The synthesis of novel pyrazole-3,4-dicarboxamides bearing 5-amino-1,3,4-thiadiazole-2-sulfonamide moiety with effective inhibitory activity against the isoforms of human cytosolic carbonic anhydrase I and II

Samet Mert [a], Zuhal Alım [b], Mehmet Mustafa İşgör [c], Şükrü Beydemir [d,e,*], Rahmi Kasımoğulları [a,*]

[a] Department of Chemistry, Faculty of Arts and Sciences, Dumlupinar University, 43100 Kutahya, Turkey
[b] Department of Chemistry, Faculty of Arts and Sciences, Ahi Evran University, 40000 Kırşehir, Turkey
[c] Department of Biochemistry, Faculty of Veterinary Sciences, Mustafa Kemal University, 31000 Hatay, Turkey
[d] Department of Chemistry, Faculty of Sciences, Atatürk University, 25240 Erzurum, Turkey
[e] Department of Chemistry, Faculty of Natural Sciences, Architecture and Engineering, Bursa Technical University, Osmangazi, 16200 Bursa, Turkey

ARTICLE INFO

ABSTRACT

A series of 1-(3-substituted-phenyl)-5-phenyl-N^3,N^4-bis(5-sulfamoyl-1,3,4-thiadiazol-2-yl)-1H-pyrazole-3,4-dicarboxamides (**4–15**) were synthesized. The structures of these pyrazole-sulfonamides were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR and elemental analysis methods. Human cytosolic carbonic anhydrase (CA, EC 4.2.1.1) isozymes (hCA I and II) were purified from erythrocyte cells by affinity chromatography. The inhibitory effects of newly synthesized derivatives (**4–15**) were investigated *in vitro* on esterase activities of these isozymes. The K_i values were determined as 0.119–3.999 μM for hCA I and 0.084–0.878 μM for hCA II. The results showed that the compound **6** for hCA I and the compound **11** for hCA II had the highest inhibitory effect. Beside that, the compound **8** had the lowest inhibition effect on both isozymes.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Carbonic anhydrase (CA) is intimately involved in the production of aqueous humor and the inhibitors of this enzyme (CAIs) are clinically used as ophthalmologic drugs for the treatment of glaucoma by acting in a systemic or topical manner [1–3]. It catalyzes a very simple essential physiological reaction: the hydration of CO_2 and dehydration of HCO_3^- [4–7]. Also, CAs play roles in pH regulation, CO_2 homeostasis, electrolyte secretion in a variety of tissues, biosynthetic reactions (such as gluconeogenesis, lipogenesis and ureagenesis), bone resorption, calcification, tumorigenicity, and many other physiological or pathologic processes [8,9]. Up to now, six genetically distinct CA families are known, α-, β-, γ-, δ-, ζ-, and η-CAs, and all of them contain Zn(II), Cd(II), or Fe(II) at their active sites [10,11]. The mammalian enzymes belonging to α-CA family consist of sixteen active members, in which several are cytosolic (CA I–III, CA VII and CA XIII), five are membrane-bound (CA IV, CA IX, CA XII, CA XIV, and CA XV), two are mitochondrial (CA VA and VB), and one (CA VI) is secreted in saliva/milk [12–16]. Three acatalytic forms are also known, called, CA-related proteins (CARPs) [16]. Inhibition of the CA is crucial in therapeutic applications as a diuretic, and an anticonvulsant, antiglaucoma and anticancer agent [9,17–20]. Sulfonamides have significant inhibitory activity against many CA classes, and these compounds still widely used as systemic antiglaucoma drugs [21]. But these systemic CAIs possess undesiderable side effects such as numbness, fatigue and metabolic acidosis [22–24]. Thus, it is required to synthesize more effectively acting topical CAIs, and keeping minimal side effects.

Pyrazole compounds are unique molecules and constitute the basic framework of drugs such as Celecoxib, and are well recognized for their multifaceted pharmacological and medical applications [25,26]. Among various pharmacological activities of pyrazole derivatives were reported as anti-inflammatory [27], antibacterial [28], antifungal [29], antipyretic [30], inhibitors of carbonic anhydrase [31], and cytotoxic [32] activities. During our research efforts of the discovery of novel pyrazole-sulfonamide derivatives [33,34], herein we describe synthesis, characterization, and evaluation of

the inhibition effects of novel coupling products (**4–15**) of 1-(3-aminophenyl)-5-phenyl-N³,N⁴-bis(5-sulfamoyl-1,3,4-thiadiazol-2-yl)-1*H*-pyrazole-3,4-dicarboxamide (**3**) on hCA I and hCA II isozymes. In our previous studies, we have synthesized a big library of coupling products that include only one sulfonamide group on the third position of the pyrazole ring [33–36]. We newly synthesized 12 novel coupling products that have two sulfonamide groups on both 3 and 4 positions. We firstly aimed to check whether the new derivatives may show higher inhibitory effect on CA isozymes with respect to the compounds previously investigated. We also aimed to discuss the selective inhibition effects of our compounds on human carbonic anhydrase isozymes I and II.

2. Results and discussion

2.1. Chemistry

Firstly, our pyrazole-3,4-dicarboxylic acid derivative (**1**) was synthesized according to the literature and its carboxyl groups were activated [37]. Then, 1-(3-nitrophenyl)-5-phenyl-N³, N⁴-bis(5-sulfamoyl-1,3,4-thiadiazol-2-yl)-1*H*-pyrazole-3,4-dicarboxamide (**2**) was synthesized from the reaction of pyrazole-3,4-dicarboxylic acid chloride with 5-amino-1,3,4-thiadiazole-2-sulfonamide [38]. The reduction of the nitro group of **2** with sodium-polysulfur hydrogenation (Na₂S/S/H₂O) afforded an arylamine derivative (**3**) as described previously (Scheme 1) [38]. Then, the arylamine derivative (**3**) was diazotized and coupled with β-naphthol, 1,3-dicarbonyl derivatives and cyclohexane-1,3-diones to generate novel series of pyrazole-sulfonamides (**4–15**). The general synthetic route shown in Scheme 2 was used to prepare the pyrazole-sulfonamide derivatives **4–15**. We purified human erythrocyte carbonic anhydrase isoenzymes, CA I and CA II, by using Sepharose-4B L-tyrosine-sulfanilamide affinity chromatography [33]. Then we investigated the inhibitory effects of newly synthesized compounds on the activities of purified human erythrocyte CA I and CA II isozymes.

The structures of the compounds were assigned by elemental analysis (C, H, N, and S) and various spectroscopic methods (IR, ¹H NMR, and ¹³C NMR). IR spectrum of **4** showed a broad absorption band at 3353 cm⁻¹ related to OH stretching of β-naphthol. And in the ¹H NMR spectrum of **4** the peak belonging to OH proton was observed as a singlet at 15.52 ppm. Further support was obtained from the ¹³C NMR spectrum of **4** which showed a peak at δ = 155.18 ppm due to the carbon atom adjacent to the OH group (=C—OH). ¹H NMR spectra displayed NH resonances in the δ = 11.25–11.92 ppm region attributed to hydrazinyl group (Ar—NH=N—C) of compounds **5–12**. According to the ¹H NMR results only **6** have tautomeric structures while the others (**5, 7–14**) preferred keto-hydrazo form. Also hydrazinyl group of compounds **13** and **14** appear as downfield shifts (~14.6 ppm) in ¹H NMR spectra, and thus, can be assigned to the intramolecular hydrogen bonds [39–41] (see Fig. 1). ¹H NMR and ¹³C NMR signals

associated with the other alkyl groups in the cyclohexanedione and dimedone rings appeared in the expected regions and the data consistent with the literature [42–44]. In the ¹H NMR spectrum of **15** SCH and OCH₃ protons were observed as singlets at 4.66 and 3.84 ppm, respectively. Also ¹³C NMR signals at δ = 99.52 and δ = 52.55 ppm belonging to SCH and OCH₃ carbons support the ¹H NMR results and consistent with the literature [45]. In general CONH protons appear between δ = 13.95 and 12.04 ppm in the ¹H NMR spectra. Therefore, all compounds have characteristic ¹³C NMR resonances at ~160 ppm related to the C-2 and C-5 positions of the thiadiazole rings. The other functional group resonances were observed at the expected chemical shifts and integral values in the ¹H NMR and ¹³C NMR spectra (see Section 3).

