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Ummuhan O. Ozdemir, Dr., Firdevs İlbiz, Ayla Balaban Gunduzalp, Neslihan Ozbek, Zuhal Karagoz Genç, Fatma Hamurcu, Suat Tekin

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Graphical abstract

The alkyl sulfonic acid hydrazides (1–4) homologous series were synthesized and characterized by theorical and spectroscopic method. Their anticancer, antibacterial, anticarbonic anhydrase activities were studied. Moleculer structures and HOMO - LUMO energy levels are given below



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

^aDepartment of Chemistry, Faculty of Science, Gazi University, Ankara, Turkey.

^bDepartment of Chemistry Faculty of Education, Ahi Evran University, Kırşehir, Turkey.

^cDepartment of Materials Engineering, Faculty of Engineering, Adıyaman University, Adıyaman, Turkey.

^dDepartment of Molecular Biology and Genetics, Faculty of Science, Bartin University, Bartin, Turkey

^eDepartment of Physiology, Faculty of Medicine, Inonu University, Malatya, Turkey

*Reprint requests to Dr. Ümmühan Ö. Özdemir Fax: 90 312 2122279 E-mail: <u>ummuhan@gazi.edu.tr</u>(Ümmühan Ö. Özdemir)

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-¹³C 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 29212and 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.

Keywords: Alkyl sulfonic acide hydrazide, sulfonamide, anticancer, antibacterial, anti carbonic anhydrase II isoenzyme, computational studies

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/ sulfonylhydrazonessignificant pharmacology applications and widespread use in medicine, these compounds have gained importance in bioinorganic and metalbased drug chemistry.

In our previous studies, we reported the antibacterial and cytotoxic effect of methane sulfonic acid hydrazide (CH₃SO₂NHNH₂) 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 1was 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, ${}^{1}H/{}^{13}C-NMR$, 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 lenghts, 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 29212and 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 IC₅₀ 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 ¹³C-NMR spectra of dimethylsulfoxide-d₆ (DMSOd₆)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 KBrdisks 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 ofalkyl 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 IC₅₀ values. The disc diffusion method were used to determine the antibacterial activity of compounds 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. The inhibition activities of synthesized compounds on carbonic anhydrase II (hCAII) were investigated by comparing K_i and IC₅₀ 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-SO₂Cl (0.12 mol) was added dropwise to the ethanol solution of hydrazine hydrate (0.62 mol), maintaining the temperature between 10-12°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 takento ether phaseusinga continuousextraction methodin 5 days. Then the etherwas removed withrotary evaporator. The resulting product recrystallised from ethylacetateandthen allowed to standin the freze and crystals were obtainedafter a few weeks.

2.3. Computational section

Molecular geometry optimizations, geometrical parameters such as bond lenghts, bond angles and torsion angles and electronic parameters consist of HOMO-LUMO energies were examined with Becke'sthree-parameterexchange functional [25] in combination with the Lee– Yang–Parrcorrelation 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% CO₂/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% CO₂–95% air mixture. Briefly, $5x10^4$ 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% CO₂ 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 IC₅₀ (μ M) 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; *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* RSKK 244, *Bacillus magaterium* RSKK 5117 and Gram negative bacteria, *Salmonella enteritidis* ATCC 13076, *Escherichia 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 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⁻¹ and sterilized by filtration by 0.45 μ m millipore filters. Antimicrobial tests were then carried out by the disc diffusion method using 100 μ L of suspension containing 10⁸ CFU mL⁻¹ bacteria spred on a nutrient agar (NA) medium. The discs (6 mm in diameter) were impregnated with 20 μ L of each compound (100 μ g/disc) at the concentration of 5.0 mg mL⁻¹ and placed on the inoculated agar. DMSO impregnated discs were used as negative control. Sulfamethoxazole (300 μ g/disk) and sulfisoxazole (300 μ g/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 =
$$\left[\frac{\text{diameter of the sample}}{\text{diameter of the positive control}}\right] \cdot 100$$

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 IC₅₀ values, 100 μ L 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 μ L of 0.05 M tris-SO₄ buffer (pH: 7.6) and 0.1 μ L enzyme solution for total volume of 300 μ L. The absorbance was determined at 348 nm after 6 min [32]. This study was repeated three times for each inhibitor. In order to determine IC₅₀ values, Graphs were drawn by using inhibition % values by a statistical packing program on a computer. The IC₅₀ 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 Fig. 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 Table 2-5 with respect to the bond lenghts, bond angles and torsion angles.

