



# Carbonic anhydrase inhibitors: Synthesis, characterization and inhibition activities of furan sulfonylhydrazones against carbonic anhydrase I (hCA I)



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## ABSTRACT

The methane sulfonic acid hydrazide (**1**) was used to obtain furan sulfonylhydrazones; 2-acetylfuranmethanesulfonylhydrazone (**2**), 2-furaldehydemethanesulfonylhydrazone (**3**), 5-nitro-2-furaldehydemethanesulfonylhydrazone (**4**). The structures of furan sulfonylhydrazones were determined by using elemental analysis, FT-IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and UV–vis methods. The structure of 5-nitro-2-furaldehydemethanesulfonylhydrazone (**4**) was also supported with X-ray diffraction method and found that compound **4** was crystallized in triclinic, space group P $\bar{1}$ . In order to gain insight into the structure of the compounds, we performed computational studies by using 6–311G(d,p) basic set in which B3LYP correlation function was implemented. The geometry of the sulfonylhydrazones were optimized at DFT method with Gaussian 09 program package and the global reactivity descriptors were also calculated by this basic set. The enzyme inhibition activities of the sulfonylhydrazones were investigated on carbonic anhydrase I (hCA I) isoenzyme and their activity parameters (Km, IC<sub>50</sub> and Ki) were calculated by spectrophotometric method. And also, their inhibitor effects were also investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods. Inhibition results show that compound **4** containing electron withdrawing group (NO<sub>2</sub>) has higher inhibition effect on hCA I isoenzyme than other's.

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## 1. Introduction

The carbonic anhydrases (CAs) are the metalloenzymes containing zinc center which classically participate in the maintenance of pH homeostasis. CAs catalyze the reversible hydration of carbon dioxide in two-step reaction to yield bicarbonate ion and proton. Sixteen CA isozymes have been described and some of them are cytosolic (CA I, CA II, CA III, CA VII and CA XIII), other CA isoenzymes are membrane bound (CA IV, CA IX, CA XII and CA XIV), two of CAs are mitochondrial (CA VA and CA VB) and one of CAs is in saliva (CA VI). The two important CA isozymes (CA I and CA II) are present at higher concentrations in the cytosol in erythrocytes [1–3].

Inhibitors of the carbonic anhydrases (CAs) have clinical usage of major diseases such as glaucoma, epilepsy, gastroduodenal ulcers, acid-base disequilibria and neurological disorders [4]. CA inhibition by sulfonamides have been reported by Mann and Keilin [5], and subsequently other inhibitors have been investigated for medical applications. The synthesis of CA inhibitors from various classes of sulfonamides (such as sulfonylhydrazide, sulfonylhydrazone, sulfonylurea etc.) have importance of specificity towards isoenzymes [6] and affect biologic systems by interacting with enzyme active sites. For this reason, new CA inhibitors interacting with CA isoenzymes have importance in bioinorganic and metallodrug chemistry. Many of metabolic processes are based on redox processes and there are many similarities between electrochemical and biological reactions relating with electron transfer mechanisms in systems [7].

In this study, methane sulfonic acid hydrazide (**1**) was synthesized as reported by us [8,9] and used to obtain furan

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sulfonylhydrazones; 2-acetylfuranmethanesulfonylhydrazone (**2**), 2-furaldehydemethanesulfonylhydrazone (**3**) and 5-nitro-2-furaldehydemethanesulfonylhydrazone (**4**). The structures of compounds **2–4** were characterized by using elemental analysis,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, FT-IR and UV–vis methods. Gaussian 09 software was used to obtain the most stable conformation of the compounds **2–4** based on DFT/B3LYP/6-311G(d,p) method and global reactivity descriptors such as the highest occupied molecular orbital ( $\epsilon_{\text{HOMO}}$ ), the lowest occupied molecular orbital ( $\epsilon_{\text{LUMO}}$ ), electronegativity ( $\chi$ ), chemical potential ( $\mu$ ), global hardness ( $\eta$ ), global softness ( $S$ ), global electrophilicity index ( $\omega$ ) were calculated by this basic set.

The inhibition effects of the heteroaromatic sulfonylhydrazones on carbonic anhydrase I (hCAI) were determined by electronic spectra. The activity parameters (Km, IC<sub>50</sub> and Ki) were calculated by Lineweaver–Burk graph, activity % graph and Cheng-Prusoff equation. Inhibition activities of furan sulfonylhydrazones were also evaluated by voltammetric techniques. The enzyme activities were investigated by using enzymatic hydrolysis of substrate (p-nitrophenylacetate, PNFA) to p-nitrophenolate, PNF via hCA I isoenzyme. In the presence of sulfonylhydrazones, the enzyme function was inhibited, resulting in a decrease in PNF formation in other words the inhibition degree of hCA I was correlated to the decrease in PNF reductive current. Biological activity results show that compound **4** containing electron withdrawing group NO<sub>2</sub> has higher inhibition effect on hCA I isoenzyme than other sulfonylhydrazones.

## 2. Experimental

### 2.1. Physical measurements

The solvents used were purified and distilled according to routine procedures. Methane sulfonyl chloride, hydrazine hydrate were commercial products (purum). Elemental analyses were performed according to standard micro analytical procedures by Leco CHNS-932,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the compounds in dimethylsulfoxide- $d_6$  (DMSO- $d_6$ ) were registered on a Bruker WM-400 spectrometer (400 MHz) using tetramethylsilane as internal standard. The infrared spectra of the compounds as KBr-disks were recorded in the range of 4000–400  $\text{cm}^{-1}$  with a Mattson 1000 FT-IR spectrometer. UV–vis spectra were recorded on UNICAM-UV 2-100 spectrophotometer. Melting points of furan sulfonylhydrazones were determined with a Gallenkamp melting point apparatus. The crystal structure of 5-nitro-2-furaldehydemethanesulfonylhydrazone (**4**) was determined by using a Bruker Kappa APEX II CCD area-detector. The inhibition activities of synthesized compounds on carbonic anhydrase I (hCAI) were investigated by measuring absorbances at 400 nm on UV–vis spectrophotometer. For electrochemical analysis, cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods were studied by using CHI 660B voltammeter.

