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NEW SULFONYLHYDRAZONES CONTAINING METHANE SULFONIC ACID HYDRAZIDE HAVING HUMAN ANTI-CARBONIC ANHYDRASE AND ANTI-MICROBIAL ACTIVITY: SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION, ELECTROCHEMICAL PROPERTIES, AND BIOLOGICAL ACTIVITIES

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In this work, new sulfonylhydrazones nomenclatured as 3,5-ditertbutylsalicylaldehyde methanesulfonylhydrazone (**II**), 3-tertbutylsalicylaldehyde methanesulfonylhydrazone (**III**), and 5-bromosalicylaldehyde methanesulfonylhydrazone (**IV**) were synthesized by the reaction of methanesulfonicacidhydrazide (**I**) with 3,5-ditertbutylsalicylaldehyde, 3-tertbutylsalicyl aldehyde, and 5-bromosalicylaldehyde. The structures of the aromatic sulfonylhydrazones were determined by using elemental analysis, UV-Vis, FT-IR, ^{1}H-NMR, and ^{13}C-NMR methods. The structure of **IV** was also supported with the X-ray diffraction method. Sulfonamides were generally investigated for their inhibitory effects on human carbonic anhydrase isoenzymes (hCAs). Synthesized alkylsulfonylhydrazones have a sulfonamide group, which is the most important pharmacophore for the carbonic anhydrase (CA) inhibition efficiency like the reference agent acetazolamide (AAZ). The enzyme inhibition trends of alkylsulfonylhydrazones on the hCA I isoenzyme were qualitatively investigated by cyclic voltammetry (CV) and differantial pulse voltammetry (DPV). Also, the inhibition activities of sulfonylhydrazones were determined by using UV-Vis spectrophotometry, and their inhibition parameters, such as K_m, IC_{50}, and K_i, were calculated. Among the tested compounds, **IV** was found to be the most active compound on the hCA I isoenzyme with an IC_{50} value of 4.86×10^{-6} M, whereas **II** and **III** were found to be the least potent compounds on hCA I with an IC_{50} value of 3.96×10^{-4} M and 5.58×10^{-5} M, respectively.

All of the compounds showed excellent inhibition activity against gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*) and gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidi*), with minimum inhibitory concentration (MIC) values less than that of standard drugs (sulfamethoxazole and sulfisoxazole). In addition, all of the compounds exhibited excellent antifungal inhibition against *C. albicans* and *A. fumigatus*, with MIC values of 8–16 μg/ml, which were 2–4 fold higher than the standard drug fluconazole (32 μg/ml).

Keywords: sulfonylhydrazones; hCA I isoenzyme; cyclic voltammetry (CV); minimum inhibitory concentrations (MICs)

НОВИ СУЛФОНИЛХИДРАЗОНИ ШТО СОДРЖАТ ХИДРАЗИД НА МЕТАНСУЛФОНСКА КИСЕЛИНА И ПОКАЖУВААТ АКТИВНОСТ ВРЗ ЧОВЕЧКА АНТИКАРБОНСКА АНХИДРАЗА И АНТИМИКРОБНА АКТИВНОСТ: СИНТЕЗА, СПЕКТРОСКОПСКА КАРАКТЕРИЗАЦИЈА, ЕЛЕКТРОХЕМИСКИ СВОЈСТВА И БИОЛОШКА АКТИВНОСТ

Во овој труд беа синтетизирани нови сулфохидразони, именувани како 3,5-дитерцбутилсалицилалдехидметансулфохидразон (**II**), 3-терцбутилсалицалдехидметансулфонил-хидразон (**III**), и 5-бромосалицилалдехидметансулфонилхидразон (**IV**) со реакција меѓу хидразид на метансулфонска кислина (**I**) со 3,5-дитерцбутилсалицилалдехид, 3-терцбутилсалицилалдехид и 5-бромосалицилалдехид. Структурите на ароматичните сулфонилхидразони беа определени со елементна анализа, UV-Vis, FT-IR, ^{1}H-NMR и ^{13}C-NMR. Структурата на **IV** беше исто така поткрепена со рендгенска дифракција. Сулфонамидите биле испитувани главно поради нивните инхибиторни влијанија врз изоензими на хуманата анхидраза (hCAs). Синтетизираните алкилсулфонилхидразони содржат сулфонамидна група која е најважниот фармакофор за ефикасноста на инхибиција на карбонската анхидраза (CA) како и референтното средство ацетазоламид (AAZ). Трендовите на ензимската инхибиција на алкилсулфонилхидразон врз изоензимот hCA I беа испитани квалитативно со циклична волтаметрија (CV) и диференцијална пулсна волтаметрија (DPV). Покрај тоа, беа определени инхибиторните активности на сулфонилхидразони со UV-Vis спектрометрија, а беа пресметани нивните инхибиторни параметри како што се K_m, IC_{50} и K_i. Меѓу тестираните соединенија **IV** се покажа како најактивно соединение врз изоензимот hCA I со IC_{50} вредност од $4{,}86 \times 10^{-6}$ M, додека **II** и **III** беа со најмала активност соодветно со IC_{50} вредности од $3{,}96 \times 10^{-4}$ M и $5{,}58 \times 10^{-5}$ M.

Сите соединенија покажаа одлично инхибиторно дејство врз грам-негативни бактерии (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Stenotrophomonas maltophilia*) и грам-позитивни бактерии (*Staphylococcus aureus, Staphylococcus epidermidi*) со минимални вредности на инхибиторни концентрации (MIC) помали од стандардни лекови (сулфаметосказол и сулфисоксазол). Покрај тоа, сите соединенија покажуваат одлични антифунгицидна инхибиција врз *C. albicans* и *A. fumigatus* со MIC, од 8–16 μg/ml што е 2–4 кратно повисоко од стандардниот лек флуконазол (32 μg/ml).

Клучни зборови: сулфохидразони, hCA I изоензим, циклична волтаметрија (CV), минималнми инхибиторни концентрации (MICs)

1. INTRODUCTION

Sulfonyl or sulfonamide based analogues have shown a variety of pharmacological properties, and its derivatives offer a high degree of structural diversity that are useful for finding new therapeutic agents. Currently, more than 150 FDA approved sulfur (SVI)-based drugs are available on the market, and they are widely used to treat various types of diseases with therapeutic power. This comprehensive review highlights the recent developments of sulfonyl or sulfonamide based compounds in a huge range of therapeutic applications, such as antimicrobial, anti-inflammatory, antiviral, anticonvulsant, antitubercular, antidiabetic, antileishmanial, carbonic anhydrase, antimalarial, anticancer, and other medicinal agents [1].

Sulfonamide is considered to be a significant moiety due to its diverse pharmacological activities [2], and these have clinical use as carbonic anhydrase inhibitors (CAIs) primarily as diuretics and anti-glaucoma agents. In many studies, sulfonamide derivatives have been reported for their wide enzyme activities against carbonic anhydrase isoforms [3–5]. Sulfonamide moieties have a chelation ability of binding zinc in the CAIs active sites, thus affording their high affinity and desired pharmacological properties [6, 7]. A family of sulfonamide based heterocycle hybrids were designed and biologically evaluated for their potent carbonic anhydrase activity against hCA II and hCA IV by Nocentini et al. [8]. The structure–activity relationship (SAR) studies showed that the presence of the sulfonamide moiety was beneficial for enhancing the carbonic anhydrase activity. Mahmood and co-workers evaluated the inhibitory effects of iminothiazolidinonesulfonamide hybrids against CAs I, II, IV, and IX [9].