2.2. Biologic activity studies

Inhibitors and activators of CAs are important for therapeutic applications in many diseases. CA I and II inhibitors are generally used for the treatment of glaucoma and epilepsy, or as diuretic drugs [36,46]. For instance, acetazolamide and brinzolamide are the most well-known drugs for the treatment of glaucoma [47], and topiramate is for the treatment of epilepsy [48] and acetazolamide and methazolamide are also used as diuretic drugs. Thus designing of novel CA inhibitors is very important for discovery of new pharmacological agents. Also carbonic anhydrase inhibitors have effective role for understanding details about protein–drug interactions at molecular level and for clarification of the mechanisms of enzyme catalysis. Because, CA enzyme is well known as a drug-target in the literature. In this study, we aimed to determine *in vitro* inhibitory effect of novel 5-amino-1,3,4-thiadiazole-2-sulfo namide containing pyrazole derivatives (**4–15**) on hCA I and II isozymes. For this purpose, hCA I and II were purified with a high yield from human erythrocytes by Sepharose-4B-L-tyrosine affinity chromatography in a single and simple step. The hCA I and II isozymes were purified with 65%, 80.2% yields and 620 and 3250 U/mg protein specific activities, respectively. The purification folds of hCA I and II isozymes were found as 85 and 520-fold, respectively. Additionally, purity of enzymes was controlled using SDS-PAGE. Purified hCA I and hCA II isozymes had single bands at 29 kDa. After the purification process, the *in vitro* effects of novel 5-amino-1,3,4-thiadiazole-2-sulfonamide containing pyrazole derivatives (**4–15**) were investigated on the esterase activity of hCAI and hCA II isozymes. All compounds exhibited inhibitory effects for hCA I and II. In addition, almost all of the synthesized compounds (except **6** and **9**) had selective inhibition against hCA II. Compound **6** had the strongest inhibitory effect on hCA I activity and K_i value of this compound was found as 0.119 μM. The compound **11** had also the strongest inhibitory effect on hCA II activity and K_i value was found in almost nanomolar concentration range (0.084 μM for hCA II). On the other hand, the compound **8** showed lowest inhibition effects on both hCA I and hCA II isozymes. By their decreasing inhibition power on hCA I, the compounds can
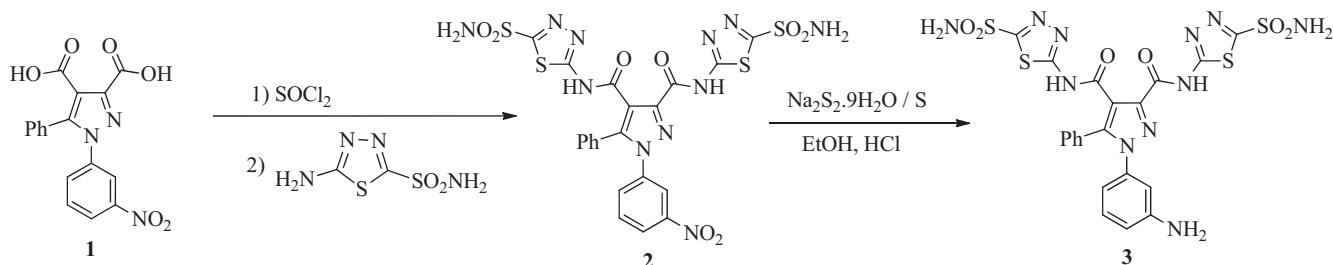
Scheme 1. Synthesis of starting compounds (**1–3**).

3

NaNO$_2$/HCl, 0°C | CH$_3$COO$^-$ Na$^+$
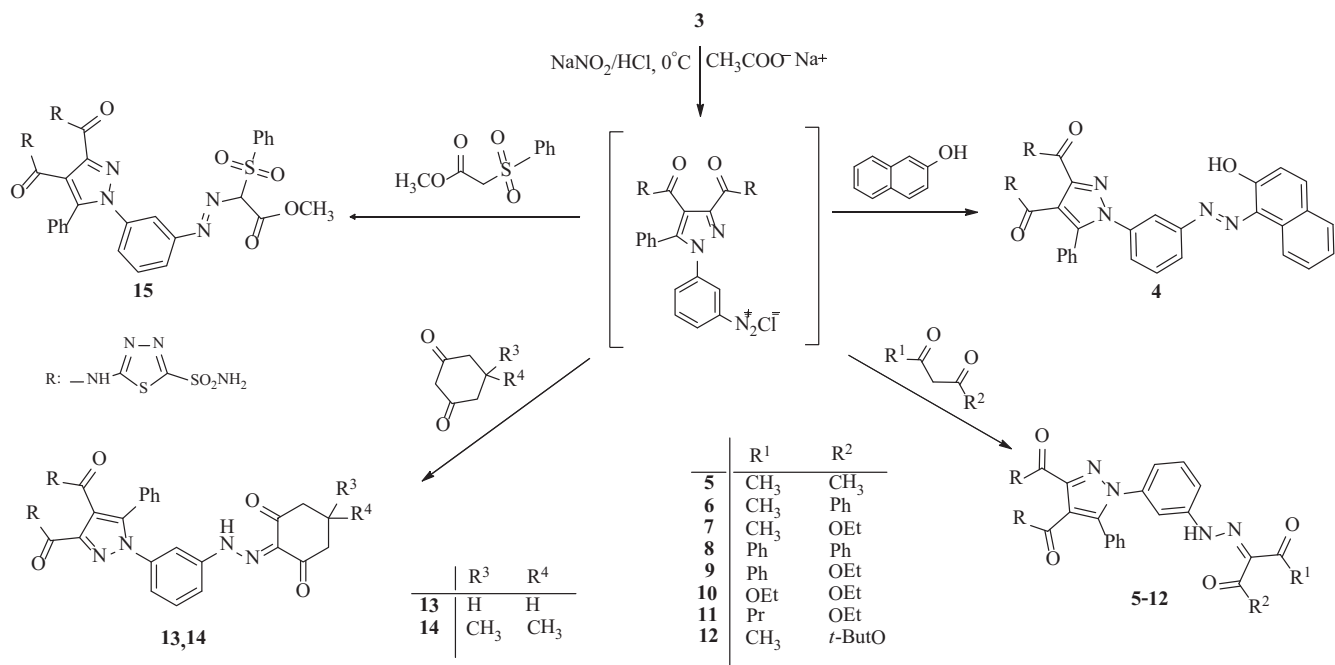
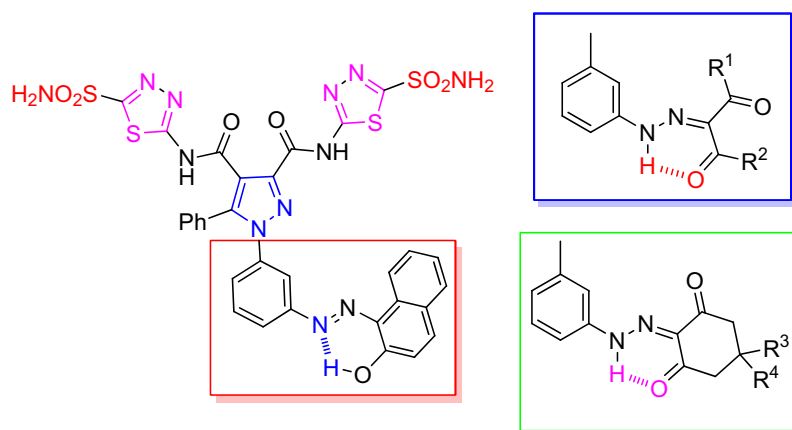**Scheme 2.** Synthetic route for the target inhibitors (**4–15**).

Fig. 1. Almost all 1,3-dicarbonyl coupling derivatives (except **6**) prefer only one tautomeric form (keto-hydrazo). It is estimated that the intramolecular hydrogen bonds cause NH and OH protons (**4–14**) appear as downfield shift in the ^{1}H NMR spectra.

Table 1
The inhibition data and K_i values of hCA I and hCA II isozymes for esterase activity.