### 2.2. General procedure for the synthesis of compounds

The nucleophilic substitution reaction of the hydrazine hydrate with methane sulfonyl chloride was carried out methane sulfonic acid hydrazide (**1**) as reported [8,10,11]. Compounds **2–4** were synthesized according to the following general procedure: The solution of methane sulfonic acid hydrazide (4.55 mmol) in 50 mL of methanol was mixed with hot solution of 4.55 mmol of the corresponding carbonyl compound (2-acetylfuran, 2-furalaldehyde and 5-nitro-2-furalaldehyde, respectively) in 30 mL of methanol and stirred for 24 h. Obtained crystals were recrystallized with ethylacetate, then washed with ethylacetate/ether and dried at 50 °C in oven.

### 2.3. X-ray structure determination of compound **4**

Crystallographic data of compound was recorded on a Bruker Kappa APEX II CCD area-detector X-ray diffractometer employing plane graphite monochromatized with MoK $\alpha$  radiation ( $\lambda = 0.71073 \text{ \AA}$ ), using  $\omega - 2\theta$  scan mode. The structures were solved by the direct methods and refined by full-matrix least-squares techniques on F<sup>2</sup> using the solution program SHELXS-97 and refined using SHELXL-97 [12]. The empirical absorption corrections were applied by multi-scan via Bruker, SADABS software [13]. The H atoms positions were calculated geometrically at distances of 0.95 Å (CH) from the parent C atoms; a riding model was used during the refinement process. The molecular structure plots were prepared using ORTEP-3 for Windows [14].

### 2.4. Computational section

Molecular geometry optimization and global reactivity descriptors such as highest occupied molecular orbital ( $\epsilon_{\text{HOMO}}$ ), lowest occupied molecular orbital ( $\epsilon_{\text{LUMO}}$ ), electronegativity ( $\chi$ ), chemical potential ( $\mu$ ), global hardness ( $\eta$ ), global softness ( $S$ ) and global electrophilicity index ( $\omega$ ) were carried out by Gaussian 09 quantum chemistry program-package examined with Becke's three-parameter exchange functional in combination with the Lee–Yang–Parr correlation functional (B3LYP) in density functional theory (DFT) method with 6-311G(d,p) basis set [15–18]. Global reactivity descriptors consist of electronegativity ( $\chi = -1/2(\epsilon_{\text{LUMO}} + \epsilon_{\text{HOMO}})$ ), chemical potential ( $\mu = 1/2(\epsilon_{\text{LUMO}} + \epsilon_{\text{HOMO}})$ ), global hardness ( $\eta = 1/2(\epsilon_{\text{LUMO}} - \epsilon_{\text{HOMO}})$ ), global softness ( $S = 1/2\eta$ ) and electrophilicity index ( $\omega = \mu^2/2\eta$ ) were highly successful in predicting global reactivity trends. These parameters were related not only to the spectral properties, but also to the reactivity properties [19].

### 2.5. Procedures for hCA I enzyme inhibitor activities

#### 2.5.1. Spectrophotometric studies

Carbonic anhydrase activities were assayed by the hydrolysis of substrate (p-nitrophenylacetate, PNFA) [20] to p-nitrophenolate, PNF via hCA I isoenzyme and activity parameters (Km, IC<sub>50</sub> and Ki) were calculated with Lineweaver–Burk graph, activity % - [inhibitor] graph and Cheng-Prusoff equation. Acetazolamide ((5-acetamido-1,3,4-thiadiazole-2-sulfonamide, AAZ) clinically used in hCA I inhibition was also been investigated as standard inhibitor.

In order to determine IC<sub>50</sub> values, 100  $\mu\text{L}$  of 3.0 mM p-nitrophenylacetate as substrate and four different concentrations ( $3 \times 10^{-2}$ ;  $3 \times 10^{-3}$ ;  $5 \times 10^{-4}$ ;  $3 \times 10^{-4}$  M) of inhibitors were used. Reaction was started by adding of 170  $\mu\text{L}$  of 0.05 M tris-SO<sub>4</sub> buffer (pH: 7.4) and 0.1  $\mu\text{L}$  enzyme solution for total volume of 300  $\mu\text{L}$ . The absorbance of the product (PNF) was determined at 400 nm after 6 min [21]. This study was repeated three times for each inhibitor. 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 ( $3 \times 10^{-2}$ ;  $3 \times 10^{-3}$ ;  $5 \times 10^{-4}$ ;  $3 \times 10^{-4}$  M). Km values were calculated from Lineweaver–Burk graphs, IC<sub>50</sub> values were calculated from activity%-[inhibitor] graphs and Ki values were calculated according to Cheng Prusoff equation using Km and IC<sub>50</sub> parameters [22,23].

