177.5230
		7C–12C–14H	111.1841	3H–1C–4C–7C	−55.8600
		7C–12C–13H	111.6905	1C–4C–7C–12C	−179.7868
		7C–12C–15H	111.6715	1C–4C–7C–8H	−57.6377
		13H–12C–15H	36.3585	1C–4C–7C–9H	58.1765
		13H–12C–14H	107.3761	6H–4C–7C–8H	64.4738
		15H–12C–14H	107.3976	6H–4C–7C–9H	−178.7120
				6H–4C–7C–12C	−57.6752
				5H–4C–7C–8H	−179.8223
				5H–4C–7C–9H	121.8672
				5H–4C–7C–12C	58.0287
				4C–7C–12C–13H	60.3041
				4C–7C–12C–14H	−179.7723
				4C–7C–12C–15H	−59.8341

DMSO-d_6 at room temperature using TMS as an internal standard (Fig. 4). $^1H-^{13}C$ NMR Chemical shifts of the compounds **1**–**4** were calculated by Gauge-Independent Atomic Orbital (GIAO) method [34] at DFT/B3LYP/6-311++G(2d, 2p) level in DMSO. The experimental and calculated $^1H-^{13}C$ NMR assignments in DMSO-d_6 are listed in Tables 6 and 7.

The terminal C**H**$_3$ protons of alkyl chain for compounds **1**–**4** as $-C\mathbf{H}_3$ (three H intensities) are observed at 2.88 (3H, singlet), 1.18 ppm (3H, triplet), 0.98 (3H, triplet), 0.87 ppm (3H, triplet) and corresponding calculation values are 3.05 ppm, 1.15 ppm, 0.98 ppm, 1.00 ppm, respectively. CH_2 protons directly linked to terminal CH3 for compounds **3** and **4** as $-C\mathbf{H}_2-CH_3$ (two H intensities) are observed at 1.65 ppm (2H, multiplet) and 1.37 ppm (2H, multiplet) and corresponding calculation values are 1.58 ppm and 1.29 ppm, respectively. C**H**$_2$ protons between two C**H**$_2$ groups for compounds **4** as $CH_2-C\mathbf{H}_2-CH_2$ (two H intensities) are observed at 1.57 ppm (2H, multiplet) and corresponding calculation values are 1.29 ppm, respectively. And also C**H**$_2$ protons directly linked to SO_2 for compounds **2**–**4** as $-C\mathbf{H}_2-SO_2-$ (two H intensities) are observed at 3.11 ppm (2H, multiplet), 3.02 ppm (2H, triplet), 3.06 ppm (2H, triplet) and corresponding calculation values are 3.02 ppm, 3.14 ppm, 3.12 ppm, respectively. In addition, the experimental primer NH_2 protons and seconder NH protons shift of compounds **1**–**4** are observed in the range of 4.32–4.82 ppm and 7.67–7.81 ppm [35,36]. This results showed poor correlation with the calculated proton shifts (3.34–3.47 ppm and 4.48–5.29 ppm) in DMSO-d_6. This suggests that NH_2 and seconder NH protons have both intramolecular and intermolecular hydrogen bonding [37].

