Full Paper

Synthesis and Carbonic Anhydrase Isoenzymes Inhibitory Effects of Brominated Diphenylmethanone and Its Derivatives

Yasin Çetinkaya[1,2], Hülya Göçer[3], İlhami Gülçin[1], and Abdullah Menzek[1]*

[1] Faculty of Science, Department of Chemistry, Atatürk University, Erzurum, Turkey
[2] Department of Food Technology, Oltu Vocational School, Atatürk University, Oltu-Erzurum, Turkey
[3] Central Researching Laboratory, Agri Ibrahim Cecen University, Agri, Turkey

Known and novel derivatives including CO, Br, and OH (benzylic and phenolic), and the corresponding benzylic alcohols of (3,4-dimethoxyphenyl)(2,3,4-dimethoxyphenyl)methanone were synthesized, and their inhibitory effects on the carbonic anhydrase (CA) isoenzymes I and II were investigated. CAs are the metalloenzymes catalyzing the reversible hydration of carbon dioxide (CO_2) to bicarbonate (HCO_3^-). The inhibitory effects of diphenylmethanone derivatives **5**–**18** were tested on human CA (hCA, EC 4.2.1.1) isoenzymes (hCA I and hCA II) and they inhibited both isoenzymes at micromolar levels. Compounds **5** and **10** were found to be the best inhibitors against both CA isoenzymes. According to our data, compound **10** was the best inhibitor for isoenzyme hCA I ($IC_{50} = 3.48\ \mu M$, $K_i = 2.19\ \mu M$) whereas compound **5** was found to be the best inhibitor for isoenzyme hCA II ($IC_{50} = 1.33\ \mu M$, $K_i = 2.09\ \mu M$). Probably, stable conformations of **5** and **10** are more convenient for interaction with CA isoenzymes than those of the other compounds.

Keywords: Benzylalcohol / Bromination / Carbonic anhydrase / Enzyme inhibition / Reduction

Received: September 15, 2013; Revised: November 27, 2013; Accepted: December 4, 2013

DOI 10.1002/ardp.201300349

Introduction

Diphenylmethanone derivatives, including Br and OH groups, are both natural and biological active compounds (Fig. 1) [1–5]. The naturally occurring bromophenols are frequently isolated from red algae of the family Rhodomelaceae [1]. Compounds **1**–**4** are also natural products and have carbonic anhydrase (CA) inhibitory properties [6–10].

Enzymes are naturally occurring biocatalysts in living organisms that regulate the metabolic activities [11]. CAs (EC 4.2.1.1) are metalloenzymes in plant and animal kingdoms and isoenzymes of CA catalyze one of the most important reactions for life, the conversion of CO_2 into bicarbonate (HCO_3^-) with a metal hydroxide in nucleophilic conversion [12]. This reaction can be summarized as follows

$$\text{Carbonic anhydrase} \sim Zn^{2+} - OH^- + CO_2$$
$$\Leftrightarrow \text{carbonic anhydrase} \sim Zn^{2+} - HCO_3^-$$
$$\text{Carbonic anhydrase} \sim Zn^{2+} - HCO_3^- + H_2O$$
$$\Leftrightarrow \text{carbonic anhydrase} \sim Zn^{2+} - H_2O + HCO_3^-$$
$$\text{Carbonic anhydrase} \sim Zn^{2+} - H_2O + X$$
$$\Leftrightarrow \text{carbonic anhydrase} \sim Zn^{2+} - OH^- + XH^+$$

In general, there are several cytosolic forms, including one mitochondrial form, four membrane-bound isoenzymes, as well as a secreted CA isoenzyme. CA I, III, and VII are cytosolic forms, CA IV, CA IX, CA XII, and CA XIV are membrane-bound, CA V is mitochondrial, and CA VI is a secreted CA isoenzyme [13, 14]. CA isoenzymes have been purified and characterized in numerous tissues. CA isoforms are found in a variety of tissues where they participate in several important biological processes such as acid–base balance, respiration, CO_2 and ion transport, bone resorption, ureagenesis, gluconeogenesis, lipogenesis, and electrolyte secretion [15]. CA is very active in the kidney, gastric mucosa, eye lens, salivary glands, brain, nerve myelin sheath, pancreas,

Correspondence: Prof. İlhami Gülçin, Faculty of Sciences, Department of Chemistry, Ataturk University, 25240 Erzurum, Turkey.
E-mail: igulcin@atauni.edu.tr
Fax: +90 4422360948

*Additional correspondence: Prof. Abdullah Menzek,
E-mail: amenzek@atauni.edu.tr,
Fax: +90 4422360948