#### 2.5.2. Electrochemical studies

Electrochemical measurements by using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were conducted on a CHI 660B electrochemical workstation (Shanghai, China). A conventional three-electrode system was employed with a glassy

carbon (GC) electrode (3 mm diameter) as working electrode, an Ag/AgCl electrode as reference electrode, and a platinum wire as counter electrode. For enzyme inhibition studies, 10 mM TRIS (pH 7.4) with 1 M H<sub>2</sub>SO<sub>4</sub> was used as supporting electrolyte. Stock solutions of 10 mM inhibitors (compounds **1–4**, **AAZ**) in acetonitrile containing 0.1 M tetrabutylammonium tetrafluoroborate (TBATFB) were prepared at room temperature. GC electrode was mechanically polished first with fine wet emery papers (Buehler) with grain size of 4000 and then with 0.3 μm and 0.05 μm alumina slurry (Baikowski Int. Corp.) on a microcloth pad (Buehler). Polished GC electrode was sonicated in ultrapure water and then with a mixture of 1:1 (v/v) isopropyl alcohol/acetonitrile (IPA/MeCN) for 10 min for removal of trace alumina from the surface. Then, GC electrode was rinsed with acetonitrile and dried under N<sub>2</sub> stream [24–26].

### 3. Results and discussion

Analytical data and some physical properties of the furan sulfonylhydrazones are summarized in Table 1. The general synthetic route used to prepare the compounds are illustrated in Fig. 1. The exothermic nucleophilic substitution reaction of corresponding methane sulfonyl chloride with hydrazine hydrate were employed to form methane sulfonic acid hydrazide (**1**) which reacted with aromatic ketone/aldehydes to form; 2-acetylfuranmethane sulfonylhydrazone (**2**), 2-furaldehydemethanesulfonylhydrazone (**3**), 5-nitro-2-furaldehydemethanesulfonylhydrazone (**4**). The geometry optimization was performed on DFT/B3LYP/6-311G(d,p) methods with Gaussian 09 program. Optimized structure of the sulfonylhydrazones are given in Fig. 2.

#### 3.1. NMR spectra

<sup>1</sup>H–<sup>13</sup>C NMR spectra of compounds **2–4** were obtained in DMSO-d<sub>6</sub> at room temperature using TMS as an internal Standard. The experimental <sup>1</sup>H–<sup>13</sup>C NMR assignments in DMSO-d<sub>6</sub> are listed in Table 2.

CH<sub>3</sub> protons bonded to SO<sub>2</sub> group (C(6)H<sub>3</sub>, three H intensities) of compounds **2–4** are easily distinguishable as a singlet and they are observed at 3.15 ppm, 3.22 ppm and 3.11 ppm, respectively. Acetyl protons (C–CH<sub>3</sub>, three H intensities) of compound **2** are observed at 2.27 ppm as singlet, the aromatic protons of furan ring systems are observed between 6.53 and 7.77 ppm, 6.64–7.82 ppm, 7.23–7.78 ppm, respectively. Azomethine protons (C(5)H=N, one H intensity) of compounds **3** and **4** are observed at 7.87 ppm and 7.93 ppm as singlet, respectively. In addition, the experimental seconder NH protons of compounds **2–4** are observed in the range of 10.22–11.84 ppm as seen in Fig. 3 [27–31].

The CH<sub>3</sub> carbons of methyl moiety bonded to SO<sub>2</sub> groups of compounds **2–4** are observed in the range of 38.38–39.79 ppm. Acetyl carbon (C–CH<sub>3</sub>) of compound **2** is observed at 14.14 ppm. The aromatic carbons of furan ring systems are observed between 111.87 and 151.56 ppm, 112.50–149.21 ppm and 115.81–153.46 ppm, respectively. Azomethine carbons (C(5) = N) of compounds **3** and **4** are observed at 145.48 ppm and 135.56 ppm as

seen in Fig. 3.

#### 3.2. FT-IR spectra

The selected vibration frequencies of furan sulfonylhydrazones are listed in Table 3. The assignment of the bands are made by taking into consideration the literature data for compounds containing appropriate structural fragments such as furan and sulfonamide derivatives [32–34]. NH vibrations in compounds **2–4** are observed between 3220 and 3231 cm<sup>-1</sup> as strong bands. And also <sup>ν</sup>SO<sub>2(as)</sub>, <sup>ν</sup>SO<sub>2(s)</sub> stretching vibrations are observed between 1333 and 1358 cm<sup>-1</sup>, 1031–1166 cm<sup>-1</sup> for compounds **2–4**. Furan rings have characteristic vibration bands (ν<sub>C–O–C</sub>) in the range of 1162–1227 cm<sup>-1</sup> as seen in Fig. 4 [35].

#### 3.3. Crystal structure of compound 4

The crystal and instrumental parameters used in the unit-cell determination and data collection are summarized in Table 4, the selected bond lengths and angles are listed in Table 5, respectively. The 5-nitro-2-furaldehydemethanesulfonylhydrazone (**4**) was crystallizes in the triclinic, P $\bar{1}$  space group and the asymmetric unit consists of two independent and similar molecules (C<sub>6</sub>H<sub>7</sub>N<sub>3</sub>O<sub>5</sub>S) as seen in Fig. 5. The S–O and S–N bond distances lie within expected range of 1.4239(18)–1.4265(19) Å and 1.647(2) Å, respectively. The S1–C6 and S2–C12 bond distances are 1.748(3) and 1.749(3) Å. The S–C bond distances still have an intermediate value between a single (1.82 Å) and double (1.56 Å) [36]. This is an indication that there is some electron delocalization in the methane sulfonic acid hydrazide chain. All bond lengths and angles for compound **4** are consistent with those found in related compound as seen in Table 5 [37].