3.1. Characterization of compounds

3.1.1 NMR spectra

 ${}^{1}\text{H}{}^{-13}\text{C}$ NMR spectra of compounds **1-4** were obtained in DMSO-d₆ at room temperature using TMS as an internal standard (Fig. 4). ${}^{1}\text{H}{}^{-13}\text{C}$ 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 ${}^{1}\text{H}{}^{-13}\text{C}$ NMR assignments in DMSO-d₆ are listed in Tables 6 and 7.

The terminal CH_3 protons of alkyl chain for compounds 1–4 as $-CH_3$ (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. CH₂ protons directly linked to terminal CH₃ for compounds **3** and **4** as -CH₂-CH₃ (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. CH₂ protons between two CH₂ groups for compounds **4** as CH₂-CH₂-CH₂ (two H intensities) are observed at 1.57 ppm (2H, multiplet) and corresponding calculation values are 1.29 ppm, respectively. And also CH₂ protons directly linked to SO₂ for compounds **2-4** as -CH₂-SO₂-(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 NH₂ 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-d₆. This suggests that NH₂ and seconder NH protons have both intramolecular and intermolecular hydrogen bonding [37].

CH₃ 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. CH₂ 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, CH₂ carbonsdirectly linked to SO₂ for compounds **2-4** are observed between 44.52-53.80 ppm and corresponding calculation values are between 49.35-56.87 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]. NH₂ vibrations in the prepared compounds **1-4** are observed between 3360- 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. v_{as} (SO₂) and v_s (SO₂) 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.

3.1.3. 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 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 compounds1-4 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 (IC₅₀) values. The compounds 1 and 2 have no significant inhibition of proliferation being observed at concentrations up to 100 μ M. The compounds 3 and 4 elicited a rather similar cytotoxicity (average IC50 values of 16.65 μ M and 15.41 μ M). Compound 4, having the highest activity in alkyl series, shows the closest activity against standard docetaxel (average IC₅₀ value of 7.24 μ M). 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 (Fig.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 alkylsulfonicacide hydrazides are active against both types of the Gram positive and Gram negative bacteria.

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, Fig. 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 6b), the compounds behave as inhibitors against hCA II enzyme. Compound **4** shows enhanged inhibition effect (Ki: 7.96×10^{-6} M, IC₅₀: 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 a 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 activies. Changing the alkyl chain increases the lipophilicity and easily passes the cell membrane.

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Compound	empound Empirical Formula (Formula Weight)		m.p. (°C)	Yield	Found (calculated)			
	(i official weight)			(70)	%C	%H	%N	%S
1	$CH_6N_2SO_2$	white	50.51	50	10,80	5,25	25,35	28,50
1	110,05 g/mol	winte	white 50-51 5	50	(10,91)	(5,45)	(25,45)	(29,09)
2	C ₂ H ₈ N ₂ SO ₂ 124,14 g/mol	white	40-42	60	19,03 (19,36)	6,15 (6,45)	(22,04)	24,65 (25.81)
3	C ₃ H ₁₀ N ₂ SO 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 ₄ H ₁₂ N ₂ SO 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)

Table 1 Analytical and physical data for alkyl sulfonic acide hidrazides

Table2

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-70	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	70-6S-80	103.4225	5H-4N-6S-7O	-157.9417
1C-2H	1.0918	70-6S-1C	106.0981	5H-4N-6S-8O	153.1209
1C-12H	1.0700	80-6S-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 leng	ths (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	100-9S-1C	108.4132	9S-1C-4C-5H	-55.2836			
1C-3H	1.1105	110-9S-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 length	ns (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-130	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	140-12S-1C	110.2971	12S-1C-4C-6H	60.8825
7C-18H	1.0976	130-12S-140	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
	X /	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 g	geometric pa	arameters of comp	pound 4 by using	g B3LYP-6-311G(d,p) ba	sic set
Bond lengths	s (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	170-16S-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
	C				

Table 6

The experimental and theoretical ¹H-NMR data (ppm) of the alkyl sulfonic acide hidrazides