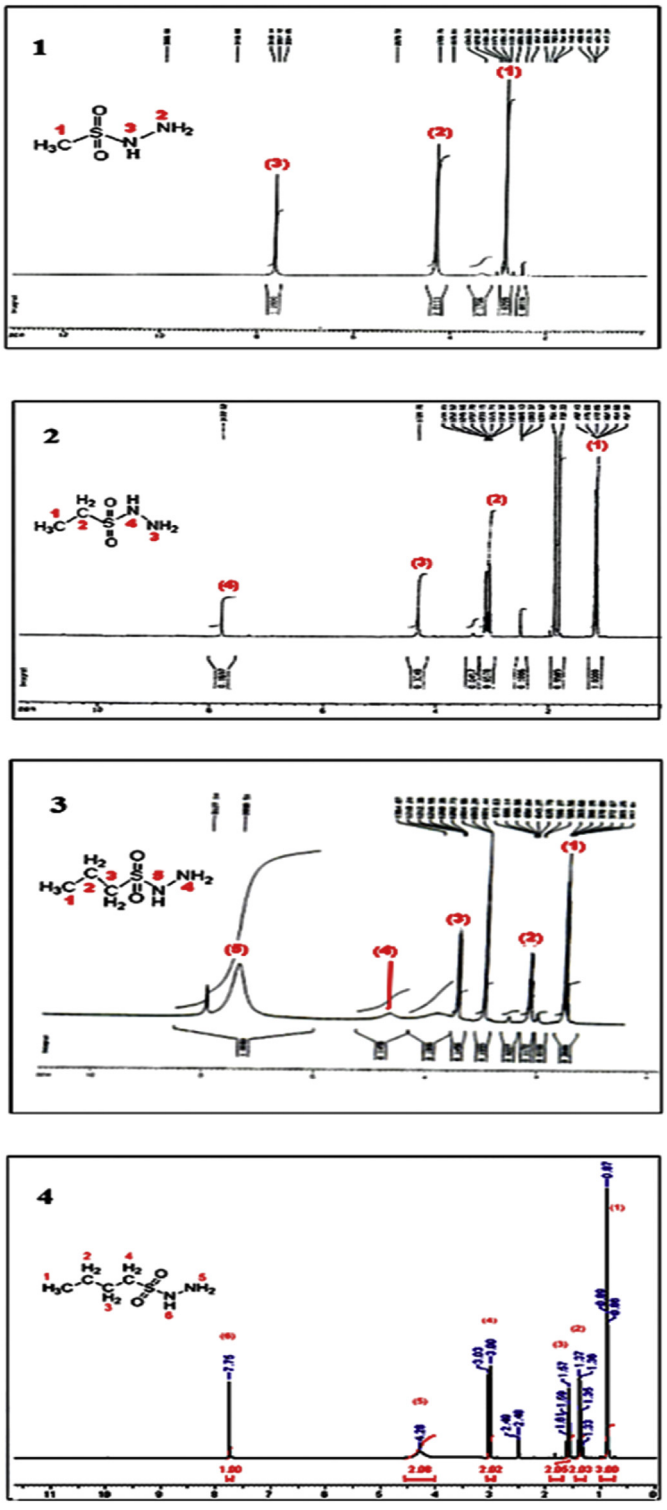

Fig. 4. ^1H NMR spectra of the alkyl sulfonic acid hydrazides (**1**–**4**).

C**H**$_3$ carbons of alkyl moiety, (C1) of compounds **1**–**4** are observed in the range of 8.24–49.50 ppm corresponding calculation values are 10.49–50.56 ppm, respectively. CH_2 carbons of alkyl moiety directly linked to terminal CH3 for compounds **3** and **4** are observed at 24.10 ppm and 21.38 ppm and corresponding calculation values are 23.13 ppm and 27.09 ppm. In addition, CH_2 carbons directly linked to SO_2 for compounds **2**–**4** are observed between 44.52 and 53.80 ppm and corresponding calculation values are between 49.35 and 56.87 ppm, respectively.

3.1.2. FT-IR spectra

The selected vibration frequencies of alkyl sulfonic acide hydrazides are listed in Table 8. The assignment of the bands was made by taking into consideration the literature data for compounds containing appropriate structural fragments; sulfonylhydrazines, methane/ethane/propane sulfonylhydrazones derivatives [7–10]. NH_2 vibrations in the prepared compounds **1**–**4** are observed between 3360 and 3249 cm^{-1} as double bonds. And also the presence of a single bands observed at 3210 cm^{-1} and 3211 cm^{-1} confirm the presence of secondary amine groups (NHs) for compounds **2** and **4**. The other NH vibrations in compound **1** and **3** could not be observed because of overlapping. υ_{as} (SO_2) and υ_s(SO_2) stretching vibrations are observed between 1321–1317 cm^{-1} and 1156–1133 cm^{-1} for compounds **1**–**4**.

3.1.3. LC/MS spectra

LC/MS shows that molecular ion peaks for compounds **1** ($[M]^+$), compound **2**($[M+H]^+$), compound **3** ($[M]^+$), ($[M-H]^+$) and compound **4** ($[2M + H]^+$) are exhibited at m/z; 110.1, 124.2, 138.3 and 304.80, respectively.

3.1.4. Frontier molecular orbital analysis

The highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) are very important for quantum chemistry [37,38]. HOMO and LUMO are the main orbitals in chemical stability. The HOMO represents the ability to donate an electron, LUMO as an electron acceptor representing the ability to obtain an electron. These orbitals play an important role in the electric and optical properties. The HOMO and LUMO are the main orbital taking part in chemical reaction. The HOMO energy is directly related to the ionization potential and LUMO energy is directly related to the electron affinity. The frontier molecular orbitals (HOMO and LUMO) are mostly the π-antibonding type molecular orbitals in the structure. The frontier molecular orbital distributions and energy levels of the HOMO-1, HOMO, LUMO and

Table 6
The experimental and theoretical ^{1}H NMR data (ppm) of the alkyl sulfonic acide hidrazides.