#### 3.4. Global reactivity descriptors analysis

The highest occupied molecular orbital (ε<sub>HOMO</sub>) and the lowest unoccupied molecular orbital (ε<sub>LUMO</sub>) are very important for quantum chemistry [38–40]. HOMO's and LUMO's are the main orbitals in chemical stability. The HOMO's as an electron donors representing the ability to donate an electron and LUMO's as an electron acceptors representing the ability to obtain an electron. The HOMO's and LUMO's are the main orbital taking part in the chemical reactions. The HOMO energy levels are directly related to the ionization potential and LUMO energy levels are directly related to the electron affinity. Frontier molecular orbitals were computed at B3LYP/6-311G(d,p) level of the title molecules as shown in Table 6. The electronegativity (χ), chemical potential (μ), global hardness (η), global softness (S) and global electrophilicity index (ω) are calculated by frontier molecular orbitals (ε<sub>HOMO</sub>, ε<sub>LUMO</sub>) energies and listed in Table 6. Compound **4** containing electron-withdrawing group (NO<sub>2</sub>) has the lowest LUMO energy level which is the most important descriptor showing higher electrophilicity of the compound **4**. The rising of electronic descriptors (χ = 0.1847 eV and ω = 0.2389 eV) causes the strongest

**Table 1**  
Analytical and physical data for furan sulfonylhydrazones.

Compound	Formula M <sub>w</sub> (g/mol)	m.p. (°C)	Yield (%)	λ (nm)	Found (calculated)			
					%C	%H	%N	%S
<b>2</b>	C <sub>7</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub> S 202.23	138–140	69	370, 305, 245	41.32 (41.60)	5.14 (4.95)	13.79 (13.86)	15.83 (15.84)
<b>3</b>	C <sub>6</sub> H <sub>8</sub> N <sub>2</sub> O <sub>3</sub> S 188.21	105	64	370, 305, 245	38.54 (38.30)	4.54 (4.25)	14.69 (14.89)	16.88 (17.02)
<b>4</b>	C <sub>6</sub> H <sub>7</sub> N <sub>3</sub> O <sub>5</sub> S 233.20	180	60	400, 245	30.57 (30.90)	3.13 (3.00)	17.62 (18.03)	13.58 (13.73)

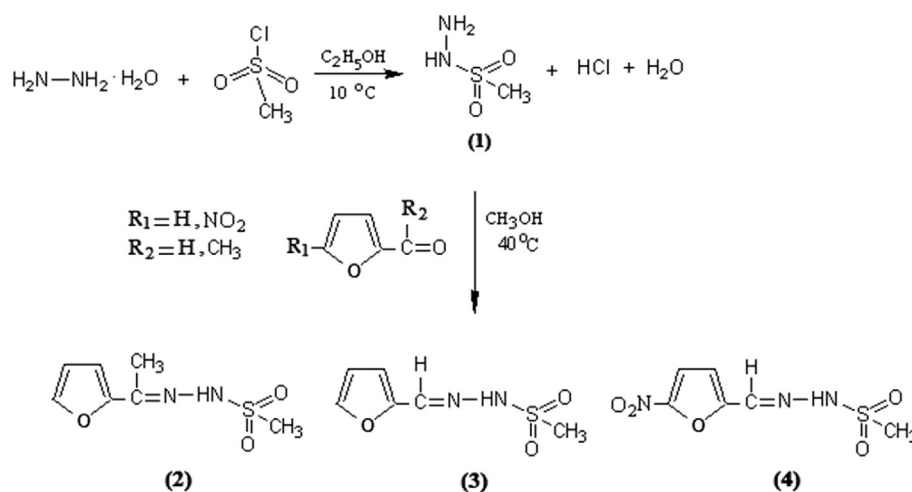


Fig. 1. Preparation of compounds (1–4).

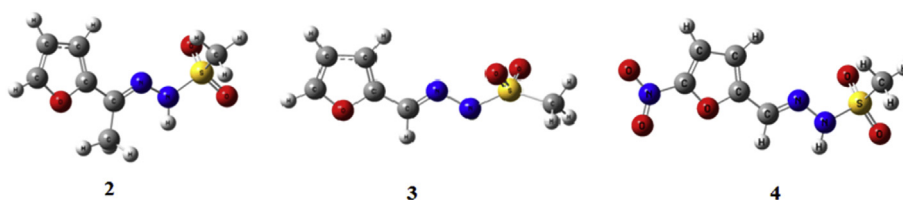


Fig. 2. Optimized geometries of furan sulfonylhydrazones (2–4).

**Table 2**  
The experimental <sup>1</sup>H NMR data of furan sulfonylhydrazones.

Assign.	<sup>1</sup> H NMR (δ, ppm)			<sup>13</sup> C NMR (δ, ppm)		
	2	3	4	2	3	4
NH (s,1H)	10.22	10.76	11.84	–	–	–
C(1)H (d,1H)	7.77	6.90	–	145.80	145.48	153.46
C(2)H (dd,1H)	6.53	6.64	7.78 <sup>a</sup>	144.99	112.50	117.86
C(3)H (d,1H)	6.94	7.82	7.23	112.26	114.15	115.81
C(4)	–	–	–	151.56	149.21	151.98
C(5)H=N (s,1H)	–	7.87	7.93	–	137.21	135.56
SO <sub>2</sub> -C(6)H <sub>3</sub> (s,3H)	3.15	3.22	3.11	38.38	37.72	39.79
C(5)-CH <sub>3</sub> (s,3H)	2.27	–	–	14.14	–	–

<sup>a</sup> C(2)H (d,1H).

electrophilicity, which plays dominant role on predicting global chemical reactivity trend.

### 3.5. Determination of hCA I inhibitory effects

#### 3.5.1. Spectrophotometric method

The inhibitory effects of the furan sulfonylhydrazones were investigated by spectrophotometric method (Fig. 6). The enzyme activity of compounds 2–4 were evaluated by using  $K_m$  (Michaelis constant),  $IC_{50}$  ( $IC_{50}$  represents the molarity of inhibition as 50% decrease of enzyme activity), and  $K_i$  (inhibitor–enzyme dissociation constant) values that are three of the most appropriate parameters of the inhibitors (Table 7) [41].