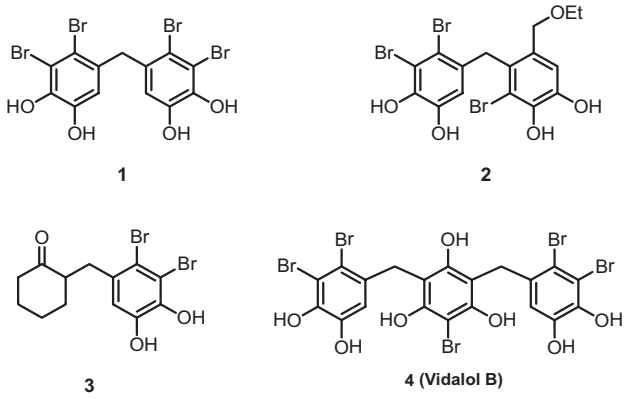


Figure 1. Some natural compounds exhibiting carbonic anhydrase inhibitory properties.

prostate, and uterus. Many studies have identified the important role of this enzyme in chloroplasts, fish branchia and secretory organs, some insects, and bacteria, and the construction of shell and egg shell formation in some of the animals, plants, and photosynthetic algae [16, 17]. In physiological conditions, the activity of hCA from human erythrocytes is quite changed, and these changes in hCA activity are related with metabolic disorders, such as diabetes and hypertension [18]. hCA enzyme inhibition studies can give insights that will help us to understand the enzyme-catalyzed reactions, and the vital functions and distribution of enzymes in these tissues. Therefore, the synthesis of enzyme inhibitors and activators of hCA are accelerated at present [19]. Specifically, CA I is located in many tissues, but some studies showed that this enzyme is inclusive in retinal and cerebral edema. Furthermore, CA II is involved in several diseases, such as glaucoma, edema, and epilepsy [19–22].

We synthesized the brominated diphenylmethanone derivatives and investigated their CA inhibitory properties [6, 7]. To compare CA inhibitory properties of diphenylmethanone, diphenylmethanol, and their derivatives with Br and OH may be important. Therefore, diphenylmethanone **5** and its derivatives **6–13** with Br and OH were synthesized [23]. However, diphenylmethanol **15** and its derivatives **16–17** with Br and OH were also synthesized. Furthermore, CA inhibitory properties of the compounds **5–18** were investigated.

Results and discussion

Synthesis

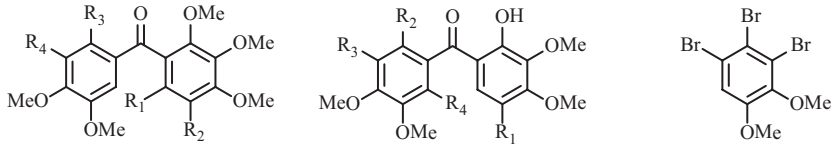
Diphenylmethanone **5** was obtained from the reaction of 3,4-dimethoxybenzoic acid and 1,2,3-trimethoxybenzene in polyphosphoric acid (PPA) and then its derivatives **6–13** and **14** with Br and OH were synthesized (Fig. 2) [23].

To obtain corresponding benzyl alcohol derivatives from diphenylmethanone **5** and its derivatives, reduction of CO groups was aimed because they have benzylic OH in case of CO. Reaction of the compound **5** with $NaBH_4$ in MeOH for 1 day (d) gave benzylic alcohol in a yield of 95% (Scheme 1). A mixture of compounds **15** and **16** was observed when HCl was added in the last of this reaction step. Column chromatography of the mixture allowed us to isolate compounds **15** and **16**. The compound **16** is an ether compound forming in the reaction condition.

In the same way, the reactions of the compounds **6** and **11** with $NaBH_4$ in MeOH were treated. It was found that the reaction rates with $NaBH_4$ in MeOH are very low. For example, the reactions of ketones **6** and **11** with $NaBH_4$ approximately were completed for 4 days. Therefore, their reduction to the corresponding benzylic alcohols **17** and **18** were realized by $LiAlH_4$ in THF (Scheme 1).

CA isoenzymes purification and activity assay

In this study, hCA I and hCA II isoenzymes were purified separately from human erythrocytes with Sepharose-4B-L-thyrosine-sulfanilamide affinity column chromatography. In the first part of our study, we have identified the inhibition effects of brominated diphenylmethanone **5–18** on the esterase activity of hCA I and hCA II. The inhibitions studies were performed with esterase activity methods [24] previously