Compound	K_ia,b (μM)		Inhibition type
	hCA I	hCA II	
4	1.663 ± 0.255	0.411 ± 0.021	Noncompetitive
5	0.767 ± 0.132	0.494 ± 0.024	Noncompetitive
6	0.119 ± 0.147	0.119 ± 0.017	Noncompetitive
7	0.710 ± 0.012	0.208 ± 0.075	Noncompetitive
8	3.999 ± 2.503	0.878 ± 0.123	Noncompetitive
9	0.487 ± 0.079	0.435 ± 0.108	Noncompetitive
10	0.461 ± 0.151	0.203 ± 0.023	Noncompetitive
11	0.246 ± 0.019	0.084 ± 0.017	Noncompetitive
12	0.522 ± 0.103	0.149 ± 0.016	Noncompetitive
13	0.508 ± 0.086	0.157 ± 0.041	Noncompetitive
14	0.787 ± 0.307	0.288 ± 0.005	Noncompetitive
15	0.318 ± 0.037	0.092 ± 0.018	Noncompetitive

^{a} Mean ± standard error, from three different assays.
^{b} $p < 0.0001$ for all analysis.

be ranked as **6, 11, 15, 10, 9, 13, 12, 7, 5, 14, 4** and **8**. By also their decreasing inhibition power on hCA II, it is in the order of **11, 15, 6, 12, 13, 10, 7, 14, 4, 9, 5** and **8** (see Table 1). The structure-activity relationship (SAR) mainly depends on R^1 and R^2 moiety of the 1,3-dicarbonyl substituents. When R moieties are hydrofobic and aromatic (R^1,R^2 = Ph, **8**), the compound shows weak inhibition effect. If R moieties are small hydrofobic groups such as R^1, R^2 = Me (**5**), the moderate inhibition effect is observed. In the case of R^1 = Me and R^2 = Ph (**6**), the inhibition effect increased significantly. Similar situation was observed in ester substituted compounds (**7, 9–12**). Having R^1 = alkyl or OR and R^2 = OR (**7, 10–12**) groups, the compounds show strong inhibitory effect. In the case of R^1 = Ph and R^2 = OR, the inhibition effect decreased dramatically. For the compounds containing cyclohexane dione moiety (**13, 14**), the methyl substituents decreased the inhibition potency. The inhibition type of all the compounds was found as noncompetitive. It is well known that noncompetitive type is a reversible inhibition. Already, enzyme-chemical interactions are generally occurring according to the reversible inhibition. However, some chemicals

or drugs may show their effects binding three-dimension structure of the enzyme as irreversible. For instance, penicillin binds to the transpeptidase enzyme, covalently and it causes the inhibition of the enzyme activity. Thus, the bacterial cell wall synthesis is inhibited. The inhibitor is known to bind to any location outside the active site. Hence, the increasing substrate concentration does not reduce the inhibition of the enzyme at the noncompetitive type. Therefore, binding mechanism is difficult to estimate for this inhibition type. On the other hand, it may be estimated by using crystallography. Many studies have been reported in the literature about effects on CA isozymes of different compounds including sulfonamide derivatives. For example, in our previous study novel pyrazole carboxamide derivatives were synthesized and *in vitro* inhibition effects of these compounds were investigated on human CA I and CA II izozymes, as mentioned previously [33]. In the present study, we used the similar chemical skeleton with the mentioned study but some R groups were changed. Additionally, two sulfonamides were connected to the chemical skeletons. However, a remarkable inhibition effect was not observed. As the cause of these findings, they are considered as bind to any location outside the active site of the enzyme-interacting groups of inhibitors. In another study, some pyrazole-3,4-dicarboxamides bearing biologically active sulfonamide moiety were synthesized and the inhibition effects of these compounds on esterase activities of hCA I and II isozymes were investigated *in vitro* [38]. The results of the inhibition types in the current study are consistent with those of our previous study. On the other hand synthesized compounds in this study (except **6**, **9** and **11**) had a bit weaker inhibitory effects than the analogs with one sulfonamide group synthesized in our previous studies [33,49]. Besides the compounds including β-naphthol substituent in this study, previous studies had at least fourfold more powerful inhibition potential on CA II isozyme [33–35,49].

3. Experimental procedures

3.1. Material and methods

Chemical compounds and solvents used in this research were purchased from Fluka, Merck and Sigma-Aldrich. All reactions were monitored by analytical thin-layer chromatography (TLC) on 0.25 mm precoated Kieselgel 60F 254 plates (E. Merck Co., Darmstadt, Germany); compounds were visualized by Camag TLC devices (Camag, Upland, CA, USA) UV (254 and 366 nm). Melting points were taken on a Barnstead Electrothermal 9200 (Electrothermal Co., Essex, UK). The structures of each compound were supported by Bruker Vertex 70 FT-IR (Bruker Optik GmbH, Ettlingen, Germany) equipped with an Attenuated Total Reflection (ATR) device and the data were reported in reciprocal centimeters (cm^{-1}). ^1H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 solutions at 298 K using a Bruker Ultrashield-400 spectrometer operating at 400.00 MHz for ^1H and 101.00 MHz for ^{13}C (Bruker BioSpin GmbH Silberstreifen D-76287, Rheinstetten, Germany). The center of the peaks of DMSO-d_6 [δ (ppm): 2.50 (^1H) and δ (ppm): 39.5 (^{13}C)] was used as an internal reference in a 5-mmNMR tube (Wilmad, No. 528-PP). The elemental analyses (C, H, N, and S) were carried out with a Leco CHNS-932 instrument (LECO Corporation, Saint Joseph, Michigan, USA).

3.2. General procedure for the synthesis of compounds 4–15

1-(3-Aminophenyl)-5-phenyl-N^3,N^4-bis(5-sulfamoyl-1,3,4-thiadiazol-2-yl)-1H-pyrazole-3,4-dicarboxamide **3** (0.647 g, 1 mmol) was dissolved in a mixture of ethanol and hydrochloric acid (60 ml, ratio 5:1) and the solution was then cooled to 0–5 °C. Sodium nitrite (0.083 g, 1 mmol) in water (10 ml) was then added

to this solution dropwise with vigorous stirring, about 30 min., while cooling at 0–5 °C. Then, the aromatic or 1,3-dicarbonyl compounds in ethanol were added in portions over 15 min to the vigorously stirred diazonium solution while keeping at 0–5 °C. The pH of the mixture, in each case, was maintained at 7–8 by simultaneous addition of sodium acetate solution. The mixture was then stirred 1 h between 0 and 5 °C and 2 h at room temperature. The precipitated colored product was filtered, washed with water several times, dried, and then recrystallized from an appropriate solvent.

3.3. 1-(3-((2-Hydroxynaphthalen-1-yl)diazenyl)phenyl)-5-phenyl-N^3,N^4-bis(5-sulfamoyl-1,3,4-thiadiazol-2-yl)-1H-pyrazole-3,4-dicarboxamide (4)

Compound (**4**) was synthesized according to general procedure from diazonium salt of **3** and β-naphthol (1 mmol, 0.144 g). The crude product was crystallized from EtOH/DMF (3:1) mixture. Red powder, 77% yield, mp: 266–268 °C; IR (v, cm^{-1}): 3353 (OH), 3288 and 3219 (NH), 3060 (ArCH), 1777 and 1664 (C=O), 1600–1413 (C=C and C=N), 1355 and 1169 (S=O); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 15.52 (s, 1H, Ar—OH), 13.38 (br, s, 2H, 2CONH), 8.37 and 8.36 (s, 4H, 2SO$_2$NH$_2$), 8.30–6.65 (m, 15H, ArH); ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 163.41 and 163.09 (C=O, amide), 162.49, 162.42, 161.78 and 161.49 (thiadiazole C-2 and C-5), 155.18 (=C—OH), 144.95 (pyrazole C-3), 144.92 (pyrazole C-5), 121.63 (pyrazole C-4), 140.86, 140.80, 132.51, 130.63, 130.51, 130.36, 130.28, 130.22, 130.08, 129.47, 129.27, 128.93, 127.98, 127.88, 127.84, 126.27, 124.53, 124.17, 115.02. Anal. Calcd. for C$_{31}$H$_{22}$N$_{12}$O$_7$S$_4$: C, 46.38; H, 2.76; N, 20.94; S, 15.98; Found: C, 46.51; H, 2.80; N, 20.87; S, 16.07.