As seen in Table 7, furan sulfonylhydrazones behave as good inhibitors against hCA I isoenzyme. Compound 4 containing  $NO_2$  group shows enhanced inhibition effect ( $IC_{50}$ :  $1.02 \times 10^{-3}$ ,  $K_i$ :  $5.88 \times 10^{-6}$  M) on hCA I than other compounds. This is probably due to the further effect of the electron withdrawing group such as  $NO_2$ . The presence of electronegative group may increase the

interaction of compounds with enzyme active sites. Acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide), **AAZ** has also been investigated as standard inhibitor which clinically used against hCA I. One of the most important findings of this study is that compound 4 containing  $NO_2$  group has the nearest activity to **AAZ**.

#### 3.5.2. Electrochemical method

Redox behaviors of methane sulfonic acid hydrazide (1) and furan sulfonylhydrazones (2–4) were investigated by CV and DPV techniques [42,43]. Two oxidation peaks belong to methane sulfonic acid hydrazide (1) were observed in the positive potential ranges in CVs. Furan sulfonylhydrazones (2–4) show redox behavior compared with free methane sulfonic acid hydrazide (1), but there are some changes including negative and/or positive shifts of redox waves and appearance/disappearance of some redox signals. In CVs of sulfonylhydrazones (2–4), the reduction peaks which belong to azomethine groups ( $C=N \rightarrow CH-NH$ ) were observed at  $-0.803$  V,  $-0.772$  V and  $-0.753$  V, respectively. Two reduction peaks of nitro group belong to compound 4 were observed at about  $1.06$  V and  $-1.42$  V, respectively. The first peak can be attributed to reduction of  $NO_2 \rightarrow NHOH$  and the second to reduction of  $NHOH \rightarrow NH_2$ .

hCA I inhibitor activities of methane sulfonic acid hydrazide (1) and furan sulfonylhydrazones (2–4) were investigated by cyclic voltammetry (CV) and also differential-pulse voltammetry (DPV). The inhibition effects of the compounds are concluded by measuring the reduction peaks of the product (PNF) produced from the enzymatic hydrolysis of substrate (PNFA) by hCA I. PNFA exhibited two peaks occurring at approximately  $-0.782$  V as reduction peak and  $0.025$  V as oxidation peak. Notably, PNF formation is also defined by a single, non-overlapping cathodic peak at approximately  $-0.2$  V (vs Ag/AgCl). In the presence of sulfonylhydrazones, the enzymatic function is inhibited resulting in a

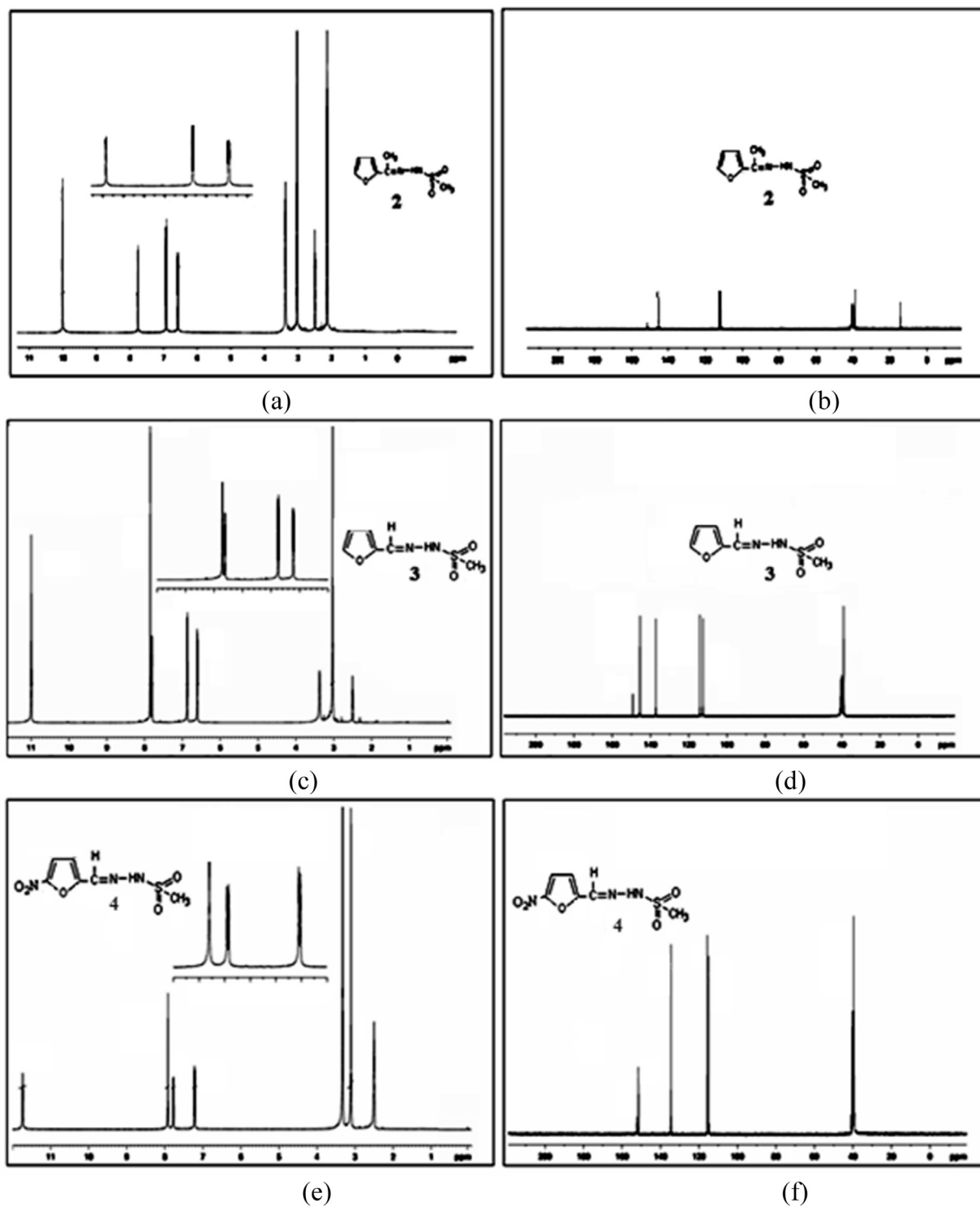


Fig. 3.  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectrum (a–f) of furan sulfonylhydrazones.

decrease in PNF formation. The inhibition degree of hCA I is therefore correlated by the decrease in PNF reductive current which depends on the increasing concentration of the sulfonylhydrazones.