Assign.	1		2		3		4	
	δ(exp.)	δ(calc.)[a]	δ(exp.)	δ(calc.)[a]	δ(exp.)	δ(calc.)[a]	δ(exp.)	δ(calc.)[a]
C**H**$_3$	2.88 (s,3H)	3.05	1.18 (t,3H)	1.15	0.98 (t,3H)	0.98	0.87 (t,3H)	1.00
C**H**$_2$–CH$_3$	–	–	–	–	1.65 (m,2H)	1.58	1.37 (m, 2H)	1.29
–C**H**$_2$–	–	–	–	–	–		1.57 (m,2H)	1.46
SO$_2$–C**H**$_2$	–	–	3.11 (m,2H)	3.22	3.02 (t,2H)	3.14	3.06 (t, 2H)	3.12
N**H**$_2$	4.32 (s,2H)	3.47	4.32 (s,2H)	3.35	4.52 (s,2H)	3.34	4.82 (s,2H)	3.42
N**H**	7.67 (s,1H)	4.48	7.67 (s,1H)	5.29	7.81 (s,1H)	5.24	7.75 (s,1H)	5.23

H: hydrogen with chemical shift in ^{1}H- NMR.
[a] Calculated using GIAO/B3LYP/6311++G(2d, 2p) method.

Table 7
The experimental and theoretical ^{13}C NMR data (ppm) of the alkyl sulfonic acide hidrazides.

Assign.	1		2		3		4	
	δ(exp.)	δ(calc.)[a]	δ(exp.)	δ(calc.)[a]	δ(exp.)	δ(calc.)[a]	δ(exp.)	δ(calc.)[a]
CH$_3$	49.50	50.56	8.24	10.49	13.50	14.13	13.94	16.17
CH$_2$–CH$_3$	–	–	–	–	24.10	23.13	21.38	27.09
CH$_2$	–	–	–	–	–	–	25.29	32.14
SO$_2$–**C**H$_2$	–	–	44.52	49.35	53.80	56.87	47.17	55.76

H: hydrogen atom with chemical shift in ^{1}H-NMR.
[a] Calculated using GIAO/B3LYP/6311++G(2d, 2p) method.

Table 8
Wave number (cm^{-1}) of selected vibration of the alkyl sulfonic acide hidrazides.

Assign.	1	2	3	4
υ_{as}(NH$_2$)	3360m	3334s	3335m	3383m
υ_{s}(NH$_2$)	3300m	3276s	3300m	3249m
υ(NH)	–	3210s	–	3211w
υ_{as}(SO$_2$)	1320s	1317s	1321s	1317s
υ_{s}(SO$_2$)	1156s	1143s	1145s	1133s
δ(NH$_2$)	1640m	1637m	1625m	1639m
δ(SO$_2$)	526s	542m	526w	525
δ(NH)	650m	577m	635w	630

Table 9
Cytotoxicity compounds (**1**–**4**), docetaxel against MCF-7 cell lines.

Compound	IC$_{50}$ (μ mol L^{-1}) for MCF-7 cell lines
1	>100
2	>100
3	16.65
4	15.41
Docetaxel	7.24

Reference: Docetaxel.

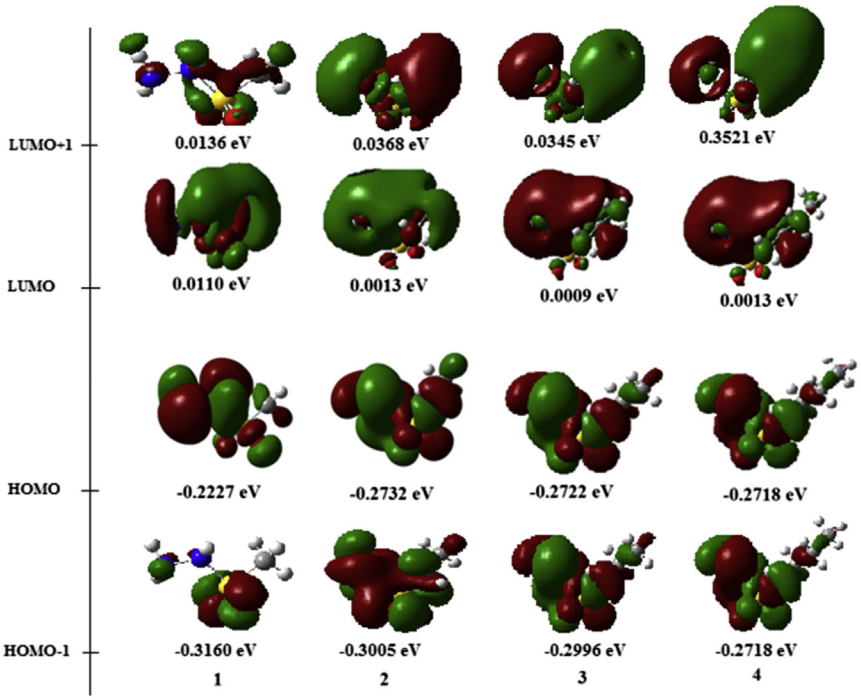

Fig. 5. HOMO and LUMO shapes of the alkyl sulfonic acid hydrazides (**1**–**4**).

Table 10
Inhibition zone values (mm, 100 μg/disc)of the alkyl sulfonic acide hydrazides by disc diffusion method.