3.4. 1-(3-(2-(2,4-Dioxopentan-3-ylidene)hydrazinyl)phenyl)-5-phenyl-N^3,N^4-bis(5-sulfamoyl-1,3,4-thiadiazol-2-yl)-1H-pyrazole-3,4-dicarboxamide (5)

Compound (**5**) was synthesized following general procedure from diazonium salt of **3** and acetylacetone (1 mmol, 0.104 ml). The crude product was crystallized from EtOH/DMF (5:1) mixture. Yellow powder, 74% yield, mp: 231–233 °C; IR (v, cm^{-1}): 3325 and 3220 (NH), 3002 (ArCH), 2968 (aliphatic CH), 1778 and 1661 (C=O), 1602–1414 (C=C and C=N), 1354 and 1165 (S=O); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.72 (s, 2H, 2CONH), 11.43 (s, 1H, Ar—NH—N=), 8.30–7.04 (m, 9H, ArH), 8.19 and 8.17 (s, 4H, 2SO$_2$NH$_2$), 2.45 and 2.32 (s, 6H, 2CH$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 197.02 and 196.33 (C=O, acetyl), 164.12 and 163.29 (C=O, amide), 163.15, 161.95, 161.84 and 161.30 (thiadiazole C-2 and C-5), 142.45 (pyrazole C-3), 139.70 (pyrazole C-5), 116.62 (pyrazole C-4), 31.19 and 26.46 (CH$_3$), 139.41, 134.13, 134.00, 130.47, 130.19, 129.40, 128.27, 127.86, 122.25, 122.07, 113.21. Anal. Calcd. for C$_{26}$H$_{22}$N$_{12}$O$_8$S$_4$: C, 41.15; H, 2.92; N, 22.15; S, 16.90; Found: C, 41.32; H, 3.01; N, 22.07; S, 16.87.

3.5. 1-(3-(2-(1,3-Dioxo-1-phenylbutan-2-ylidene)hydrazinyl)phenyl)-5-phenyl-N^3,N^4-bis(5-sulfamoyl-1,3,4-thiadiazol-2-yl)-1H-pyrazole-3,4-dicarboxamide (6)

Starting from **3** (1 mmol, 0.647 g) and benzoylacetone (1 mmol, 0.164 g), the title compound **6** was obtained by general method. The crude product was crystallized from DMF/H$_2$O (1:1). Yellow powder, 66% yield, mp: 209–211 °C; IR (v, cm^{-1}): 3331 and 3220 (NH), 3062 (ArCH), 2925 (aliphatic CH), 1777 and 1649 (C=O), 1603–1418 (C=C and C=N), 1355 and 1169 (S=O); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 14.14 (s, 1H, C=C—OH enol tautomer), 13.77 and 13.75 (s, 2H, 2CONH), 11.25 (s, 1H, Ar—NH—N= keto tautomer), 8.30 and 8.21 (s, 4H, 2SO$_2$NH$_2$), 8.33–6.82 (m, 14H,

ArH), 2.43 (s, 3H, COC$\underline{H}_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 196.02 (C=O, acetyl), 194.89 (C=O, benzoyl), 164.33 and 164.17 (C=O, amide), 162.95, 162.88, 162.07 and 161.22 (thiadiazole C-2 and C-5), 143.77 (pyrazole C-3), 139.63 (pyrazole C-5), 119.65 (pyrazole C-4), 24.96 (CH$_3$), 139.51, 139.11, 135.36, 134.54, 134.47, 130.28, 130.13, 129.98, 129.67, 129.06, 128.71, 128.49, 128.04, 127.97, 114.99. Anal. Calcd. for C$_{31}$H$_{24}$N$_{12}$O$_8$S$_4$: C, 45.36; H, 2.95; N, 20.48; S, 15.63; Found: C, 45.41; H, 3.02; N, 20.45; S, 15.57.

3.6. Ethyl 3-oxo-2-(2-(3-(5-phenyl-3,4-bis((5-sulfamoyl-1,3,4-thiadiazol-2-yl)carbamoyl)-1H-pyrazol-1-yl)phenyl)hydrazono) butanoate (7)

Compound (7) was synthesized according to general procedure from diazonium salt of 3 and ethyl acetoacetate (1 mmol, 0.127 ml). The crude product was crystallized from methanol. Orange powder, 72% yield, mp: 222–224 °C; IR (υ, cm^{-1}): 3327 and 3196 (NH), 3045 (ArCH), 2977 (aliphatic CH), 1727 and 1668 (C=O), 1606–1414 (C=C and C=N), 1354 and 1165 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.95 (br, s, 2H, 2CONH), 11.52 (s, 1H, Ar—NH—N=), 8.28 and 8.17 (s, 4H, 2SO$_2$NH$_2$), 7.45–6.89 (m, 9H, ArH), 4.30 (q, J = 7.2 Hz, 2H, OCH$_2$), 2.32 (s, 3H, COCH$_3$), 1.27 (t, J = 7.1 Hz, 3H, CH$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 193.74 (C=O, acetyl), 164.07 (C=O, ester), 163.91 and 163.34 (C=O, amide), 162.34, 162.31, 161.94 and 161.35 (thiadiazole C-2 and C-5), 143.10 (pyrazole C-3), 142.93 (pyrazole C-5), 120.41 (pyrazole C-4), 61.29 (OCH$_2$), 25.36 (COCH$_3$), 13.84 (CH$_3$), 139.35, 133.54, 132.46, 130.47, 130.18, 129.98, 129.32, 128.22, 127.85, 115.28, 112.24. Anal. Calcd. for C$_{27}$H$_{24}$N$_{12}$O$_9$S$_4$: C, 41.11; H, 3.07; N, 21.31; S, 16.26; Found: C, 40.96; H, 3.01; N, 21.37; S, 16.34.

3.7. 1-(3-(2-(1,3-Dioxo-1,3-diphenylpropan-2-ylidene)hydrazinyl) phenyl)-5-phenyl-N³,N⁴-bis(5-sulfamoyl-1,3,4-thiadiazol-2-yl)-1H-pyrazole-3,4-dicarboxamide (8)

Title compound (8) was obtained by general method from diazonium salt of 3 and dibenzoylmethane (1 mmol, 0.228 g). The crude product was crystallized from DMF/H$_2$O (1:1). Bright yellow powder, 80% yield, mp: 226–228 °C; IR (υ, cm^{-1}): 3373 and 3277 (NH), 3065 (ArCH), 1777 and 1639 (C=O), 1594–1410 (C=C and C=N), 1342 and 1169 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.74 (br, s, 2H, 2CONH), 11.58 (s, 1H, Ar—NH—N=), 7.87 and 7.84 (s, 4H, 2SO$_2$NH$_2$), 7.57–6.67 (m, 19H, ArH); ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 196.99 and 193.20 (C=O, benzoyl), 165.26 and 164.04 (C=O, amide), 163.53, 162.65, 161.46 and 160.92 (thiadiazole C-2 and C-5), 144.54 (pyrazole C-3), 143.56 (pyrazole C-5), 119.87 (pyrazole C-4), 139.75, 136.57, 135.69, 134.45, 131.86, 129.95, 129.85, 129.14, 128.73, 128.47, 128.30, 127.92, 127.85, 127.72, 127.48, 127.38, 126.67, 114.82, 99.49. Anal. Calcd. for C$_{36}$H$_{26}$N$_{12}$O$_8$S$_4$: C, 48.97; H, 2.97; N, 19.04; S, 14.53; Found: C, 49.11; H, 3.05; N, 18.97; S, 14.61.