To determine the enzyme inhibition activity of substrate (PNFA), CVs were taken first in tris buffer media (pH 7.4) containing 3 mM substrate (PNFA) and after adding 5  $\mu\text{L}$  carbonic anhydrase I enzyme (hCA I) into the cell, respectively. The CV potential wave

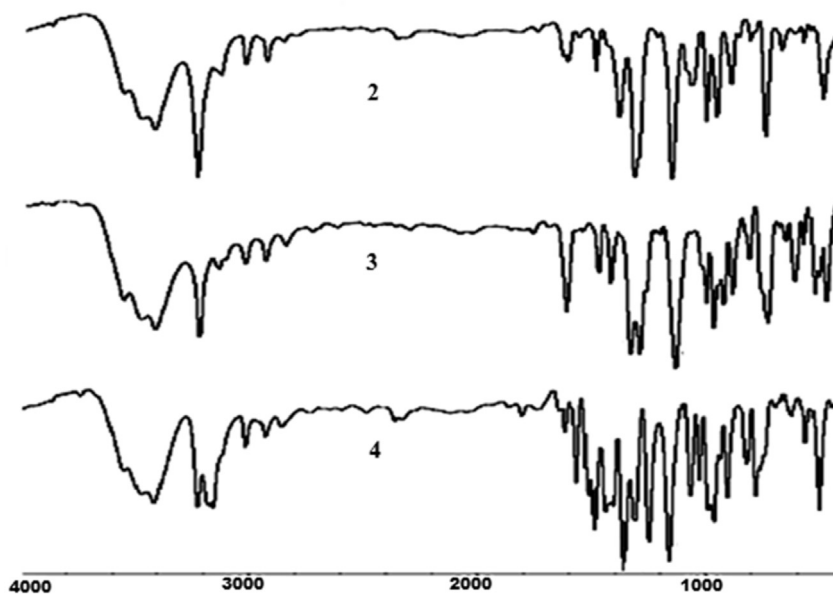
form was applied from  $-1.4$  V to  $0.70$  V. Fig. 7 shows that hCA I activity is related with the decrease of reduction peak current of substrate (PNFA) and the increase of reduction peak current of product (PNF). In other approach, inhibition degree of inhibitor can be correlated to the decrease in PNF reductive current which depends on the increasing concentration of the inhibitor in the sample.

The effects of different concentration ( $0.1$ – $100$   $\mu\text{M}$ ) of inhibitors



**Table 3**  
Selected FT-IR vibration bands of furan sulfonylhydrazones ( $\text{cm}^{-1}$ ).

Compound	$\nu_{\text{(NH)}}$	$\nu_{\text{CH(ar)}}$	$\nu_{\text{CH(alph)}}$	$\nu_{\text{C=N}}$	$\nu_{\text{SO}_2(\text{as})}$	$\nu_{\text{C-O-C}}$	$\nu_{\text{SO}_2(\text{s})}$	$\delta_{\text{NH}}$	$\delta_{\text{SO}_2}$	$\delta_{\text{CH(ar)}}$
2	3231	3018	2929 2852	1622	1333	1227	1166	637	510	757
3	3224	3028	2937 2853	1634	1346	1164	1031	639	541	755
4	3220	3021	2930 2860	1642 1623	1358	1162	1067	626	564	779

**Fig. 4.** FT-IR spectrum of furan sulfonylhydrazones (2–4).**Table 4**  
Crystal data and structure refinement details for compound 4.

Chemical formula	$\text{C}_6 \text{H}_7 \text{N}_3 \text{O}_5 \text{S}$
Formula weight	233.22
Temperature (K)	293(2)
Wavelength (Å)	0.71073
Crystal system, space group	Triclinic, $P\bar{1}$
Unit cell dimensions:(Å)	
a	7.6005(15)
b	8.2485(17)
c	16.226(3)
$\alpha$	77.718(5)
$\beta$	86.492(5)
$\gamma$	78.812(5)
Volume (Å <sup>3</sup> )	974.9(3)
Z	4
Absorption coefficient ( $\text{mm}^{-1}$ )	0.339
Calculated density ( $\text{Mg m}^{-3}$ )	1.589
F(000)	480
Crystal size (mm)	$0.25 \times 0.17 \times 0.11$
Theta range for data collection(°)	2.57–28.49
Limiting indices	$-9 \leq h \leq 10, -10 \leq k \leq 11, -21 \leq l \leq 21$
Reflections collected	11,455
Independent reflections	4952
Number of reflections used	3617
Number of parameters	273
Max. and min. transmission	0.933, 0.963
Refinement method	Ful-matrix least-squares on $F^2$
Final R indices [ $I \geq 2\sigma(I)$ ]	$R_1 = 0.0514, wR_2 = 0.1552$
R indices (all data)	$R_1 = 0.0660, wR_2 = 0.1720$
Goodness-of-fit (GOF) on $F^2$	1.092
Largest difference in peak and hole ( $e \text{ Å}^{-3}$ )	0.488 and $-0.571$