Electrochemical methods have been preferred because of their simplicity, high sensitivity, less time-consuming, and rapidity without any pre-treatment in enzyme inhibition studies [10–13]. The dramatic increase in multi-drug resistance for microbial pathogens is considered a global problem. Therefore, working toward developing novel antimicrobial agents will always remain an important and critical issue. In this regard, sulfonylhydrazone derivatives are important compounds for drug

design due to their various biological properties [14,15]. In this study, alkylsulfonylhydrazones (II, III, IV) derived from methane sulfonic acid hydrazide (I) were newly synthesized and characterized by using elemental analysis, UV-Vis, FT-IR, ^{1}H-NMR, and ^{13}C-NMR methods. The crystal structure of IV was also presented by using X-ray diffraction. We developed a method using differential pulse voltammetry for the activity of alkylsulfonylhydrazones on the carbonic anhydrase I (CA I) with p-nitrophenol (PNP) as an electrochemical mediator. The inhibition effects of the alkylsulfonylhydrazones were also determined by using UV-Vis spectrophotometry. The inhibition parameters (K_m, IC_{50}, and K_i) were calculated by a Lineweavere Burk graph, an activity % graph, and the Cheng-Prusoff equation. Additionally, the antimicrobial activities of the sulfonylhydrazone derivatives were evaluated against bacterial and fungal pathogens.

2. EXPERIMENTAL

2.1. *Materials and physical measurements*

Methane sulfonyl chloride, hydrazine hydrate, 3,5-ditertbutylsalicylaldehyde, 3-tertbutylsalicyl aldehyde, 5-bromosalicylaldehyde, ethanol, methanol, diethylether and dimethylsulfoxide (all from Sigma-Aldrich) and solvents (all from Merck) were used without further purification. All chemicals and solvents used in the synthesis were of analytical grade.

The UV-Vis spectra (200–1100 nm) were recorded on a UNICAM-UV 2-100 model. The IR spectra (4000–400 cm^{-1}) were recorded on a Mattson 1000 FT-IR spectrophotometer with samples prepared as KBr pellets. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Agilent Spectrospin Avance VNMRS-500 Ultra-Shıeld. Trimethylsilane (TMS) was used as an internal standard and deuteriated DMSO as the solvent. For single crystal X-ray analysis, the crystal was mounted on a micromount and was attached to a goniometer head on a Bruker D8 VENTURE diffractometer equipped with a PHOTON100 detector. It was measured with graphite monochromated Mo-Kα radiation (λ = 0.71073 Å) using 1.0° Ω and Φ rotation frames at room temperature (296 K). The structure was solved by the direct method and refined by full-matrix least-squares methods with SHELXL-2013 [16]. All non-hydrogen atoms were refined anisotropically. All the hydrogen atoms were placed in their calculated positions and refined in the riding model. SADABS [17] was used to perform absorption correction. Molecular drawings were generated using OLEX2. Ver. 1.2-dev [18]. The melting points were measured using an Opti Melt apparatus. Thin-layer chromatography (TLC) was conducted on 0.25 mm silica gel plates (60F254, Merck).

All of the electrochemical studies were performed by using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) on a CHI 660B electrochemical workstation (Shanghai, China). A typical three electrode system was used: a glassy carbon as working electrode, a platinum wire as counter-electrode, and an Ag/AgCl electrode in aqueous solution and an Ag/Ag^{+} in nonaqueous solution as reference electrodes. The glassy carbon (GC) electrode was mechanically polished with 0.05 mm alumina slurry (Baikowski Int. Corp.) on a microcloth pad (Buehler). The polished GC electrode was sonicated in ultrapure water and then with acetonitrile (ACN) for 5 min to remove trace alumina from the surface. Then, the GC electrode was rinsed with ACN.

The enzyme inhibition activities of the synthesized compounds on carbonic anhydrase I (hCA I) were investigated by measuring absorbances at 400 nm on a UV-Vis spectropohotometer.

Sulfonylhydrazones were screened for their *in vitro* antimicrobial activities against isolates of gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia*), gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis)*, and fungi (*Candida albicans* and *Aspergillus fumigatus)* by using the minimum inhibitory concentration (MIC) method. The MIC values were determined by a broth microdilution method according to the procedures recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [19]. Also, minimum bactericidal/fungicidal concentration (MBC/MFC) values were determined for each isolate. All test microorganisms were obtained from culture collections of Nigde Omer Halisdemir University, School of Medicine.

2.2. *General procedure for the synthesis of sulfonylhydrazones*

The reaction of the hydrazine hydrate with methane sulfonyl chloride formed methanesulfonic acid hydrazide (**I**) as mentioned in Ref 20 [20]. The sulfonylhydrazones were synthesized according to the following general procedure [15];

The solution of methanesulfonicacidhydrazide (0.01 mol) in ethanol was mixed with a hot solution of the corresponding salicylaldehyde derivatives (0.015 mol) in ethanol and stirred for 1 h. Upon cooling, crystalline precipitates were filtered, washed with ethanol–ether, recrystallized from water, and dried in vacuo over P_2O_5.

3,5-ditertbutylsalicylaldehyde methanesulfonylhydrazone **(II)***;* $C_{16}H_{26}N_2SO_3$ (*M* = 326 g/mol); Yield 78 %; mp: 148 ºC; λ_{max} = 240 nm, 345 nm.

3-tertbutylsalicylaldehyde methanesulfonylhydrazone **(III);** $C_{12}H_{18}N_2O_3S$ (*M* = 270 g/mol); Yield 72 %; mp: 175–176 ºC; λ = 245 nm, 315 nm.

5-bromosalicylaldehyde methanesulfonylhydrazone **(IV);** $C_8H_9N_2O_3SBr$ (*M* = 293 g/mol); Yield 67%; λ_{max} = 245 nm, 310 nm; mp: 183–184 ºC. Elemental analysis: Calcd for C: 32.60; H: 3.06; N: 9.52; S: 10.88. Found: C: 32.00; H: 2.97; N: 9.34; S: 9.30.

2.3. *Electrochemistry measurements*

All electrochemical studies were performed by using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) on a CHI 660B electrochemical workstation. CV experiments were recorded in ACN under nitrogen atmosphere using a sweep rate of 0.1 V s^{-1} between 1.5 and −1.7 V vs. a nonaqueous reference electrode.

2.4. *Procedure for enzyme inhibition*

2.4.1. *Carbonic anhydrase I enzyme inhibition with the electrochemical method*

A DPV technique was developed for the assessment of the impact of sulfonamide compounds (inhibitors) on the carbonic anhydrase I (CA I). The inhibition of CA I was assessed by measuring the enzyme activity before and after incubating with the inhibitors. Stock solutions of 10 mM inhibitors (compounds **I-IV** and AAZ as reference) in ACN containing 0.1 M tetrabutylammonium tetrafluoroborate (TBATFB) were prepared at room temperature.