Assign.	1		2	2		3		4	
	<u>δ(exp.)</u>	$\delta(calc.)^{a}$	<u>δ(exp.)</u>	δ (calc.) ^a	<u>δ(exp.)</u>	δ (calc.) ^a	<u>δ(exp.)</u>	δ (calc.) ^a	
C <u>H</u> 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 <u>H</u> ₂ -CH ₃	-	-	-	-	1.65(m,2H)	1.58	1.37(m, 2H)	1.29	
-C <u>H</u> 2-	-	-	-	-	-		1,57(m,2H)	1.46	
SO ₂ -C <u>H</u> ₂	-	-	3.11 (m,2H)	3.22	3.02 (t,2H)	3.14	3.06 (t, 2H)	3.12	
N <u>H</u> 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 <u>H</u>	7,67(s,1H)	4.48	7,67(s,1H)	5.29	7,81(s,1H)	5.24	7,75(s,1H)	5.23	

^a Calculated using GIAO/B3LYP/6311++G(2d,2p) method.

Table 7

The experimental and theoretical ¹³C-NMR data (ppm) of the alkyl sulfonic acide hidrazides

Assion	1		2		3			4
TIBBILIN	<u>δ(exp.)</u>	$\delta(calc.)^a$	<u>δ(exp.)</u>	$\delta(calc.)^{a}$	<u>δ(exp.)</u>	$\delta(\text{calc.})^{a}$	<u>δ(exp.)</u>	$\delta(calc.)^{a}$
<u>C</u> H ₃	49.50	50.56	8.24	10.49	13.50	14.13	13,94	16.17
<u>C</u> H ₂ -CH ₃	-	-		-	24.10	23.13	21,38	27.09
<u>C</u> H ₂	-	-	-	-	-	-	25,29	32.14
SO ₂ - <u>C</u> H ₂	-	-	44.52	49.35	53.80	56.87	47,17	55.76

^a Calculated using GIAO/B3LYP/6311++G(2d,2p) method.

Assign.	1	2	3	4
$v_{as}(NH_2)$	3360m	3334s	3335m	3383m
$v_{s}(NH_{2})$	3300m	3276s	3300m	3249m
υ(NH)	-	3210s	-	3211w
$v_{as}(SO_2)$	1320s	1317s	1321s	1317s
$\upsilon_s(SO_2)$	1156s	1143s	1145s	1133s
$\delta(NH_2)$	1640m	1637m	1625m	1639m
$\delta(SO_2)$	526s	542m	526w	525
δ(NH)	650m	577m	635w	630

Table 8
Wave number (cm ⁻¹) of selected vibration of the alkyl sulfonic acide hidrazides.

Table 9

Cytotoxicity compounds (1-4), Docetaxel against MCF-7 cell lines

	IC ₅₀ (µmolL ⁻¹) for MCF-7
Compound	cell lines
1	>100
2	>100
3	16.65
4	15.41
Docetaxel	7.24
Refererence : Docetaxel	

Table 10

Inhibition zone values (mm,100 $\mu g/\text{disc}) of$ the alkyl sulfonic acide hydrazides by disc diffusion method

	Gram				Gram		
Compound		positive			negative		
Compound	B.subtilis	B. cereus	E. faecalis	S. aureus	P.aeruginosa	K.pneuonia	E. coli
	ATCC	NRRL-B-	ATCC	ATCC	ATCC	ATCC	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
	12	10	10	10		10	10
4	13	12	10	10	14	10	12
Sulficovozolo	25	19		17	e)	20	28
Sumsoxazoie	23	10	-	17	0	20	28
Sulfamethaxazole	15	15	_	18	17	17	24
Summentaxazore	15	15		10		1/	<i>2</i> - f

Reference : Sulfisoxazole, Sulfamethaxazole

Table 11

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

	hCA II	
Compound	Esterase activity	
Q	$IC_{50}(M)$	Ki (M)
1	3,78×10 ⁻⁴	6,94.10 ⁻⁵
2	3.44×10 ⁻⁴	6,44.10 ⁻⁵
3	3.17×10 ⁻⁴	5,96.10 ⁻⁵
4	3.10×10 ⁻⁴	5,80.10-5
Acetazolamide	1.13×10 ⁻⁴	2,11.10 ⁻⁵

Reference : Acetazolamide



 $R = CH_3, C_2H_5, C_3H_7, C_4H_9$

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 the alkyl sulfonic acid hydrazides (1-4).









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



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



Fig. 6. Comparison of antibacterial activite 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

Highlights

- Homolog series were synthesized
- Computational studies were evaluated
- Biological activities were studied