**Table 5**  
Selected bond distances [Å] and angles [°] of compound **4**.

S1–O4	1.4239 (18)	S2–O9	1.4326 (18)
S1–O5	1.4265 (19)	S2–O10	1.4162 (18)
S1–N3	1.647 (2)	S2–N6	1.649 (2)
N2–N3	1.385 (3)	N5–N6	1.385(2)
S1–C6	1.748 (3)	S2–C12	1.749 (3)
O4–S1–O5	119.75 (12)	O9–S2–O10	119.38 (12)
O4–S1–N3	107.14 (11)	O10–S2–N6	107.22 (11)
N2–N3–S1	115.20 (15)	N5–N6–S2	115.50 (7)
N3–S1–C6	106.32 (13)	N6–S2–C12	109.27 (7)

on the reduction peak of product (PNF) formed by the interaction of the enzyme with the substrate were investigated by DPV method. As seen in Fig. 7, the reduction peak (at about  $-0.2$  V) belongs to PNF is observed by negative scan in DPV and so, the decreasing rate of PNF reduction peak is employed to assay the hCA I inhibition activity of compound **4**. Accordingly, analysis over a wider range of inhibitor concentrations ( $0.1$ – $100$   $\mu$ M) show that the gradual decrease in PNF peak current intensity with increasing inhibitor concentration reveals the inhibition effects of the inhibitors against hCA I isoenzyme [44]. When the inhibitory activities of the compounds (**1**–**4**) compared with the standard (acetazolamide, **AAZ**), compound **4** having  $\text{NO}_2$  group is found to has good inhibitory properties than others. Sulfonylhydrazone containing acetyl  $\text{CH}_3$  group has lower enzyme inhibition activity than other furan sulfonylhydrazones. Methane sulfonic acid hydrazide has poor activity which resulting of absence of active sites such as imine, furan, nitro etc. [45].

#### 4. Conclusion

In this study, the synthesis of new furan sulfonylhydrazones from methane sulfonic acid hydrazide is reported. The structural characterization of the synthesized compounds (**2**–**4**) was made by using the elemental analyses and spectroscopic methods. Based on physicochemical evidence, the proposed structure of compounds **2**–**4** are exhibited in Fig. 2. The structure of 5-nitro-2-furaldehydemethanesulfonylhydrazone is also supported by X-ray crystal diffraction. The enzyme inhibition effects of the compounds are evaluated using activity parameters ( $K_m$ ,  $\text{IC}_{50}$  and  $K_i$ ) found by spectrophotometric method. The remarkable inhibition efficiency

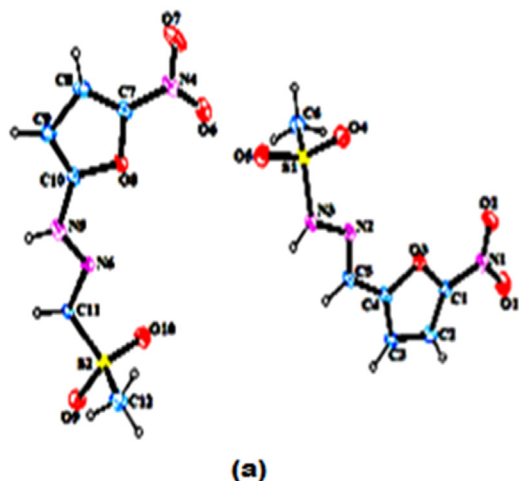
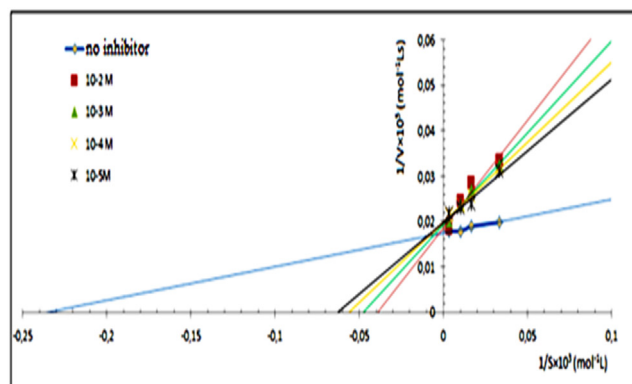


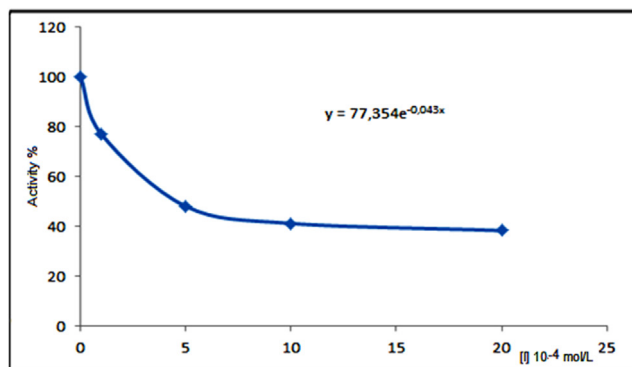
Fig. 5. Crystal structure of compound **4** (ORTEP diagram (a) and package model (b)).