2.4.2. *Carbonic anhydrase I enzyme inhibition with the spectroscopic method*

Enzyme activities were spectrophotometrically determined at 400 nm by following the absorbance changes during the conversion of 4-nitrophenyl-acetate (PNPA) to 4-nitrophenylate (PNP) over a period of 6 min at 25 ºC [21]. The enzymatic reaction contained 1.4 ml 0.05 M Tris-SO_4 buffer (pH: 7.4), 1.0 ml 4-nitrophenylacetate, 0.5 ml H_2O, and 0.1 ml enzyme solution in a total volume of 3.0 ml. The inhibitory effects of the compounds were compared with AZA (acetazolamide). Different inhibitor concentrations (10^{-2} M, 10^{-3} M, 10^{-4} M, and 10^{-5} M) were used, and all compounds were tested in triplicate at each concentration used. In this experiment, 4-nitrophenyl-acetate (PNPA) was used as a substrate at five different concentrations (0.3 mM, 0.6 mM, 1.0 mM, 3.0 mM). Then, Lineweaver–Burk curves were drawn to calculate the inhibition parameters [22].

Control cuvette activity was acknowledged as 100 % in the absence of the inhibitor. An activity %-[Inhibitor] graph was drawn for each inhibitor [23, 24]. In order to determine IC_{50} values, graphs were drawn by using inhibition % by a statistical package program on a computer. The IC_{50} values of the compounds were determined at a 1.0 mM substrate concentration. K_i values reflect the binding affinity of the compounds to both carbonic anhydrase isoenzymes. K_i values were calculated according to the Cheng Prusoff equation using K_m and IC_{50} parameters [25–27].

2.5. *Procedure for antimicrobial activity*

All compounds were screened for their *in vitro* antimicrobial activities against bacterial and fungal isolates using the broth microdilution method. The MIC values were determined for the bacterial strains using Muller Hinton Broth (MHB) medium with an inoculum of 5×10^5 cfu/ml and RPMI 1640 2 % glucose medium for fungal strains with an inoculum of 2×10^5 cfu/ml, incubated aerobically at 35 ºC for 24–48 h. After incubation, MIC values were detected as the lowest inhibition concentration of the compounds that inhibited the growth of microorganisms. The concentrations of the compounds ranged from 256 µg/ml to 0.5 µg/ml. In order to determine the MBC/MFC values, 100 µl of MIC, 2 × MIC, and 4 × MIC dilutions were subcultured onto Mueller Hinton agar (MHA) plates and incubated at 35 ºC for 24 h. MBCs/MFCs were defined as the lowest concentration of the compound that kills the microorganism. As reference drugs, sulfamethoxazole and sulfisoxazole were used for the bacterial strains, and fluconazole was used for the fungal strains. Antimicrobial activity measurements against each isolate were repeated twice in our biological studies.

3. RESULTS AND DISCUSSION

The methanesulfonylhydrazones (**II–IV**) were synthesized by the reaction of methane sulfonic acid hydrazide (**I**) with salicylaldehyde derivatives (Fig. 1) and characterized using general spectroscopic methods (FT-IR, $^{1}H/^{13}C$ NMR) (Supplementary Figs. S1-S9).

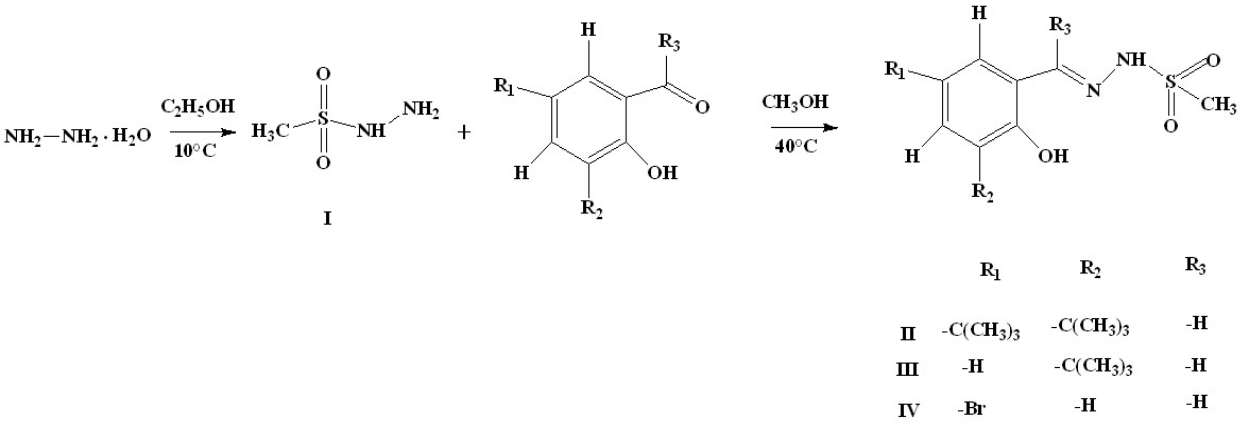


Fig. 1. General synthesis method of the sulfonylhydrazones

3.1. *The characterization of the compounds*

3.1.1. *Experimental FT-IR spectroscopy*

The FT-IR spectra of **II–IV** (Supplementary Fig. S1, Fig. S2, Fig. S3) were recorded in KBr pellets. The selected vibration frequencies of the methanesulfonylhydrazone derivatives are listed in Table 1. The assignment of the bands are made by taking into consideration the literature data for compounds containing appropriate structural fragments, such as salicylaldehyde and sulfonamide derivatives [28, 29]. NH vibrations in compounds (**II–IV**) are observed between 3021–3038 cm^{-1} as strong bands. Also, $\upsilon_{SO2(as)}$ and $\upsilon_{SO2(s)}$ stretching vibrations are observed at 1327 cm^{-1} and 1060 cm^{-1} for compound **II**, 1313 cm^{-1} and 1155 cm^{-1} for compound **III**, and 1317 cm^{-1} and 1156 cm^{-1} for compound **IV** [30, 31].