3.8. Ethyl 3-oxo-3-phenyl-2-(2-(3-(5-phenyl-3,4-bis((5-sulfamoyl-1,3,4-thiadiazol-2-yl)carbamoyl)-1H-pyrazol-1-yl)phenyl)hydrazono) propanoate (9)

Compound (9) was synthesized following general procedure from diazonium salt of 3 and ethyl benzoylacetate (1 mmol, 0.173 ml). The crude product was crystallized from methanol. Pale yellow powder, 81% yield, mp: 165–167 °C; IR (υ, cm^{-1}): 3225 (NH), 3062 (ArCH), 2981 (aliphatic CH), 1727 and 1671 (C=O), 1603–1416 (C=C and C=N), 1362 and 1169 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.85 (br, s, 2H, 2CONH), 11.92 (s, 1H, Ar—NH—N=) and 8.27 and 8.15 (s, 4H, 2SO$_2$NH$_2$), 7.97–6.81 (m,

14H, ArH), 4.33 (q, J = 7.1 Hz, 2H, OCH$_2$), 1.26 (t, J = 7.1 Hz, 3H, CH$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 193.48 (C=O, benzoyl), 167.67 (C=O, ester), 162.20 and 162.13 (C=O, amide), 162.04, 161.92, 161.29 and 160.28 (thiadiazole C-2 and C-5), 146.96 (pyrazole C-3), 141.35 (pyrazole C-5), 119.12 (pyrazole C-4), 60.60 (OCH$_2$), 13.98 (CH$_3$), 136.82, 135.78, 133.78, 132.69, 129.86, 128.81, 128.51, 128.38, 128.28, 128.08, 127.73, 127.36, 126.71, 115.19, 112.55. Anal. Calcd. for C$_{32}$H$_{26}$N$_{12}$O$_9$S$_4$: C, 45.17; H, 3.08; N, 19.75; S, 15.07; Found: C, 45.06; H, 3.02; N, 19.86; S, 14.96.

3.9. Diethyl 2-(2-(3-(5-phenyl-3,4-bis((5-sulfamoyl-1,3,4-thiadiazol-2-yl)carbamoyl)-1H-pyrazol-1-yl)phenyl)hydrazono)malonate (10)

Starting from 3 (1 mmol, 0.647 g) and diethyl malonate (1 mmol, 0.154 ml), the title compound 10 was obtained by general method. The crude product was crystallized from ethanol. Pale orange powder, 70% yield, mp: 203–205 °C; IR (υ, cm^{-1}): 3223 (NH), 3063 (ArCH), 2985 (aliphatic CH), 1668 (C=O), 1603–1414 (C=C and C=N), 1354 and 1167 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.89 (br, s, 2H, 2CONH), 11.88 (s, 1H, Ar—NH—N=), 8.27 and 8.17 (s, 4H, 2SO$_2$NH$_2$), 8.15–7.14 (m, 9H, ArH), 4.32 (q, J = 6.9 Hz, 2H, OCH$_2$), 4.25 (q, J = 6.9 Hz, 2H, OCH$_2$), 1.29 (t, J = 6.6 Hz, 3H, CH$_3$), 1.19 (t, J = 7.1 Hz, 3H, CH$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 164.10 and 163.38 (C=O, ester), 162.16 and 161.81 (C=O, amide), 161.74, 161.61, 161.40 and 161.34 (thiadiazole C-2 and C-5), 147.81 (pyrazole C-3), 145.66 (pyrazole C-5), 120.51 (pyrazole C-4), 61.40 and 60.91 (OCH$_2$), 14.09 and 13.84 (CH$_3$), 139.29, 138.36, 130.14, 129.81, 129.68, 129.01, 128.17, 125.84, 123.07, 116.41, 112.41. Anal. Calcd. for C$_{28}$H$_{26}$N$_{12}$O$_{10}$S$_4$: C, 41.07; H, 3.20; N, 20.53; S, 15.66; Found: C, 40.95; H, 3.14; N, 20.41; S, 15.81.

3.10. Ethyl 3-oxo-2-(2-(3-(5-phenyl-3,4-bis((5-sulfamoyl-1,3,4-thiadiazol-2-yl)carbamoyl)-1H-pyrazol-1-yl)phenyl)hydrazono) hexanoate (11)

Title compound (11) was obtained by general method from diazonium salt of 3 and ethyl butyrylacetate (1 mmol, 0.163 ml). The crude product was crystallized from ethanol. Pale yellow powder, 77% yield, mp: 205–207 °C; IR (υ, cm^{-1}): 3213 (NH), 3065 (ArCH), 2963 (aliphatic CH), 1778 and 1672 (C=O), 1606–1415 (C=C and C=N), 1355 and 1167 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.88 (s, 2H, 2CONH), 11.41 (s, 1H, Ar—NH—N=), 8.23 and 8.13 (s, 4H, 2SO$_2$NH$_2$), 8.35–6.90 (m, 9H, ArH), 4.30 (q, J = 6.2 Hz, 2H, OCH$_2$), 2.76 (t, J = 7.1 Hz, 2H, COCH$_2$CH$_2$), 1.58 (m, 2H, CH$_2$C$\underline{H}_2$CH$_3$), 1.27 (t, J = 6.7 Hz, 3H, OCH$_2$C$\underline{H}_3$), 0.92 (t, J = 6.8 Hz, 3H, CH$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 195.94 (C=O, ketone), 163.91 (C=O, ester), 163.57 and 163.12 (C=O, amide), 163.01, 162.72, 162.40 and 161.47 (thiadiazole C-2 and C-5), 147.45 (pyrazole C-3), 143.11 (pyrazole C-5), 120.42 (pyrazole C-4), 61.25 (OCH$_2$), 38.38 (C$\underline{H}_2$CH$_2$CH$_3$), 17.35 (CH$_2$C$\underline{H}_2$-CH$_3$), 13.85 and 13.69 (CH$_3$), 142.98, 139.68, 139.48, 130.52, 130.31, 129.76, 128.74, 128.02, 127.76, 114.93, 112.36. Anal. Calcd. for C$_{29}$H$_{28}$N$_{12}$O$_9$S$_4$: C, 42.64; H, 3.45; N, 20.58; S, 15.70; Found: C, 42.55; H, 3.38; N, 20.64; S, 15.77.

3.11. tert-Butyl 3-oxo-2-(2-(3-(5-phenyl-3,4-bis((5-sulfamoyl-1,3,4-thiadiazol-2-yl)carbamoyl)-1H-pyrazol-1-yl)phenyl)hydrazono) butanoate (12)

Starting from 3 (1 mmol, 0.647 g) and t-butyl acetoacetate (1 mmol, 0.168 ml), the title compound 12 was obtained by general method. The crude product was crystallized from methanol. Orange powder, 75% yield, mp: 248–250 °C; IR (υ, cm^{-1}): 3209 (NH), 3059 (ArCH), 2975 (aliphatic CH), 1778 and 1674 (C=O),

1607–1418 (C=C and C=N), 1359 and 1168 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.75 (br, s, 2H, 2CONH), 11.56 (s, 1H, Ar—NH—N=), 8.32 and 8.20 (s, 4H, 2SO$_2$NH$_2$), 7.82–6.92 (m, 9H, ArH), 2.29 (s, 3H, COCH$_3$), 1.51 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 197.79 (C=O, acetyl), 165.82 (C=O, ester), 164.39 and 164.30 (C=O, amide), 161.55, 161.42, 161.15 and 160.64 (thiadiazole C-2 and C-5), 147.78 (pyrazole C-3), 146.39 (pyrazole C-5), 120.92 (pyrazole C-4), 83.08 (OC(CH$_3$)$_3$), 27.89 (OC(CH$_3$)$_3$), 24.35 (CH$_3$), 143.20, 141.89, 139.51, 139.36, 131.85, 129.97, 128.52, 126.80, 125.16, 122.78, 112.53. Anal. Calcd. for C$_{29}$H$_{28}$N$_{12}$O$_9$S$_4$: C, 42.64; H, 3.45; N, 20.58; S, 15.70; Found: C, 42.48; H, 3.34; N, 20.65; S, 15.61.