Compound	Gram positive				Gram negative		
	B. subtilis ATCC	*B. cereus NRRL-B-*	*E. faecalis ATCC*	*S. aureus ATCC*	*P. aeruginosa ATCC*	*K .pneuonia ATCC*	*E. coli ATCC*
1	10	8	8	9	9	8	8
2	8	9	10	9	9	9	9
3	8	9	9	10	10	10	9
4	13	12	10	10	14	10	12
Sulfisoxazole	25	18	–	17	8	20	28
Sulfamethaxazole	15	15	–	18	17	17	24

Reference: Sulfisoxazole, Sulfamethaxazole.

Table 11
The result of inhibition studies for alkyl sulfonic acide hidrazides on carbonic anhydrase II.

Compound	hCA II	
	Esterase activity	
	IC_{50} (M)	Ki (M)
1	3.78×10^{-4}	$6.94 \cdot 10^{-5}$
2	3.44×10^{-4}	$6.44 \cdot 10^{-5}$
3	3.17×10^{-4}	$5.96 \cdot 10^{-5}$
4	3.10×10^{-4}	$5.80 \cdot 10^{-5}$
Acetazolamide	1.13×10^{-4}	$2.11 \cdot 10^{-5}$

Reference: Acetazolamide.

LUMO+1 orbitals, which computed at B3LYP/6-311G (d, p) level of the title molecule are shown in Fig. 5.

3.2. Biological studies

3.2.1. In vitro antitumor activity

To study the growth inhibitory effects of compounds **1**–**4** on breast cancer, MCF-7 cells were treated with compounds and the growth of the cells were examined with MTT assay. Table 9 illustrates the cytotoxic activities as 50% inhibitory concentration (IC_{50}) values. The compounds **1** and **2** have no significant inhibition of proliferation being observed at concentrations up to 100 μM. The compounds **3** and **4** elicited a rather similar cytotoxicity (average IC50 values of 16.65 μM and 15.41 μM). Compound **4**, having the highest activity in alkyl series, shows the closest activity against

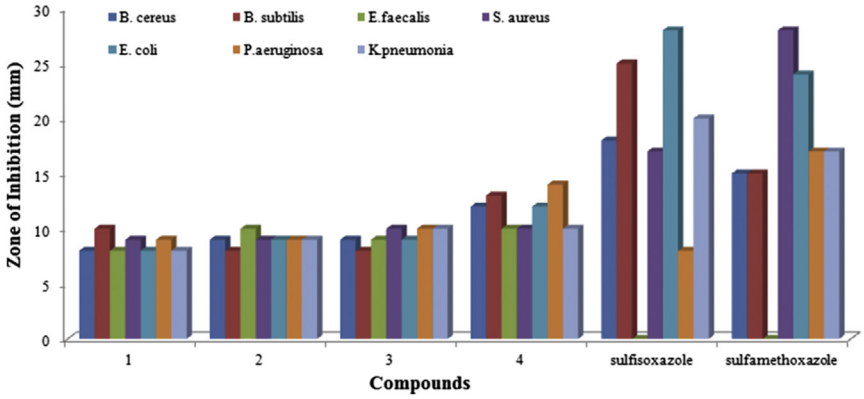

Fig. 6. Comparison of antibacterial activity of the alkyl sulfonic acid hydrazides (**1**–**4**) and antibiotics.

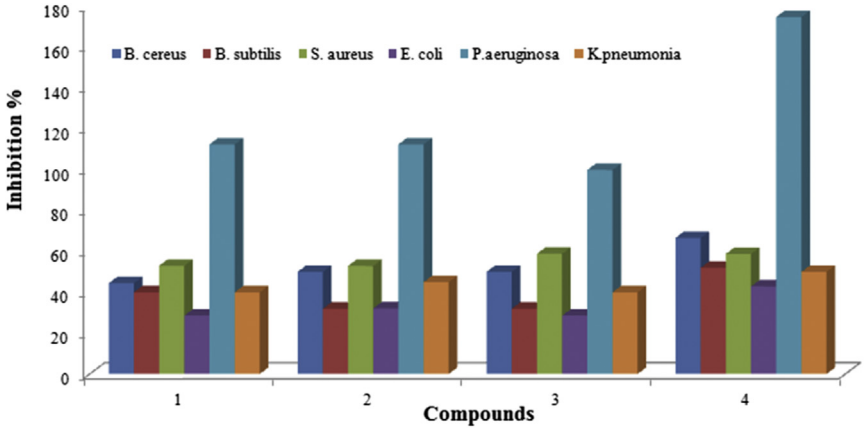

Fig. 7. Percentage of inhibition of the alkyl sulfonic acid hydrazides (**1**–**4**) against sulfisoxazol.