**Table 6**  
Calculated global reactivity descriptors in eV.

Compound	$\epsilon_{\text{LUMO}}$	$\epsilon_{\text{HOMO}}$	$\chi$	$\mu$	$\eta$	S	$\omega$
<b>2</b>	-0.0556	-0.2165	0.1361	-0.1361	0.0805	0.0403	0.1151
<b>3</b>	-0.1000	-0.2234	0.1617	-0.1617	0.0617	0.0309	0.2119
<b>4</b>	-0.1133	-0.2560	0.1847	-0.1847	0.0714	0.0357	0.2389

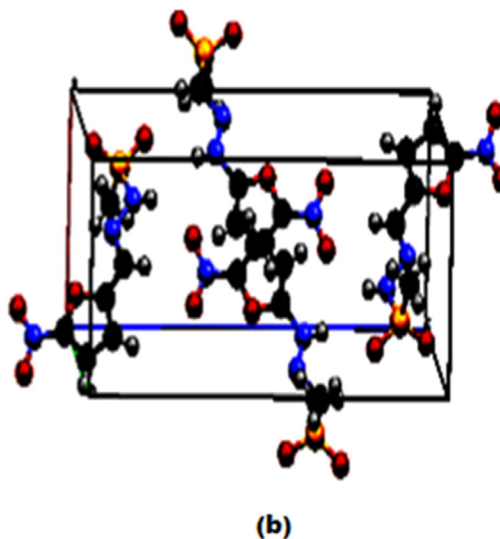


(a)

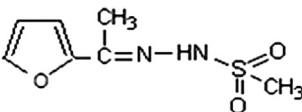
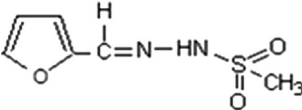
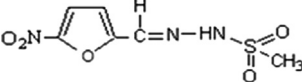
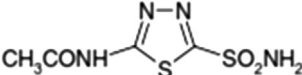


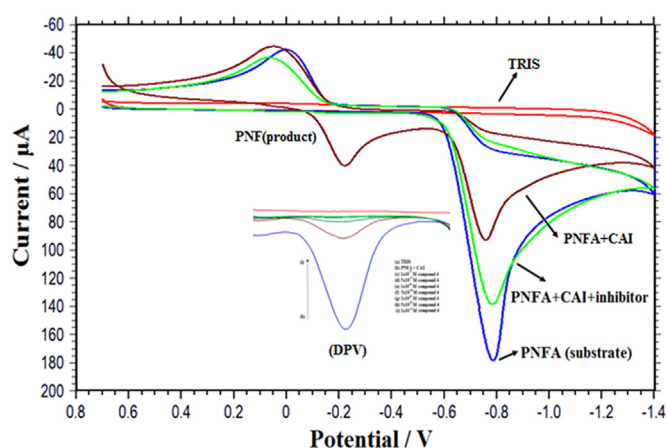
(b)

Fig. 6. Lineweaver–Burk (a) and Activity % (b) graphs of compound **4**.



**Table 7**  
Enzyme inhibition results of furan sulfonylhydrazones (2–4) and AAZ.

Compound	Inhibitor	K <sub>m</sub> (M)	I <sub>C50</sub> (M)	K <sub>i</sub> (M)	Inhibition type
2		24.21 × 10 <sup>-5</sup>	1.12 × 10 <sup>-3</sup>	2.55 × 10 <sup>-5</sup>	Competitive
		20.78 × 10 <sup>-5</sup>			
		17.51 × 10 <sup>-5</sup>			
		16.24 × 10 <sup>-5</sup>			
3		24.74 × 10 <sup>-5</sup>	1.10 × 10 <sup>-3</sup>	1.28 × 10 <sup>-5</sup>	Competitive
		21.30 × 10 <sup>-5</sup>			
		18.04 × 10 <sup>-5</sup>			
		16.23 × 10 <sup>-5</sup>			
4		25.30 × 10 <sup>-5</sup>	1.02 × 10 <sup>-4</sup>	5.88 × 10 <sup>-6</sup>	Competitive
		20.80 × 10 <sup>-5</sup>			
		17.60 × 10 <sup>-5</sup>			
		15.80 × 10 <sup>-5</sup>			
AAZ		64.94 × 10 <sup>-5</sup>	5.47 × 10 <sup>-5</sup>	7.03 × 10 <sup>-6</sup>	Competitive
		55.96 × 10 <sup>-5</sup>			
		46.97 × 10 <sup>-5</sup>			
		39.64 × 10 <sup>-5</sup>			



**Fig. 7.** CVs of CA I samples comparing the non-overlapping cathodic peak potential of PNF (−0.2 V) relative to PNFA (−0.782 V; 0.025 V). Samples were prepared using 5 µL CA I, 3 mM PNFA and 1 × 10<sup>-4</sup> M inhibitor in 10 mM pH 7.4 on GC electrode vs Ag/AgCl. DPVs of PNF reduction at GC in the presence of different concentrations of inhibitor (compound 4) were measured from 100 mV to −500 mV vs Ag/AgCl in 10 mM TRIS containing 3 × 10<sup>-3</sup> M PNFA, 5 µL hCA I and increasing concentrations of inhibitor (1 × 10<sup>-7</sup>–1 × 10<sup>-4</sup> M) at pH 7.4.

of compound 4 may be arising from electron withdrawing group (NO<sub>2</sub>) which may play an important role in biological activities. And also, electrochemical methods (CV and DPV) are applied to determine the inhibition effects of the compounds on hCA I isoenzyme. DPVs show that the inhibition properties of compounds against hCA I decrease in following order: 4 > 3 > 2 > 1. When the inhibition activities of the compounds are compared with the standard (AAZ) used as a strong inhibitor in glucoma treatment, they are found to have good inhibitory properties. The high similarities in enzyme inhibition effects determined from comparative studies promote the utility of this electrochemical detection system as an alternative method for simple and efficient screening of novel hCA I inhibitors which have importance in bioinorganic and metallodrug chemistry.

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#### Appendix A. Supplementary data

CCDC 1409649 contain the supplementary crystallographic data for complex. This data can be obtained free of charge via [http://www.ccdc.cam.ac.uk/data\\_request/cif](http://www.ccdc.cam.ac.uk/data_request/cif), (or from the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223-336-033; e-mail: [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk) or [www://www.ccdc.cam.ac.uk](http://www.ccdc.cam.ac.uk)).

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