T a b l e 1

The selected vibration frequencies of salicylaldehyde derivatived methanesulfonylhydrazones (cm^{-1})

Compounds	$\nu_{(NH)}$	$\nu_{(CH)ar}$	$\nu_{(CH)as}$	$\nu_{(C=N)}$	$\nu_{(SO2)as}$	$\nu_{(CO)}$	$\nu_{(SO2)s}$	$\delta_{(NH)}$	δ_{SO2}
II	3176s	3029w	2957s	1652s	1327s	1262m	1060sh	652w	517m
III	3226s	3038 w	2960s	1649s	1313sh	1251m	1155sh	675m	514m
IV	3154s	3021w	2937w	1666m	1317s	1269s	1156sh	626s	542s

w: weak , m: medium, s: strong , sh: sharp

3.1.2. *NMR spectra*

^{1}H-^{13}C NMR spectra of compounds **II–IV** were obtained in DMSO-d_6 at room temperature using TMS as an internal standard. The experimental and theorical ^{1}H-^{13}C NMR assignments in DMSO-d_6 of compounds **II–IV** are listed in Table 2. In the ^{1}H NMR spectrum, CH_3 protons bonded to the SO_2 group (SO_2-$\underline{CH_3}$, 3H) of compounds **II–IV** are easily distinguishable as a singlet, and they are observed at 3.16 ppm, 3.09 ppm, and 2.92 ppm, respectively (Supplementary Fig. S4, Fig. S5, Fig. S6). Aromatic protons are observed at 7.36 ppm (C(4)H, 1H) and 7.29 ppm (C(6)H, 1H) for compound II; 7.28 ppm (C(4)H and C(6)H, 2H) and 6.87 ppm (C(5)H, 1H) for compound III; 7.76 ppm (C(6)H, 1H), 7.41 ppm (C(3)H, 1H), and 6.88 ppm (C(4)H, 1H) for compound IV. Azomethine protons (CH=N, 1H) of compounds **II–IV** are observed at 8.21 ppm, 8.19 ppm, and 8.29 ppm as a singlet, respectively. The secondary NH and OH protons of compounds **II–IV** are observed in the range of 10.35–11.27 ppm, the aliphatic protons bonded to aromatic ring are observed at 1.45 ppm and 1.21 ppm for compound **II**, 1.35 ppm for

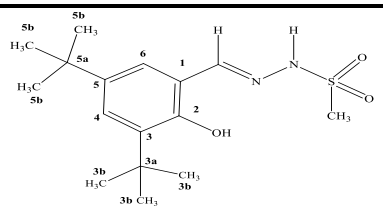
compound **III,** as seen in Supplementary Fig. S4, Fig. S5, Fig. S6 [32–34].

In the ^{13}C NMR spectrum, the SO_2-$\underline{C}H_3$ carbons of compounds **II–IV** are observed at 39.0 ppm, 39.1 ppm, and 40.2 ppm, respectively (Supplementary Fig. S7, Fig. S8, Fig. S9). The aromatic ring carbons of compounds **II–IV** are observed between 111.5–141.3 ppm in the weak field. Azomethine carbons ($\underline{C}$H=N) of compounds **II–IV** are observed at 152.4 ppm, 154.3 ppm, and 144.6 ppm; the aliphatic carbons bonded to the aromatic ring are observed between 29.8–35.1 ppm for compound **II** and 29.7–35.0 ppm for compound **III,** as seen in Table 2 [35, 36].

T a b l e 2

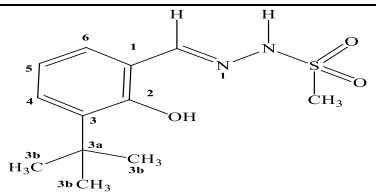
*Experimental ^{1}H and ^{13}C- NMR chemical shifts δ(ppm) f or the compounds **II–IV***

II



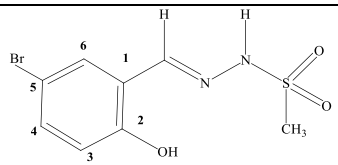
^{1}H-NMR	**δ(ppm)**	**^{13}C- NMR**	**δ(ppm)**
O**H** (br, ^{1}H)	11.27	**C**(2)	154.5
N**H** (s, ^{1}H)	11.13	**C**H=N	152.4
C**H**=N (s, ^{1}H)	8.21	**C**(3)	141.3
C(4)**H** (d, ^{1}H)	7.36	**C**(5)	136.1
		C(4)H	126.4
C(6)**H** (d, ^{1}H)	7.29	**C**(6)H	126.1
SO_2-C**H**$_3$ (s, ^{3}H)	3.16	**C**(1)	117.4
		SO_2-**C**H$_3$	39.0
C(3b)**H**$_3$ (s, ^{9}H)	1.45	**C**(3a)	35.1
		C(5a)	34.4
C(5b)**H**$_3$ (s, ^{9}H)	1.21	**C**(3b)H$_3$	31.7
		C(5b)H$_3$	29.8

III



^{1}H-NMR	**δ(ppm)**	**^{13}C-NMR**	**δ(ppm)**
N**H** (s, ^{1}H)	11.20	**C**(2)	157.4
O**H** (s, ^{1}H)	11.27	**C**H=N	154.3
C**H**=N (s, ^{1}H)	8.19	**C**(3)	137.7
C(4)**H,** C(6)**H** (m, ^{2}H)	7.28	**C**(4), **C**(6)H	130.1
C(5)**H** (t, ^{1}H)	6.87	**C**(1)H	119.1
SO_2C**H**$_3$ (m, ^{3}H)	3.09	**C**(5)H	116.7
		SO_2C**H**$_3$	39.1
C(3b)**H** (m, ^{9}H)		**C**(3a)	35.0
		C(3b)**H**$_3$	29.7

T a b l e 2 continue

IV			
^{1}H-NMR	**δ(ppm)**	**^{13}C-NMR**	**δ(ppm)**
O**H** (br, ^{1}H)	11.06	C(2)	154.7
N**H** (s, ^{1}H)	10.35	**C**H=N	144.6
		C(5)H	134.1
C**H**=N (s, ^{1}H)	8.29	**C**(6)	128.7
C(6)**H** (d, ^{1}H)	7.76	**C**(3)H	121.5
C(3)**H** (d, ^{1}H)	7.41	**C**(4)H	118.9
C(4)**H** (d, ^{1}H)	6.88	**C**(1)	111.5
SO_2-CH_3 (s, ^{3}H)	2.92	SO_2-**C**H_3	40.2

3.1.3. *Crystal structure of 5-bromosalicyl aldehydemethanesulfonylhydrazone (**IV**)*

The parallelepiped single crystal of compound **IV** with dimensions 0.10 mm×0.10 mm×0.30 mm was grown by slow evaporation of its chloroform solution. Crystal data and structure refinement parameters are given in Table 3. Table 4 shows the selected bond lengths and bond and torsion angles for the compound. Supplementary Table S1 gives the hydrogen bonding geometry of structure **IV**. Thermal ellipsoids are plotted in Fig. 2, intermolecular hydrogen bonding can be seen in Fig. 3, and the packing motif of the molecule can be seen in Supplementary Fig. S10. Further details on crystal data, data collection, and refinements are included in the supporting information.

Compound **IV** was crystallized in a monoclinic crystal system with P 1 21/c 1 space group. Each unit cell contains four molecules (Z = 4). The molecule is strongly stabilized by the intermolecular hydrogen bonding occurring between hydrazine of N-H of the hydrazine unit and oxygen of the sulfonyl group. These NH…O intermolecular interactions form one dimensional framework along the a-axis. The geometry around the S atoms of the SO_2 moiety is a distorted tetrahedral, as expected, with O2-S1-O3 = 118.7 (2)º [37]. The O-H…N type intramolecular hydrogen bonding forms a six membered ring: this six membered ring is tilted at a dihedral angle of 0.48º, which indicates that this ring motif is in the same plane with the aromatic system (C2 – C8) [38]. The N-N bond distance of 1.40 (6) Å hydrazine moiety and S = O bond distance of 1.43 (4) Å are in good agreement with the literature corresponding to sulfonyl hydrazines [39, 40].