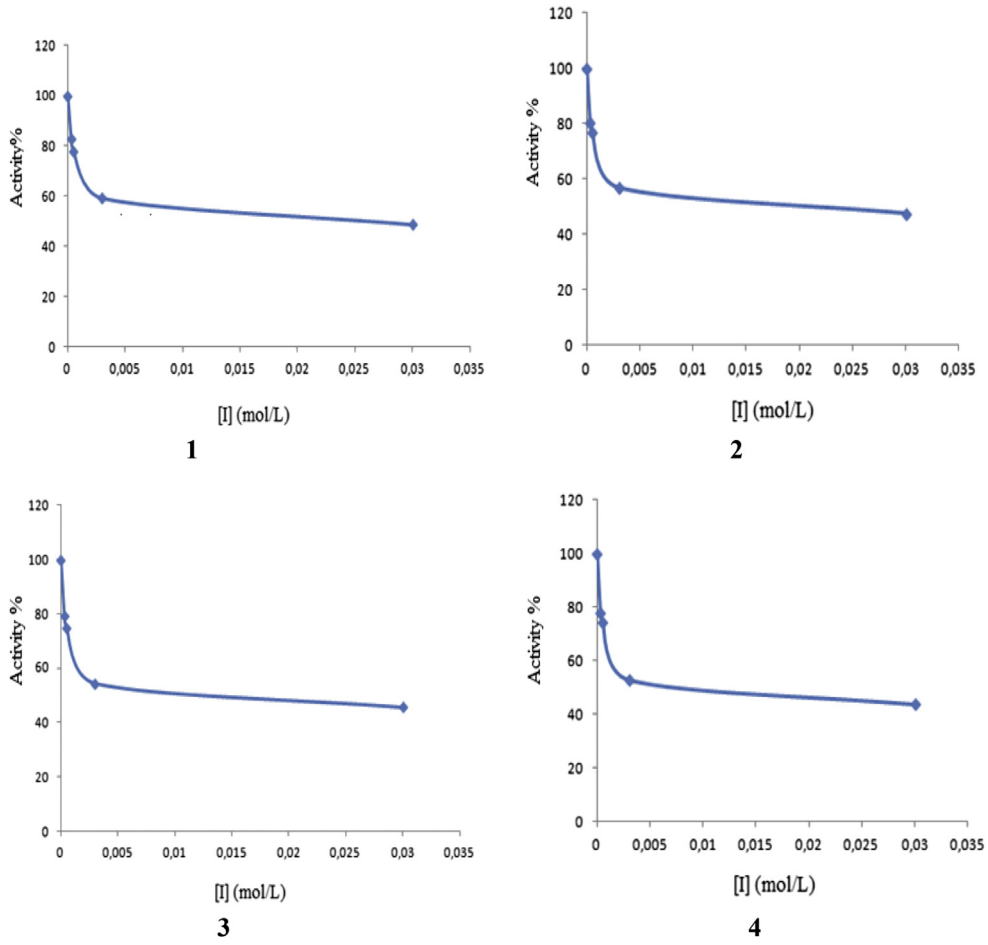

Fig. 8. Activity% vs [I] regression analysis graphs for hCA II in the presence of different concentrations of **1**–**4**.

standard docetaxel (average IC_{50} value of 7.24 μM). Viewing their structures, it can be assumed that the increasing methyl units provides enhanced activity.

3.2.2. Antibacterial activity results

The test compounds were screened in vitro for their antibacterial activities against four Gram-positive species (*S. aureus, B. subtilis B. cereus* and *E. faecalis*) and three Gram-negative species (*E. Coli, P. Aeruginosa and K. pneumonia*) by the disc diffusion method. The antibacterial results were given in Table 10 by disc diffusion and Table 11. The results were compared with those of the standard drugs as sulfamethoxazole and sulfisoxazole (Figs. 6 and 7). The size of the inhibition zone depends upon the culture medium, incubation conditions, rate of diffusion and the concentration of the antibacterial agent (the activity increases as the concentration increases). In the present study, the alkyl sulfonic acide hydrazides are active against both types of the Gram positive and Gram negative bacteria (Fig. 8).

As the disc diffusion assay results evidently show that compound **4** exhibits generally strong inhibition effect against tested bacteria whereas the other compound **1**–**3** have moderate activity (Table 10, Figs. 6 and 7). The alkyl sulfonic acide hydrazides show the highest activities against Gram positive bacteria; *E. Faecalis* in the diameter zone of 8–10 mm whereas sulfisoxazole and sulfamethaxazole, the drug used as standard, have been found inactive. This result is interesting that the compounds **1**–**4** have selectivity against *E. Faecalis.*

Percentage of inhibition for the compounds exhibited in Fig. 7 that is expressed as excellent activity (120–200% inhibition), good activity (90–100% inhibition), moderate activity (75–85% inhibition), significant activity (50–60% inhibition), negligible activity (20–30% inhibition) and no activity [39]. As seen in Fig. 7, the compound **4** (200%) Show excellent activity against bacteria especially *P. aeruginosa* (Sulfisoxazole is accepted 100% inhibition).