3.12. 1-(3-((2-Hydroxy-6-oxocyclohex-1-en-1-yl)diazenyl)phenyl)-5-phenyl-N^3,N^4-bis(5-sulfamoyl-1,3,4-thiadiazol-2-yl)-1H-pyrazole-3,4-dicarboxamide (13)

Compound (13) was synthesized following general procedure from diazonium salt of 3 and cyclohexane-1,3-dione (1 mmol, 0.115 g). The crude product was crystallized from EtOH/DMF (3:1) mixture. Yellowish green powder, 67% yield, mp: 235–237 °C; IR (υ, cm^{-1}): 3210 (NH), 3041 (ArCH), 2957 (Aliphatic CH), 1776 and 1668 (C=O), 1600–1506 (C=C and C=N), 1355 and 1167 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 14.65 (br, s, 1H, Ar—NH—N=, keto-hydrazo), 13.61 (br, s, 2H, 2CONH), 8.36 and 8.16 (s, 4H, 2SO$_2$NH$_2$), 7.79–6.91 (m, 9H, ArH), 2.69 and 2.62 (t, J = 7.1 Hz, 4H, H-3 and H-5), 2.03–1.96 (m, 2H, H-4); ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 197.79 and 189.98 (C=O, ketone), 163.36 and 163.24 (C=O, amide), 161.73, 161.29, 160.64 and 160.44 (thiadiazole C-2 and C-5), 144.22 (pyrazole C-3), 139.39 (pyrazole C-5), 119.50 (pyrazole C-4), 99.51 (—N=N—C, C-1), 38.61 and 29.10 (2CH$_2$, C-5 and C-3), 17.68 (CH$_2$, C-4), 137.27, 136.99, 130.44, 130.20, 130.14, 129.84, 129.64, 128.42, 127.91, 122.98. Anal. Calcd. for C$_{27}$H$_{22}$N$_{12}$O$_8$S$_4$: C, 42.07; H, 2.88; N, 21.81; S, 16.64; Found: C, 42.21; H, 2.93; N, 21.73; S, 16.56.

3.13. 1-(3-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)diazenyl)phenyl)-5-phenyl-N^3,N^4-bis(5-sulfamoyl-1,3,4-thiadiazol-2-yl)-1H-pyrazole-3,4-dicarboxamide (14)

Compound (14) was synthesized according to general procedure from diazonium solution of 3 and dimedone (1 mmol, 0.143 g). The crude product was crystallized from ethanol. Light brown powder, 71% yield, mp: 241–243 °C; IR (υ, cm^{-1}): 3226 (NH), 3063 (ArCH), 2960 (Aliphatic CH), 1776 and 1671 (C=O), 1601–1458 (C=C and C=N), 1353 and 1167 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 14.67 (br, s, 1H, Ar—NH—N=, keto-hydrazo), 13.61 (br, s, 2H, 2CONH), 8.26 and 8.15 (s, 4H, 2SO$_2$-NH$_2$), 7.80–6.90 (m, 9H, ArH), 2.66 and 2.58 (s, 4H, H-3 and H-5), 1.04 (s, 6H, C(CH$_3$)$_2$); ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 197.01 and 192.69 (C=O, ketone), 164.07 and 162.97 (C=O, amide), 161.98, 161.43, 161.35 and 160.00 (thiadiazole C-2 and C-5), 142.42 (pyrazole C-3), 139.49 (pyrazole C-5), 117.39 (pyrazole C-4), 99.50 (—N=N—C, C-1), 51.98 and 51.77 (2CH$_2$, C-3 and C-5), 30.20 (C(CH$_3$)$_2$, C-4), 27.97 (C(CH$_3$)$_2$), 132.17, 130.61, 130.52, 130.25, 130.01, 128.19, 126.64, 126.61, 123.07, 114.42. Anal. Calcd. for C$_{29}$H$_{26}$N$_{12}$O$_8$S$_4$: C, 43.60; H, 3.28; N, 21.04; S, 16.06; Found: C, 43.76; H, 3.34; N, 20.97; S, 15.99.

3.14. Methyl 2-((3-(5-phenyl-3,4-bis((5-sulfamoyl-1,3,4-thiadiazol-2-yl)carbamoyl)-1H-pyrazol-1-yl)phenyl)diazenyl)-2-(phenylsulfonyl) acetate (15)

Title compound (15) was obtained by general method from diazonium salt of 3 and methyl phenylsulfonyl acetate (1 mmol,

0.169 ml). The crude product was crystallized from DMF/H$_2$O (1:1). Cream powder, 64% yield, mp: 198–200 °C; IR (υ, cm^{-1}): 3224 (NH), 3062 (ArCH), 2959 (aliphatic CH), 1760 and 1669 (C=O), 1604–1415 (C=C and C=N), 1355 and 1168 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.04 (br, s, 2H, 2CONH), 8.28 and 8.21 (s, 4H, 2SO$_2$NH$_2$), 8.16–6.83 (m, 14H, ArH), 4.66 (s, 1H, SCH(C=O)), 3.84 (s, 3H, OCH$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 164.47 (C=O, ester), 164.20 and 163.15 (C=O, amide), 161.34, 160.15, 159.78 and 158.75 (thiadiazole C-2 and C-5), 142.62 (pyrazole C-3), 139.51 (pyrazole C-5), 121.88 (pyrazole C-4), 99.52 (SCH(C=O)), 52.55 (OCH$_3$), 139.22, 139.12, 138.92, 134.21, 133.78, 130.44, 130.23, 130.11, 129.29, 128.49, 128.01, 125.82, 123.97, 116.41. Anal. Calcd. for C$_{30}$H$_{24}$N$_{12}$O$_{10}$S$_5$: C, 41.28; H, 2.77; N, 19.26; S, 18.37; Found: C, 41.17; H, 2.84; N, 19.37; S, 18.29.

3.15. Affinity chromatography

The purification process of the isozymes was performed by the Sepharose-4B L-tyrosine sulfanilamide affinity chromatography column. The column was prepared according to our previous studies [50,51]. Erythrocytes were obtained from human blood, which was obtained from the Hospital Blood Center of Erzurum Atatürk University. The hemolysate was prepared from these samples and the purification process of the hCA I and hCA II isozymes was performed according to the previous study [52]. All procedures were performed at 4 °C.

3.16. Enzyme activity assay

The activity of CA isozymes can be assayed by two different methods: the first is CO$_2$ hydratase activity, which is the physiological activity of the CA, and the second one is the esterase activity which is non-physiological activity under *in vitro* condition of the CA. The hydratase activity was carried out according to the method described by Wilbur and Anderson [53]. On the other hand, esterase activity was assayed by following the changing the absorbance at 348 nm, spectrophotometrically. This assay was performed according to the method described by Verpoorte et al. The basis of this method is the conversion of 4-nitrophenyl acetate to 4-nitrophenolate ion a period of 3 min at 25 °C [54].

3.17. Protein determination

During all purification steps, protein quantity was determined at 595 nm spectrophotometrically according to the Bradford method. The bovine serum albumin was used as a standard [55].

3.18. SDS–polyacrylamide gel electrophoresis

The purity of the isozymes was controlled by carrying out SDS-PAGE according to the Laemmli's procedure. The gel includes two different acrylamide concentrations, 3% and 8% for running and stacking gel, respectively [56].

3.19. In vitro inhibition studies

CA activity was measured according to the method described by Verpoorte et al. in inhibition studies, spectrophotometrically [54]. The reaction mixture contains 50 mM Tris-SO$_4$ buffer (pH = 7.4), 3 mM 4-nitrophenylacetate and enzyme solution in 1 ml total volume. A reference measurement was done by using the same cuvette without enzyme solution. The measurement was repeated in triplicate at each concentration of 5-amino-1,3,4-thiadiazole-2-sulfonamide containing pyrazole derivatives. K_i experiments were

performed by measuring enzyme activity at three different inhibitor concentrations with five different substrate concentrations. Lineweaver–Burk curves were used for the determination of K_i and inhibition type [33,38].

4. Conclusions

We report here the synthesis, characterization, and biologic activity evaluation of novel 5-amino-1,3,4-thiadiazole-2-sulfona mide containing pyrazole derivatives (**4–15**). All newly synthesized compounds exhibited satisfactory analytical and spectral data consistent with their structures. Inhibition studies on carbonic anhydrases are crucial for designing new drugs and clarification of the drug-enzyme interaction at molecular level. Thus, *in vitro* inhibitory effects of novel 5-amino-1,3,4-thiadiazole-2-sul fonamide containing pyrazole derivatives have been studied in this study. Moreover, all of these compounds showed effective inhibitory activity against the human cytosolic carbonic anhydrase isoforms I and II. Compound **6** exhibited remarkable inhibition effects on hCA I, and the compound **11** has the highest inhibitory effects on hCA II. Compound **8** exhibited lowest inhibition effects on both hCA I and hCA II activities. Consequently, **6, 11** and **15** were better inhibitors than the other and they can be used in the future development of new potent CAIs.