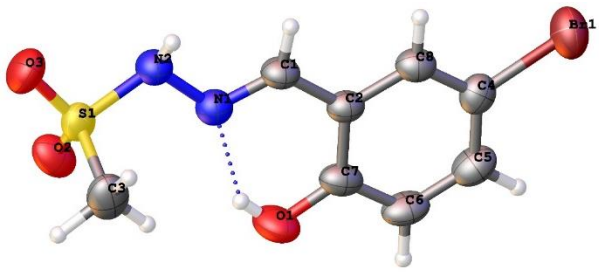


Fig. 2. Thermal ellipsoids of 1 are drawn at 50% probability.

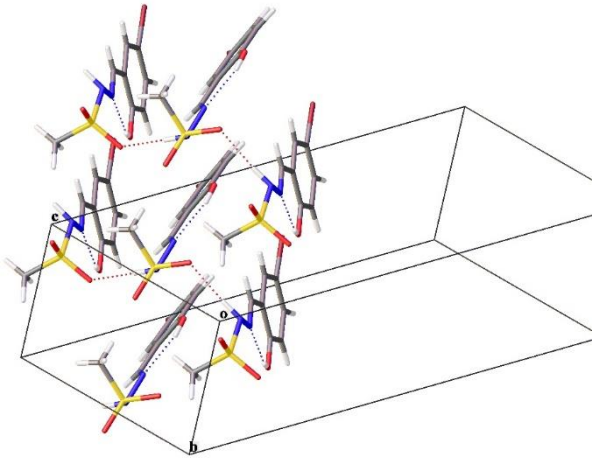


Fig. 3. Plot of the intermolecular hydrogen bonding

T a b l e 3

*Crystal data and structure refinement parameters for **IV***

	IV
Empirical formula	$C_8H_9BrN_2O_3S$
Formula weight (g/mol)	293.14
T(K)	296(2)
λ(Å)	0.71073
Crystal system	Monoclinic
Space group	P 1 21/c1
Unit cell dimensions: (Å, º)	
a	22.444(3)
b	5.8589(8)
c	8.5426(11)
V(Å^3)	1117.6(3)
α	90
β	95.776(4)
γ	90
Z	4
Absorption coefficient (mm^{-1})	3.853
Dcalc (g/ cm^3)	1.742
F(000)	584
Crystal size (mm)	0.10×0.10×0.30
θ range for data collection (º)	2.74 to 26.42
Index ranges	–28 ≤ h ≤ 28 –7 ≤ k ≤ 7 –10 ≤ l ≤ 10
Reflections collected	36206
Independent reflections	2293
Coverage of independent reflections (%)	99.7
Data/parameters	2293 / 139
Max. and min. transmission	0.681 – 0.886
Final R indices [I ≥ 2σ(I)]	R1 = 0.0506 wR2 = 0.1430
R indices (all data)	R1 = 0.0658 wR2 = 0.1540
Goodness-of-fit on F^2	0.932

T a b l e 4

Selected bond lengths (Å) , bond angles (º), and torsion angles (º) for ***IV***

Assig.	Bond lengths (Å)	Assig.	Bond angles (º)	Assig	Torsion angles (º)
N2-N1	1.402(6)	S1-N2-N1	113.9(3)	C3-S1-N2-N1	61.3(4)
C1-N1	1.283(6)	N1-C1-C2	121.3(4)	N1-C1-C2-C7	–2.1(7)
C1-C2	1.445(6)	C2-C7-C6	119.5(5)	N1-N2-S1-O3	178.6(3)
C7-C2	1.411(7)	C6-C5-C4	119.0(5)	N1-N2-S1-O2	–54.8(4)
C7-O1	1.349(6)	C5-C4-Br1	119.4(4)	C2-C8-C4-Br1	175.5(4)
C7-C6	1.386(8)	O1-C7-C2	122.4(4)		
C4-Br1	1898(5)	O2-S1-O3	118.7(2)		
		C3-S1-N2	108.5(3)		
S1-N2	1.641(4)	N2-N1-C1	116.3(4)		
S1-O3	1.430(4)				
S1-O2	1.426(4)				
C3-S1	1.743(7)				
C6-C5	1.386(7)				

3.1.4. *Electrochemical behaviors of compounds*

The electrochemical behaviors of methane sulfonic acid hydrazide (**I**) and methane sulfonyl-hydrazones (**II–IV**) were investigated by the CV technique (Fig. 4).

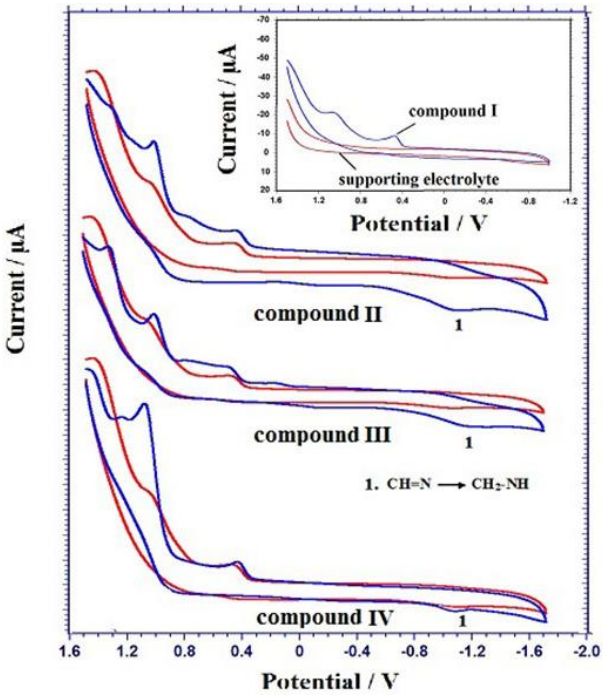


Fig. 4. Electrochemical behaviors of the compounds in ACN containing TBATFB as a supporting electrolyte with a sweep rate of 0.1 V s^{-1} between 1.5 and –1.7 V by the CV technique

Methane sulfonylhydrazones (**II–IV**) showed smilar redox behaviors as free methane sulfonic acid hydrazide (**I**), but there were some differences, including shifts to a negative and/or positive direction and the appearence of new peaks in the anodic redox waves, which were observed at about 0.4 V and 1 V, respectively. In CVs of sulfonylhydrazones (**II–IV**), the reduction peaks belonging to the azomethine groups (C=N→CH-NH) were observed at −1.09 V, −1.169 V, and −1.05 V, respectively [41, 42].

3.2. *Biological studies*

3.2.1. *hCA I inhibition result*

A DPV technique was developed for the assessment of the impact of the sulfonamide compounds (inhibitors) on the carbonic anhydrase I (CA I). The inhibition of CA I was assessed by measuring the enzyme activity before and after incubating with inhibitors. Stock solutions of 10 mM inhibitors (compounds **I–IV** and AAZ as standard) in ACN containing 0.1 M tetrabutylammonium tetra–fluoroborate (TBATFB) were prepared at room temperature. For the enzyme inhibition studies, 10 mM Tris (pH 7.4) with 1 M H_2SO_4 was used as a supporting electrolyte. P-nitrophenyl acetate (PNPA) was used as a substrate and exhibited two peaks occurring at approximately 0.782 V as a reduction peak and 0.025 V as an oxidation peak, as shown in the inset in Fig. 5.