The results obtained by the disc diffusion method indicates that the number of carbon atoms in alkyl sulfonic acide hydrazide (Fig. 7) may play an important role in the antibacterial activity. The highest antibacterial activity was observed with an aliphatic carbon chain of compound **4**.

3.2.3. hCA II inhibition result

In this study, the aim was to determine the inhibitory effects of the alkyl sulfonic acide hydrazides. The inhibitory effects of new compounds were evaluated by using IC_{50} (IC_{50} represents the molarity of inhibition as 50% decrease of enzyme activity) and K_i (inhibitor–enzyme dissociation constant) values which are two of the most appropriate parameters of the inhibitors (Table 8) [40]. Acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide) (AAZ) has also been investigated as standard inhibitor, clinically used against CA II. As seen in Table 8 (Fig. 6a and b), the compounds behave as inhibitors against hCA II enzyme. Compound **4** shows enhanced inhibition effect (Ki: 7.96×10^{-6} M, IC_{50}: 3.10×10^{-4}) on hCA II than the other compound. This is probably due to the further effect of the length of an alkyl chain that alters the lipophilicity of a

lead and its ability to cross the cell membrane on the histidine residue in the active site of CA II enzyme [41].

Alkyl groups are very often investigated as different R-groups during drug discovery. The various alkyl groups tend to be easy to prepare and provide useful information. Analog series are made by repeatedly adding an extra carbon to an alkyl chain and are called homologues. If the homologues have a longer and longer chain with no branching, they are called a homologous series. Within a homologous series, activity often increases to a maximum as a side chain is lengthened. This behavior is often seen in both biochemical and cell-based analysis.

Increasing the length of an alkyl chain changes the lipophilicity of a lead and its ability to cross the cell membrane. In many of the homologous series, the lipophilicity has an optimal value. If lipophilicity is too low, the compound hardly crosses the membranes and if the lipophilicity is too high, the compound enters the membrane easily. Testing a homologous series can help discover the suitable lipophilic properties of a lead. This activity trend has less to do with target binding, which can be determined through a biochemical assay. Instead, the trend is related to the ability of the lead to reach the target [40].

4. Conclusions

In this study, the researchers have reported the synthesis of alkyl sulfonic acide hydrazides. The structural characterizations of the synthesized compounds were made by using the elemental analyses and spectroscopic methods. Based on physicochemical evidence, the proposed structure of compounds **1**–**4** are exhibited in Fig. 2. The biological activity screening shows that compound **4** has high inhibition effect against breast cancer cell lines MCF-7, tested bacteria and hCA II enzyme. The remarkable activity of compound **4** may be arising from increasing methyl units, which may play an important role in biological activities. Changing the alkyl chain increases the lipophilicity and easily passes the cell membrane.

Acknowledgments

This research was supported by Gazi University Research Fund under Project No 05/2010-92. We thank Ankara University, Faculty of Pharmacy. Central Labs. for allocation of time at the Mass Spectra and Elemental Analyses.