References

[1] F. Mincione, A. Scozzafava, C.T. Supuran, The development of topically acting carbonic anhydrase inhibitors as anti-glaucoma agents, Curr. Top. Med. Chem. 7 (2007) 849–854.

[2] A. Innocenti, J. Antel, M. Wurl, A. Scozzafava, C.T. Supuran, Carbonic anhydrase inhibitors: inhibition of human cytosolic isozyme II and mitochondrial isozyme V with a series of benzene sulfonamide derivatives, Bioorg. Med. Chem. Lett. 14 (2004) 5703–5707.

[3] T.H. Maren, Carbonic anhydrase: general perspective and advances in glaucoma research, Drug Develop. Res. 10 (1987) 255–276.

[4] F. Abbate, C.T. Supuran, A. Scozzafava, P. Orioli, M.T. Stubbs, G. Klebe, Nonaromatic sulfonamide group as an ideal anchor for potent human carbonic anhydrase inhibitors: role of hydrogen-bonding networks in ligand binding and drug design, J. Med. Chem. 45 (2002) 3583–3587.

[5] N. Gencer, C. Bilen, D. Demir, A. Atahan, M. Ceylan, M. Kücükislamoglu, In vitro inhibition effect of some chalcones on erythrocyte carbonic anhydrase I and II, Artif. Cells Nanomed. Biotechnol. 41 (2013) 384–388.

[6] B.L. Wilkinson, L.F. Bornaghi, T.A. Houston, A. Innocenti, D. Vullo, C.T. Supuran, Carbonic anhydrase inhibitors: inhibition of isozymes I, II, and IX with Triazole-linked O-glycosides of benzene sulfonamides, J. Med. Chem. 50 (2007) 1651–1657.

[7] S.R. Krungkrai, N. Suraveratum, S. Rochanakij, J. Krungkrai, Characterisation of carbonic anhydrase in *Plasmodium falciparum*, Int. J. Parasitol. 31 (2001) 661–668.

[8] C.T. Supuran, A. Scozzafava, Carbonic anhydrases as targets for medicinal chemistry, Bioorg. Med. Chem. 15 (2007) 4336–4350.

[9] C.T. Supuran, Carbonic anhydrase inhibitors, Bioorg. Med. Chem. Lett. 20 (2010) 3467–3474.

[10] A. Yildirim, U. Atmaca, A. Keskin, M. Topal, M. Celik, I. Gülcin, C.T. Supuran, *N*-Acylsulfonamides strongly inhibit human carbonic anhydrase isoenzymes I and II, Bioorg. Med. Chem. 23 (2015) 2598–2605.

[11] N. Dedeoglu, V. De Luca, S. Isik, H. Yildirim, F. Kockar, C. Capasso, C.T. Supuran, Cloning, characterization and anion inhibition study of a β-class carbonic anhydrase from the caries producing pathogen *Streptococcus mutans*, Bioorg. Med. Chem. 23 (2015) 2995–3001.

[12] S. Parkkila, A. Innocenti, H. Kallio, M. Hilvo, A. Scozzafava, C.T. Supuran, The protein tyrosine kinase inhibitors imatinib and nilotinib strongly inhibit several mammalian α-carbonic anhydrase isoforms, Bioorg. Med. Chem. Lett. 19 (2009) 4102–4106.

[13] Y. Akbaba, H.T. Balaydin, A. Menzek, S. Göksu, E. Sahin, D. Ekinci, Synthesis and biological evaluation of novel bromophenol derivatives as carbonic anhydrase inhibitors, Arch. Pharm. Chem. Life Sci. 346 (2013) 447–454.

[14] O. Ozensoy, M. Arslan, C.T. Supuran, Carbonic anhydrase inhibitors: purification and inhibition studies of pigeon (*Columba livia* var. *domestica*) red blood cell carbonic anhydrase with sulfonamides, J. Enzyme Inhib. Med. Chem. 26 (2011) 749–753.

[15] G. Nasr, A. Cristian, M. Barboiu, D. Vullo, J.-Y. Winum, C.T. Supuran, Carbonic anhydrase inhibitors. Inhibition of human cytosolic isoforms I and II with (reduced) Schiff's bases incorporating sulfonamide, carboxylate and carboxymethyl moieties, Bioorg. Med. Chem. 22 (2014) 2867–2874.

[16] A. Thiry, J.-M. Dogné, B. Masereel, C.T. Supuran, Targeting tumor-associated carbonic anhydrase IX in cancer therapy, Trends Pharmacol. Sci. 27 (2006) 566–573.

[17] V. Alterio, A. Di Fiore, K. D'Ambrosio, C.T. Supuran, G. De Simone, Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms?, Chem Rev. 112 (2012) 4421–4468.

[18] C.T. Supuran, Carbonic anhydrases: novel therapeutic applications for inhibitors and activators, Nat. Rev. Drug Discov. 7 (2008) 168–181.

[19] D. Neri, C.T. Supuran, Interfering with pH regulation in tumours as a therapeutic strategy, Nat. Rev. Drug Discov. 10 (2011) 767–777.

[20] C.T. Supuran, A. Scozzafava, Carbonic anhydrase inhibitors and their therapeutic potential, Expert Opin. Ther. Pat. 10 (2000) 575–600.

[21] A.M. Marini, A. Maresca, M. Aggarwal, E. Orlandini, S. Nencetti, F. Da Settimo, S. Salerno, F. Simorini, C. La Motta, S. Taliani, E. Nuti, A. Scozzafava, R. McKenna, A. Rosello, C.T. Supuran, Tricyclic sulfonamides incorporating benzothiopyrano [4,3-*c*]pyrazole and pyridothiopyrano[4,3-*c*]pyrazole effectively inhibit α- and β-carbonic anhydrase: X-ray crystallography and solution investigations on 15 isoforms, J. Med. Chem. 55 (2012) 9619–9629.

[22] F. Mincione, M. Starnotti, L. Menabuoni, A. Scozzafava, A. Casini, C.T. Supuran, Carbonic anhydrase inhibitors: 4-sulfamoyl-benzenecarboxamides and 4-chloro-3-sulfamoyl-benzenecarboxamides with strong topical antiglaucoma properties, Bioorg. Med. Chem. Lett. 11 (2001) 1787–1791.

[23] G.E. Granero, M.R. Longhi, Promising complexes of acetazolamide for topical ocular administration, Expert Opin. Drug Deliv. 7 (2010) 943–953.

[24] F. Carta, C.T. Supuran, A. Scozzafava, Novel therapies for glaucoma: a patent review 2007–2011, Expert Opin. Ther. Pat. 22 (2012) 79–88.

[25] F. Chimenti, R. Fioravanti, A. Bolasco, F. Manna, P. Chimenti, D. Secci, O. Befani, P. Turini, F. Ortuso, S. Alcaro, Monoamine oxidase isoform-dependent tautomeric influence in the recognition of 3,5-diaryl pyrazole inhibitors, J. Med. Chem. 50 (2007) 425–428.

[26] B.A. Bhat, S.C. Puri, M.A. Qurishi, K.L. Dhar, G.N. Qazi, Synthesis of 3,5-diphenyl-1H-pyrazoles, Synth. Comm. 35 (2005) 1135–1142.

[27] S.A.H. El-Feky, Z.K. Abd El-Samii, N.A. Osman, J. Lashine, M.A. Kamel, H.K. Thabet, Synthesis, molecular docking and anti-inflammatory screening of novel quinoline incorporated pyrazole derivatives using the Pfitzinger reaction II, Bioorg. Chem. 58 (2015) 104–116.

[28] L.-L. Xu, C.-J. Zheng, L.-P. Sun, J. Miao, H.-R. Piao, Synthesis of novel 1,3-diaryl pyrazole derivatives bearing rhodanine-3-fatty acid moieties as potential antibacterial agents, Eur. J. Med. Chem. 48 (2012) 174–178.