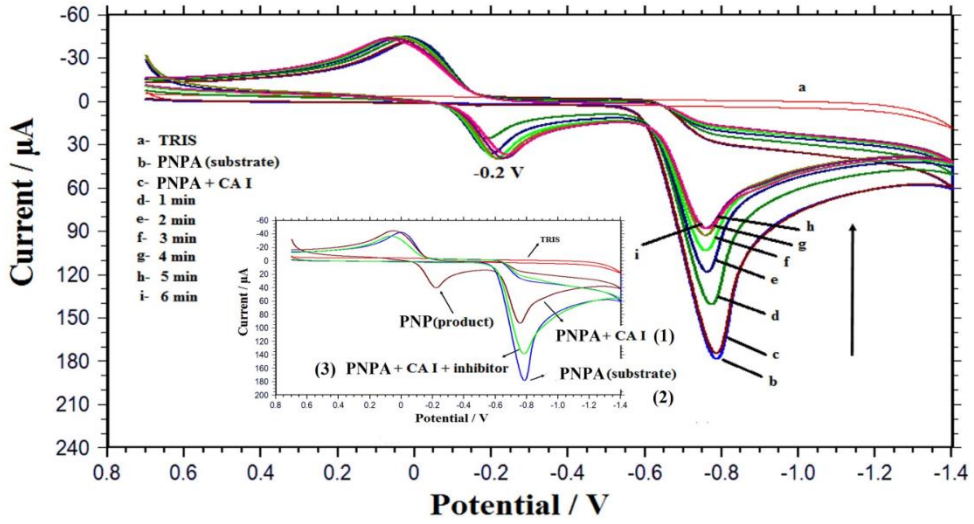


Fig. 5. CVs that show the effect of time on the PNPA (3×10^{-3} M) peak current in the presence of 5 µl CA I. CVs of PNP inhibition in 0.1 M Tris buffer at pH 7.4 were seen in Fig. 5. [(1) 3×10^{-3} M substrate alone; (2) substrate in the presence of 5 µl hCA I; and (3) addition of 1×10^{-4} M inhibitor to (2)].

To determine the enzyme inhibition activity of the inhibitors, DPVs were taken in Tris buffer media (pH 7.4) containing 3 mM substrate (PNPA) and after adding 5 ml of carbonic anhydrase I enzyme (hCA I) to the cell. Since a whole enzyme reaction was completed in 6 min, we waited this amount of time before adding the inhibitor [43]. PNPA released p-nitrophenol (PNP) by the enzymatic hydrolysis by the carbonic anhydrase I (CA I) and gave a non-overlapping cathodic peak at approximately –0.2 V (vs. Ag/AgCl). PNP reacted with the sulfonamides (inhibitors) with different concentrations (0.1–10 µM) to give an obvious decrease in the electrochemical signal. The results showed that the PNP peak decreases with increasing concentration of the inhibitors due to inhibition of the enzymatic function (Fig. 6) [11].

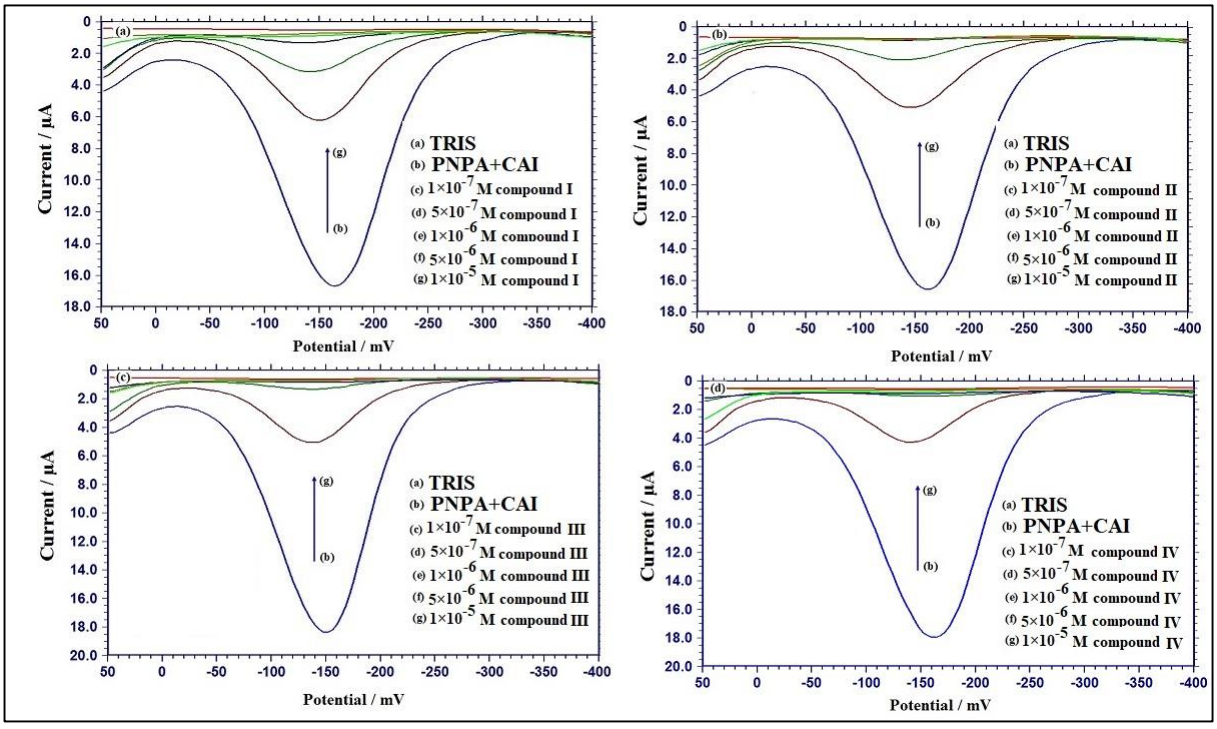


Fig. 6. DPVs of PNP inhibition at the GC electrode in the presence of different concentrations of inhibitors (compound I-IV) were measured from 50 mV to –400 mV vs. Ag/AgCl in 10 mM Tris buffer containing 3×10^{-3} M PNPA, 5 µl hCA I, and increasing concentrations of inhibitors (1×10^{-7}–1×10^{-4} M) at pH 7.4.

When the inhibitory activities of the compounds **II–IV** are compared with a standard drug (acetazolamide, AAZ), compound **IV** having a substitued Br is found to have good inhibitory properties compared to the others.

We also examined the inhibitory actions of the sulfonylhydrazones on the CA I from human by assaying the inhibition of the esterase activity, and their activities were compared to the standard reference drug acetazolamide (AAZ) by using the spectroscopic method. Lineweaver-Burk plots and activity % regression analysis graphs are given in Figure 7 and Figure 8, respectively. Inhibition data are shown in Table 5.