References

[1] H.A. Seow, P.G. Penketh, K. Shyam, S. Rockwell, A.C. Sartorelli, 1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-[[1-(4-nitrophenyl)ethoxy]carbonyl] hydrazine: an anticancer agent targeting hypoxic cells, Proc. Natl. Acad. Sci. 102 (2005) 9282–9287.
[2] A. Kamal, M.N.A. Khan, K.S. Reddy, K. Rohini, Synthesis of a new class of 2-anilino substituted nicotinyl arylsulfonylhydrazides as potential anticancer and antibacterial agents, Bioorg. Med. Chem. 15 (2007) 1004–1013.
[3] A.M. Al-Azzawi, M.S.A. Razzak, Synthesis, characterization and antibacterial activity of several new Schiff bases linked to phthalimide Moiety Kerbala, J. Pharm. Sci. 2 (2011) 124–133.
[4] S.M. Sondhi, M. Dinodia, A. Kumar, Synthesis, anti-inflammatory and analgesic activity evaluation of some amidine and hydrazone derivatives, Bioorg. Med. Chem. 14 (2006) 4657–4663.
[5] A. Ignat, V. Zaharia, C. Mogoşan, N. Palibroda, C. Cristea, L. Silaghi-Dumitrescu, Heterocycles, microwave assisted synthesis of some p-toluenesulfonyl-hydrazinothiazoles with analgesic and anti-inflammatory activity, Farmacia 58 (2010) 290–302.
[6] M.R. Abdo, D. Vullo, M.C. Saada, J.L. Montero, A. Scozzafava, J.Y. Winum, et al., Carbonic anhydrase activators: activation of human isozymes I, II and IX with phenylsulfonylhydrazido-l-histidine derivatives, Bioorg. Med. Chem. Lett. 19 (2009) 2440–2443.
[7] C.T. Supuran, A. Casini, A. Mastrolorenzo, A. Scozzafava, COX-2 selective inhibitors, carbonic anhydrase inhibition and anticancer properties of sulfonamides belonging to this class of pharmacological agents, Mini Rev. Med. Chem. 4 (2004) 625–632.
[8] S.M.I. Morsy, A.M. Badawi, A. Cecchi, A. Scozzafava, C.T. Supuran, Carbonic anhydrase inhibitors. Biphenylsulfonamides with inhibitory action towards the transmembrane, tumor-associated isozymes IX possess cytotoxic activity against human colon, lung and breast cancer cell lines, J. Enzyme Inhib. Med. Chem. 24 (2009) 499–505.
[9] S.M. Marques, E.A. Enyedy, C.T. Supuran, N.I. Krupenk, S.A. Krupenko, Pteridine-sulfonamide conjugates as dual inhibitors of carbonic anhydrases and dihydrofolate reductase with potential antitumor activity, Bioorg. Med. Chem. 18 (2010) 5081–5089.
[10] W. deKeizer, M.E. Bienenmann-Ploum, A.A. Bergwerff, W. Haasnoot, Flow cytometric immunoassay for sulfonamides in raw milk, Anal. Chim. Acta 620 (2008) 142–149.
[11] A. Innocenti, A. Maresca, A. Scozzafava, C.T. Supuran, Carbonic anhydrase inhibitors: thioxolone versus sulfonamides for obtaining isozyme-selective inhibitors, Bioorg. Med. Chem. Lett. 18 (2008) 3938–3941.
[12] K.K. Sahu, V. Ravichandran, V.K. Mourya, R.K. Agrawal, QSAR analysis of caffeoyl naphthalene sulfonamide derivatives as HIV-1 integrase inhibitors, Med. Chem. Res. 15 (2007) 418–430.
[13] M.E. Wolff, Burger's Medicinal Chemistry and Drug Discovery, Wiley, Laguna Beach, 1996.
[14] N.I. Dodoff, U. Ozdemir, N. Karacan, M. Georgieva, S.M. Konstantinov, M.E. Stefanova, Schiff Bases of methanesulfonylhydrazine. Synthesis, spectroscopic characterization, conformational analysis biological activity, Z. Naturforsch 54 (1999) 1553–1562.
[15] U.O. Ozdemir, N. Akkaya, N. Özbek, New Ni(II), Pd(II), Pt(II) complexes with aromatic methanesulfonylhydrazone based ligands. Synthesis, spectroscopic characterization and in vitro antibacterial evaluation, Inorg. Chim. Acta 400 (2013) 13–19.
[16] U. Ozdemir, O.S. Senturk, S. Sert, N. Karacan, F. Uğur, Reaction of metal Carbonyls with 2-hydroxy-1-napthaldehyde methanesulfonylhydrazone and characterization of the substitution products, J. Coord. Chem. 59 (2006) 1905–1911.
[17] U. Ozdemir, O.S. Senturk, S. Sert, N. Karacan, F. Uğur, Photochemical reactions of metal carbonyls [M(CO)(6) (M=Cr, Mo and W), Re(CO)(5)Br, Mn(CO)(3)Cp] with 2-hydroxyacetophenone methanesulfonylhydrazone (apmsh), Trans. Met. Chem. 28 (2003) 443–446.
[18] O.S. Senturk, S. Sert, U. Ozdemir, Synthesis and characterization of metal carbonyls [$M(CO)_6$ (M = Cr, Mo, W), $Re(CO)_5Br$ and $Mn(CO)_3Cp$] with 2-hydroxy-1-napht aldehyde ethanesulfonylhydrazone (nafesh), Z. Naturforsch 58 (2003) 1124–1127.
[19] O.S. Senturk, U. Ozdemir, S. Sert, N. Karacan, F. Uğur, Photochemical reactions of metal carbonyls [M(CO)6 (M = Cr, Mo, W), Re(CO)5Br, Mn(CO)3Cp] with salicyl- aldehydeethanesulfonylhydrazone (Hsalesh), J. Coord. Chem. 60 (2007) 229–235.
[20] U. Ozdemir, P. Güvenç, E. Şahin, F. Hamurcu, Synthesis, characterization and antibacterial activity of new sulfonamide derivatives and their nickel(II), cobalt(II) complexes, Inorg. Chim. Acta 362 (2009) 2613–2618.
[21] U. Ozdemir, F. Arslan, F. Hamurcu, Synthesis, characterization, antibacterial activities and carbonic anhydrase enzyme inhibitor effects of new arylsulfonylhydrazone and their Ni(II), Co(II) complexes, Spectrochim. Acta Part A 75 (2010) 121–126.
[22] U.O. Ozdemir, G. Olgun, Synthesis, characterization and antibacterial activity of new sulfonyl hydrazone derivatives and their nickel(II) complexes, Spectrochim. Acta Part A 70 (2008) 641–645.
[23] U.Ozmen Ozdemir, A. Altuntaş, A. Balaban Gündüzalp, F. Arslan, F. Hamurcu, New aromatic/heteroaromatic propanesulfonylhydrazone compounds: synthesis, physical properties and inhibition studies against carbonic anhydrase II (CAII) enzyme, Spectrochim. Acta Part A 128 (2014) 452–460.
[24] A. Ienco, C. Mealli, P. Paoli, N. Dodoff, Z. Kantarci, N. Karacan, Structure and vibrational spectroscopy of methanesulfonic acid hydrazide: an experimental and theoretical study, New J. Chem. 23 (1999) 1253–1260.
[25] A.D. Becke, Density-functional thermochemistry. III. The role of exact exchange, J. Chem. Phys. 98 (1998) 5648–5652.
[26] C. Lee, W. Yang, R.G. Parr, Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density, Phys. Rev. B Condens. Matter. 37 (1988) 785–789.
[27] M.J. Frisch, et al., Gaussian 03 Revision D. 01, Gaussian Inc., Wallingford, CT, 2004.
[28] Hamit Alyar, Arslan Ünal, Neslihan Özbek, Saliha Alyar, Nurcan Karacan, Conformational analysis, vibrational and NMR spectroscopic study of the methane sulfonamide-N, N′-1,2-ethanediylbis, Spectrochim. Acta Part A 91 (2012) 39–47.
[29] F. Denizot, R. Lang, Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability, J. Immunol. Methods 89 (1986) 271–277.
[30] R.B. Mulaudzi, A.R. Ndhlala, M.G. Kulkarni, J.F. Finnie, J. Van Staden, Antimicrobial properties and phenolic contents of medicinal plants used by the Venda people for conditions related to venereal diseases, J. Ethnopharmacol. 135 (2011) 330–337.
[31] J. McD Armstrong, D.V. Myers, J.A. Verpoorte, J.T. Edsall, Purification and properties of human erythrocyte carbonic anhydrases, J. Biol. Chem. 241 (1966) 5137–5149.
[32] S.L. Bradbury, The carboxymethylation of human carbonic anhydrase B. I. The nature of the reaction, J. Biol. Chem. 244 (1969) 2002–2009.