[29] J. Ramírez, M.V. Rodríguez, J. Quiroga, R. Abonia, M. Sortino, S.A. Zacchino, B. Insuasty, Efficient synthesis of novel 3-aryl-5-(4-chloro-2-morpholinothiazol-5-yl)-4,5-dihydro-1H-pyrazoles and their antifungal activity alone and in combination with commercial antifungal agents, Arch. Pharm. Chem. Life Sci. 347 (2014) 566–575.

[30] D.C. Malvar, R.T. Ferreira, R.A. De Castro, L.L. De Castro, A.C.C. Freitas, E.A. Costa, I.F. Florentino, J.C.M. Mafra, G.E.P. De Souza, F.A. Vanderlinde, Antinociceptive, anti-inflammatory and antipyretic effects of 1.5-diphenyl-1H-Pyrazole-3-carbohydrazide, a new heterocyclic pyrazole derivative, Life Sci. 95 (2014) 81–88.

[31] I.N. Cvijetić, M. Tanc, I.O. Juranić, T.Ž. Verbić, C.T. Supuran, B.J. Drakulić, 5-Aryl-1H-pyrazole-3-carboxylic acids as selective inhibitors of human carbonic anhydrases IX and XII, Bioorg. Med. Chem. 23 (2015) 4649–4659.

[32] P. Puthiyapurayil, B. Poojary, C. Chikkanna, S.K. Buridipad, Design, synthesis and biological evaluation of a novel series of 1,3,4-oxadiazole bearing N-methyl-4-(trifluoromethyl)phenyl pyrazole moiety as cytotoxic agents, Eur. J. Med. Chem. 53 (2012) 203–210.

[33] H. Balseven, M.M. Isgor, S. Mert, Z. Alim, S. Beydemir, S. Ok, R. Kasimogullari, Facile synthesis and characterization of novel pyrazole-sulfonamides and their inhibition effects on human carbonic anhydrase isoenzymes, Bioorg. Med. Chem. 21 (2013) 21–27.

[34] R. Kasimogullari, M. Bulbul, S. Mert, H. Guleryuz, Synthesis of 5-amino-1,3,4-thiadiazole-2-sulphonamide derivatives and their inhibition effects on human carbonic anhydrase isozymes, J. Enzyme Inhib. Med. Chem. 26 (2011) 231–237.

[35] R. Kasimogullari, M. Bulbul, H. Gunhan, H. Guleryuz, Effects of new 5-amino-1,3,4-thiadiazole-2-sulfonamide derivatives on human carbonic anhydrase isozymes, Bioorg. Med. Chem. 17 (2009) 3295–3301.

[36] E. Sen, Z. Alim, H. Duran, M.M. Isgor, S. Beydemir, R. Kasimogullari, S. Ok, Inhibitory effect of novel pyrazole carboxamide derivatives on human carbonic anhydrase enzyme, J. Enzyme Inhib. Med. Chem. 28 (2013) 328–336.

[37] S. Mert, R. Kasimogullari, T. Ica, F. Colak, A. Altun, S. Ok, Synthesis, structure–activity relationships, and in vitro antibacterial and antifungal activity evaluations of novel pyrazole carboxylic and dicarboxylic acid derivatives, Eur. J. Med. Chem. 78 (2014) 86–96.

[38] S. Mert, Z. Alim, M.M. Işgor, B. Anil, R. Kasimogullari, S. Beydemir, Novel pyrazole-3,4-dicarboxamides bearing biologically active sulfonamide moiety as potential carbonic anhydrase inhibitors, Arabian J. Chem. (2015), http://dx.doi.org/10.1016/j.arabjc.2015.05.020.

[39] M.N. Kopylovich, A.C.C. Nunes, K.T. Mahmudov, M. Haukka, T.C.O. Mac Leod, L.M.D.R.S. Martins, M.L. Kuznetsov, A.J.L. Pombeiro, Complexes of copper(II) with 3-(ortho-substituted phenylhydrazo)pentane-2,4-diones: syntheses, properties and catalytic activity for cyclohexane oxidation, Dalton Trans. 40 (2011) 2822–2836.

[40] J. Marten, W. Seichter, J. Wagler, E. Weber, Unusual behaviour during the route of a Japp-Klingemann reaction, Z. Naturforsch. B 65 (2010) 745–752.

[41] J. Marten, W. Seichter, E. Weber, 3-(Arylhydrazono)pentane-2,4-diones and their complexes with copper(II) and nickel(II)-synthesis and crystal structures, Z. Anorg. Allg. Chem. 631 (2005) 869–877.

[42] K.T. Mahmudov, A.M. Maharramov, R.A. Aliyeva, I.A. Aliyev, M.N. Kopylovich, A.J.L. Pombeiro, Ion pairs of 5,5-dimethyl-2-(2-hydroxy-3,5-disulfophenylhydrazo) cyclohexane-1,3-dione with cationic surface-active substances as analytical reagent for determination of copper(II), Anal. Lett. 43 (2010) 2923–2938.

[43] P. Šimůnek, A. Lyčka, V. Macháček, Synthesis, ^{1}H, ^{13}C and ^{15}N NMR study of azo coupling products from enaminones, Eur. J. Org. Chem. 2002 (2002) 2764–2769.

[44] P. Livant, Y. Jie, X. Wang, Solvent effects in a carbenoid N—H insertion route to triarylamines via 2-diazo-1,3-cyclohexanedione, Tetrahedron Lett. 46 (2005) 2113–2116.

[45] M. Hutchby, C.E. Houlden, M.F. Haddow, S.N.G. Tyler, G.C. Lloyd-Jones, K.I. Booker-Milburn, Switching pathways: room-temperature neutral solvolysis and substitution of amides, Angew. Chem. Int. Ed. 51 (2012) 548–551.

[46] B. Siesky, A. Harris, L.B. Cantor, L. Kagemann, Y. Weitzman, L. McCranor, C. Marques, A. Werne, E. Stefansson, A comparative study of the effects of brinzolamide and dorzolamide on retinal oxygen saturation and ocular microcirculation in patients with primary open-angle glaucoma, Br. J. Ophthalmol. 92 (2008) 500–504.

[47] E. Kockelmann, C.E. Elger, C. Helmstaedter, Significant improvement in frontal lobe associated neuropsychological functions after withdrawal of Topiramate in epilepsy patients, Epilepsy Res. 54 (2003) 171–178.

[48] Z. Alim, N. Kilinc, M.M. Işgor, B. Sengul, S. Beydemir, Some anti-inflammatory agents inhibit esterase activities of human carbonic anhydrase isoforms I and II: an in vitro study, Chem. Biol. Drug Des. 86 (2015) 857–863.

[49] R. Kasimogullari, M. Bulbul, B.S. Arslan, B. Gokce, Synthesis, characterization and antiglaucoma activity of some novel pyrazole derivatives of 5-amino-1,3,4-thiadiazole-2-sulfonamide, Eur. J. Med. Chem. 45 (2010) 4769–4773.

[50] O. Arslan, O.I. Kufrevioglu, B. Nalbantoglu, Synthesis and investigation of inhibition effects of new carbonic anhydrase inhibitors, Bioorg. Med. Chem. 5 (1997) 515–518.

[51] S. Beydemir, M. Ciftci, I. Ozmen, M.E. Buyukokuroglu, H. Ozdemir, O.I. Kufrevioglu, Effects of some medical drugs on enzyme activities of carbonic anhydrase from human erythrocytes in vitro and from rat erythrocytes in vivo, Pharmacol. Res. 42 (2000) 187–191.

[52] D. Ekinci, S. Beydemir, Z. Alim, Some drugs inhibit in vitro hydratase and esterase activities of human carbonic anhydrase-I and II, Pharmacol. Rep. 59 (2007) 580–587.

[53] K.M. Wilbur, N.G. Anderson, Electrometric and colorimetric determination of carbonic anhydrase, J. Biol. Chem. 176 (1948) 147–154.

[54] J.A. Verpoorte, S. Mehta, J.T. Edsall, Esterase activities of human carbonic anhydrases B and C, J. Biol. Chem. 242 (1967) 4221–4229.

[55] M.M. Bradford, Anal. Biochem. 72 (1976) 248–254.

[56] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.