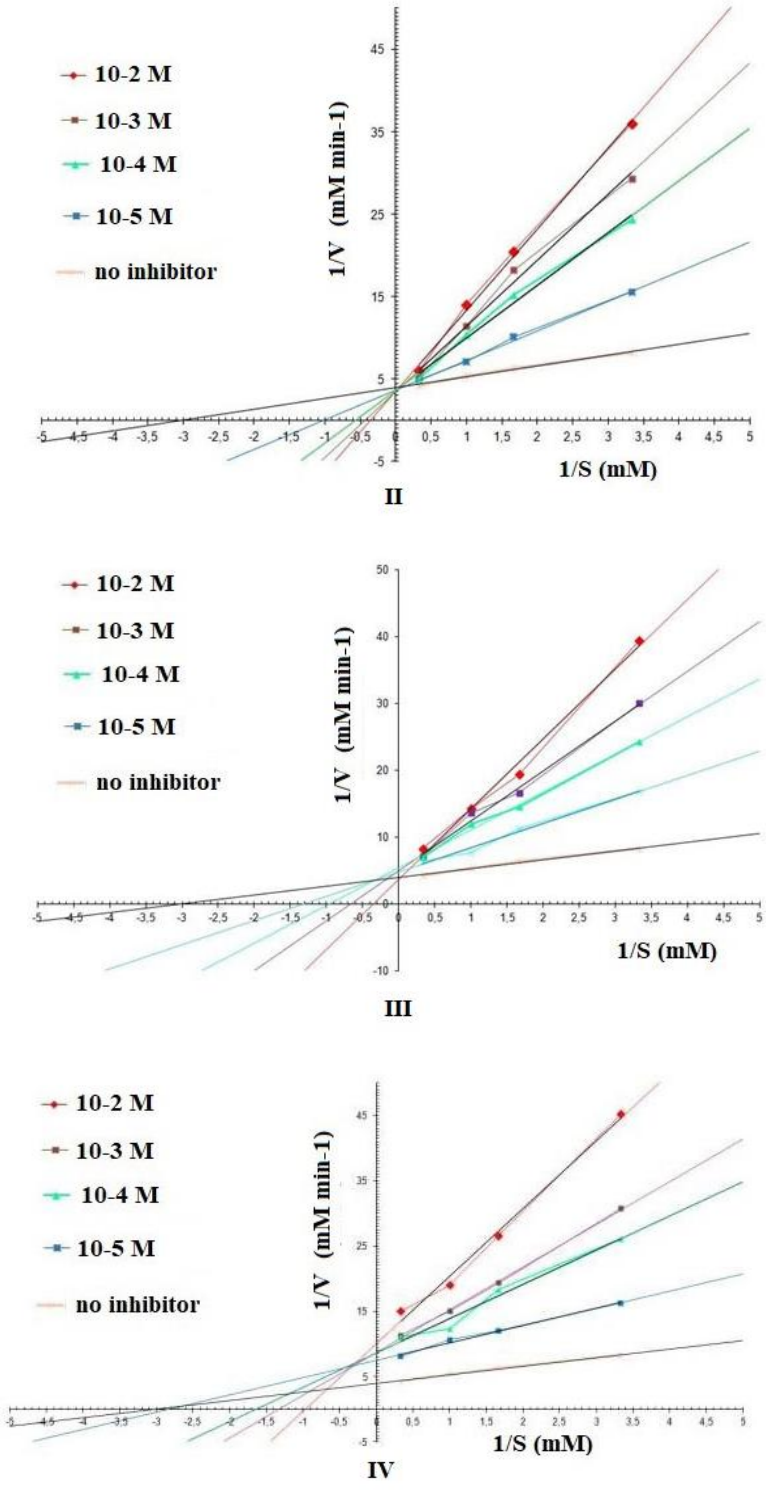
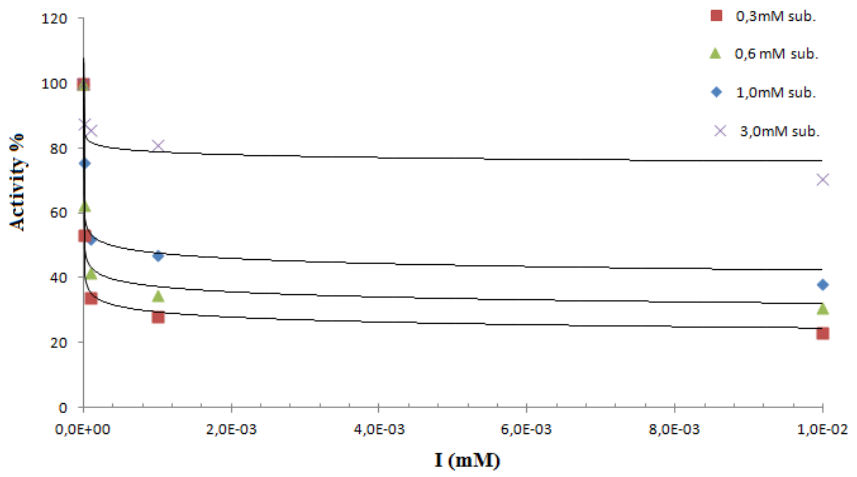
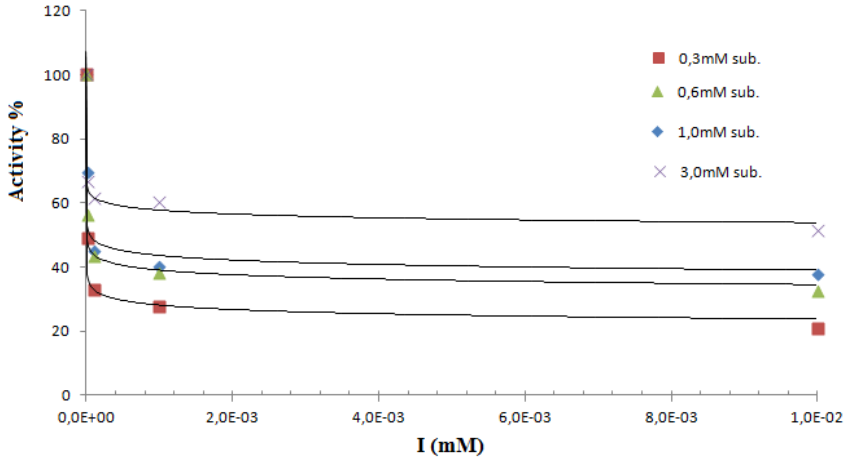