[33] C. Temperini, A. Scozzafava, L. Puccetti, C.T. Supuran, Carbonic anhydrase activators: X-ray crystal structure of the adduct of human isozyme II with L-histidine as a platform for the design of stronger activators, Bioorgan. Med. Chem. Lett. 15 (2005) 5136.
[34] R. Dichfield, Self-consistent perturbation theory of diamagnetism. 1. Gauge-invariant LCAO method for N.M.R, Chem. Shifts Mol. Phys. 27 (1974) 789–807.
[35] A. Balaban, N. Çolak, H. Ünver, B. Erk, T.N. Durlu, D.M. Zengin, Synthesis, spectral studies and crystal structure of N,N-bis((thiophene-2-carboxamido) propyl) piperazine, J. Chem. Cryst. 38 (5) (2008) 369–372.
[36] A. Gündüzalp Balaban, B. Erk, Copper(II) and zinc(II) complexes of thiophene/furan carboxamides: synthesis, structure and properties, Russ. J. Inorg. Chem. 55 (2010) 1094–1102.
[37] Ü.Ö. Özdemir, E. Aktan, F. İlbiz, A.B. Gündüzalp, N. Özbek, M. Sarı, Ö. Çelik, S. Saydam, Characterization, antibacterial, anticarbonic anhydrase II isoenzyme, anticancer, electrochemical and computational studies of sulfonic acide hydrazide derivative and its Cu(II) complex, Inorg. Chim. Acta 423 (2014) 194–203.
[38] A.B. Gündüzalp, U.O. Ozmen, B.S. Çevrimli, S. Mamaş, S. Çete, Synthesis, characterization, electrochemical behavior and antimicrobial activities of aromatic/heteroaromatic sulfonylhydrazone derivatives, Med. Chem. Res. 23 (2014) 3255.
[39] N. Sultana, A. Naz, M.S. Arayne, M.A. Mesaik, Synthesis, characterization, antibacterial, antifungal and immunomodulating activities of gatifloxacin–metal complexes, J. Mol. Struct. 969 (2010) 17–24.
[40] O. Arslan, İ.Ö. Küfrevioğlu, B. Nalbantoğlu, Synthesis and investigation of inhibition effects of new carbonic anhydrase inhibitors, Bioorg. Med. Chem. 5 (1997) 515–518.
[41] F. Hamurcu, A. Balaban Gündüzalp, S. Çete, B. Erk, The synthesis, characterization and antimicrobial activity of N,N-bis(2-thiophenecarbox amido)-1,3-diaminopropane and N, N′-bis(2-furancarboxamido)-1,3-diaminopropane and their Cu(II), Zn(II), Co(III) complexes, Trans. Met. Chem. 33 (2008) 137–141.