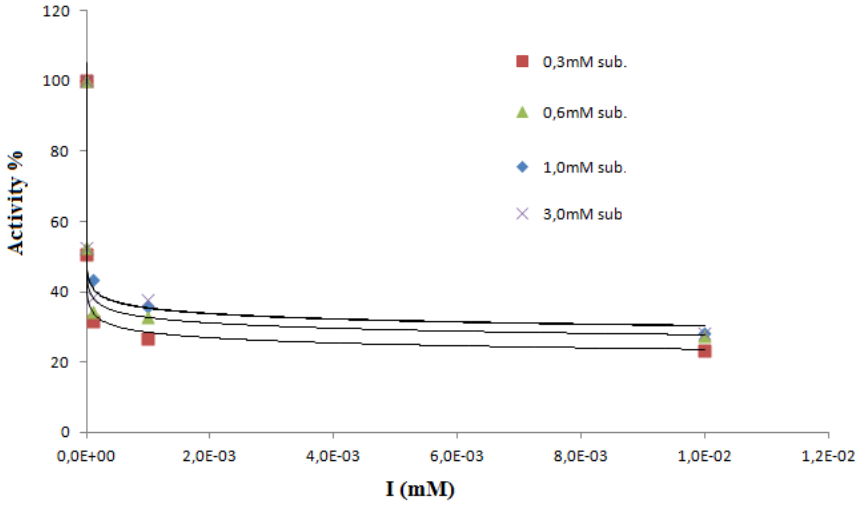
Fig. 7. Lineweaver-Burk plots for the inhibiton of hCA I by sulfonylhydrazones at different concentrations of the substrate



II



III



IV

Fig. 8. Activity % regression analysis graphs for hCA I in the presence four different substrate concentrations

T a b l e 5

Inhibition effects of sulfonylhydrazones on the carbonic anhydrase (hCA I) enzyme

Compounds	hCA I Esterase Activity		
	IC_{50} (mM)	K_i (mM)	Inhibition type
II	3.96×10^{-4}	9.90×10^{-5}	Competitive
III	5.58×10^{-5}	1.40×10^{-5}	Competitive
IV	4.86×10^{-6}	1.22×10^{-6}	Competitive
AZA	2.85×10^{-7}	1.42×10^{-7}	Competitive

The sulfonylhydrazone derivatives investigated here showed moderate inhibitory properties against the slow cytosolic isoform hCA I. Compound **II** exhibited the lowest inhibition against this isoform with a K_i value of 9.90×10^{-5} mM. The activity results showed that compounds **III** and **IV** were much more effective inhibitors against hCA I with K_i in the range of 1.40×10^{-5} to 1.22×10^{-6} mM. These results demonstrate the contribution of hydroxyl and bromide groups to the inhibition efficacy, especially the hydroxyl group [44]. The presence of electron-donating groups as methyl moieties reduces the enzyme inhibition activities of the compounds. Since compound **II** has more methyl groups [24, 45], its enzyme inhibition activity is the lowest. As expected, compound **IV (**K_i: 1.22×10^{-6} mM) is the strongest hCA I inhibitor of all synthesized compounds and has a comparable potency to the standard drug AAZ (K_i: 1.42×10^{-7} mM). Consequently, the increasing inhibition order of the synthesized compounds is as follows: **II < III < IV**.

3.2.2. Antimicrobial activity results

The antibacterial and antifungal activities of the sulfonylhydrazone derivatives were evaluated *in vitro* by the broth microdilution method and compared with reference drugs (sulfamethoxazole, sulfisoxazole, and fluconazole) in terms of the MIC values. The results of these screenings are summarized in Table 6 for the MICs that inhibited more than 90 % bacterial and fungal growth. Our data indicated that all of the synthesized compounds had broad spectrum antimicrobial activity in the range of 2–64 μg/ml against bacterial and fungal strains.

All compounds showed an excellent inhibition activity against the bacterial strain with MIC values less than that of the standard drugs sulfamethoxazole and sulfisoxazole. However, compound **IV** displayed a similar inhibition activity with sulfamethoxazole against *S. aureus* at the MIC of 64 μg/ml. Among the series, compound **III** had excellent inhibition (MIC = 2 μg/ml) against *S. epidermidis*, which was 64-fold higher than sulfamethoxazole and sulfisoxazole (128 μg/ml).

From the antifungal activity results, all compounds (**II–IV**) exhibited excellent inhibition against *C. albicans* and *A. fumigatus* with MIC 8–16 μg/ml, which was 2–4 fold higher than the standard drug fluconazole (32 μg/ml). However, compound **III** showed a similar activity as the fluconazole agent against *C. albicans* at MIC = 32 μg/ml.

T a b l e 6

MIC values of compounds against the bacterial and fungal strains tested

Compounds	**MIC (μg/ml)**							
	Gram-positive bacteria		**Gram-negative bacteria**				**Fungi**	
	S. aureus	*S. epidermidis*	*E. coli*	*P. aeruginosa*	*S. maltophilia*	*K.pneumoniae*	*C.albicans*	*A. fumigatus*
II	32	16	32	16	16	32	16	8
III	32	2	32	16	16	32	32	8
IV	64	32	32	32	16	32	16	16
Sulfamethoxazole	64	128	64	128	64	128	–	–
Sulfisoxazole	256	128	256	128	256	256	–	–
Fluconazole	–	–	–	–	–	–	32	32

In addition, all of the compounds possessed bactericidal and fungicidal activities against the tested microorganisms with MBCs/MFCs ranging between 8–256 μg/ml (Supplementary Table S2).

Our antimicrobial assay results indicated that the synthesized compounds have effective and strong antimicrobial activity against clinical isolates of gram-positive and gram-negative bacteria and fungi.

4. CONCLUSION

Alkylsulfonylhydrazones (**II–IV**) were newly synthesized and characterized by using the elemental analyses and spectroscopic methods. Their structures are presented in Figure 1, and also, the exact structure of 5-bromosalicylaldehyde-methanesulfonylhydrazone (**IV**) (Fig. 2) was supported by X-ray diffraction.

The sulfonamide family is specialized as potent enzyme inhibitors against carbonic anhydrase isoenzymes like acetazolamide (AAZ) used in glucoma treatment. Differential pulse voltammetry (DPV) methods were applied to qualitatively evaluate the inhibition of alkylsulfonylhydrazones (inhibitors) on hCA I. As the concentrations of inhibitors increased, the decrease observed in the product (PNP) peak indicated that the substrate-enzyme interaction decreased and CA I inhibition increased. The inhibitors showed high inhibition ability even at low concentrations. DPVs showed that the inhibition properties of compounds against CA I decreased in the order of **IV > III** > **II**, which supports the inhibition activity results obtained by the spectroscopic method. The enzyme inhibition activities of the compounds **II–IV** were evaluated using activity parameters (K_m, IC_{50}, and K_i) calculated by the spectrophotometric method. The highest inhibition efficiency of compound **IV** with the lowest IC_{50} (4.86×10^{-6} mM) may arise from the electron withdrawing property of the Br atom. When the inhibition activities of compound **IV** were compared with the standard (AAZ), it could be concluded that it is as good an hCA I inhibitor, having importance in bioinorganic and metallodrug chemistry. Sulfa drugs are the oldest chemically known antimicrobial agents, and they are still widely used for the treatment of various bacterial, protozoal, and fungal infection diseases [46]. The antmicrobial activities of the alkylsulfonylhydrazones were investigated by the broth microdilution method and compared with the MICs of standards (sulfamethoxazole, sulfisoxazole, and fluconazole). Antimicrobial activity results showed that compound **III** had an excellent inhibition (MIC = 2 μg/ml) against *S. epidermidis*, which was 64-fold higher than sulfamethoxazole and sulfisoxazole (128 μg/ml) .

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