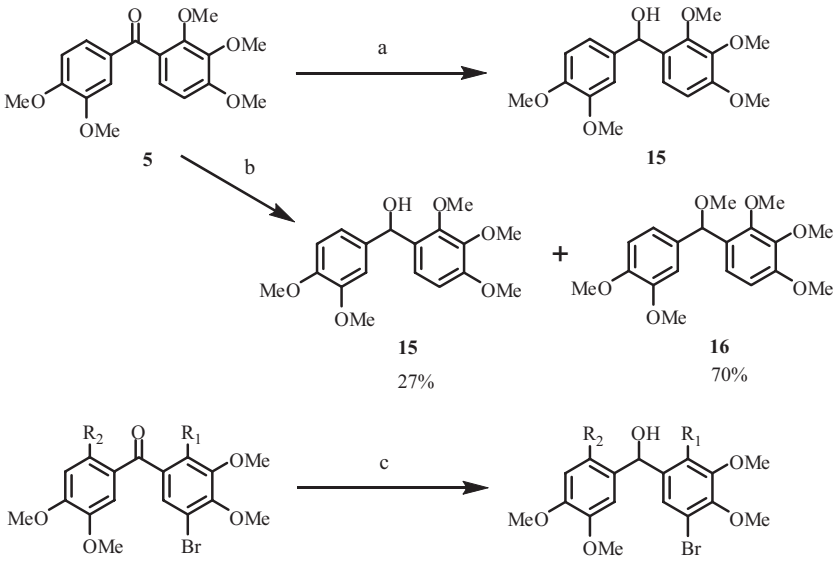


5 $R_1 = R_2 = R_3 = R_4 = H$
6 $R_1 = R_3 = R_4 = H$, $R_2 = Br$
7 $R_1 = R_4 = H$, $R_2 = R_3 = Br$
8 $R_3 = R_4 = H$, $R_1 = R_2 = Br$
9 $R_4 = H$, $R_1 = R_2 = R_3 = Br$
10 $R_1 = R_2 = R_3 = R_4 = Br$

11 $R_3 = R_4 = H$, $R_1 = R_2 = Br$
12 $R_4 = H$, $R_1 = R_2 = R_3 = Br$
13 $R_3 = H$, $R_1 = R_2 = R_4 = Br$

14

Figure 2. Synthesized compounds without benzylic OH or OMe.



Scheme 1. Reagents and conditions: (a) $NaBH_4$, MeOH, RT, 1 d, 95%; (b) 1: $NaBH_4$, MeOH, RT, 1 d, 2: HCl, 10 min; (c) $LiAlH_4$, THF, RT, 20 min.

described by Gocer and Gülçin [25]. This method is based on the destruction of CA ester bonds. In the process, the CA enzyme hydrolyzes the phenyl acetate and the resulting product is composed at 348 nm absorption. In our study, this method was preferred due to its high sensitivity compared to methods of Wilbur–Anderson. The isoenzyme purities were controlled by SDS–PAGE and single band was observed for each isoenzyme.

CA isoenzymes inhibition effects

As is known, there are many studies in the scientific world on the interactions of various compounds and the CA isoenzymes. In recent years, the CA isoenzymes have become an interesting target for the design of inhibitors or activators with biomedical applications. The CA inhibitors have been essentially used as diuretics, antiglaucoma and antiepileptic agents, while novel compounds are subject to clinical research as antiobesity and antitumor drugs/diagnostic tools [19, 26–29].

For this purpose, we have investigated the inhibitory effects of brominated diphenylmethanone compounds **5–18** on the hCA I and hCA II *in vitro*. Initially inhibition effects of investigated compounds on hCA I and hCA II were determined by drawing Lineweaver–Burk charts. Then, IC_{50} and the average of K_i values were calculated for **5–18** based on the drawn charts (Table 1).

In the first step, we determined the inhibitory effects of the synthesized brominated diphenylmethanone derivatives **5–18** on the esterase activity of the cytosolic isoform hCA I, and physiologically dominant isoform II. The inhibition results are presented in Table 1. It was reported that CA isoenzyme inhibitors coordinate to the zinc ion from the active site of CA isoenzyme [30]. Among the investigated brominated diphenylmethanone compounds, the compounds coded as **5** and **10** were shown to be effective inhibitors of cytosolic isoenzymes hCA I and hCA II (Table 1). According to the data obtained from this study, it is clear that the brominated diphenylmethanone

Table 1. Human carbonic anhydrase isoenzymes (hCA I and II) inhibition values with some brominated diphenylmethanone derivatives by an esterase assay with 4-nitrophenylacetate as substrate.

	IC_{50} (μM)		K_i (μM)	
Compounds	**hCA I**	**hCA II**	**hCA I**	**hCA II**
5	10.59	1.33	6.68	2.09
6	8.50	9.94	3.17	2.66
7	38.51	67.95	12.71	33.06
8[a)]	–	–	–	–
9	19.41	29.37	14.34	18.66
10	3.48	4.91	2.19	4.51
11	52.91	31.79	6.67	6.28
12	93.54	99.44	21.99	41.93
13[a)]	–	–	–	–
14	203.26	171.14	55.74	12.96
15	362.90	297.48	84.54	197.36
16	582.47	563.53	293.13	115.06
17	391.60	239.84	152.31	126.72
18[a)]	–	–	–	–

[a)] Human carbonic anhydrase isoenzymes results belonging to these compounds could not be determined.

derivatives compound **10** showed the strongest inhibitory activity on hCA I with K_i of 2.19 μM and compound **5** showed the strongest inhibitory activity on hCA II with K_i of 2.09 μM (Table 1). Furthermore, the other remaining brominated diphenylmethanone derivatives have quite strong inhibition effect on cytosolic isoenzyme hCA I with IC_{50}, which ranged from 3.48 to 582.47 μM and on hCA II with IC_{50} in the range of 1.33–563.53 μM (Table 1). It is well known that the nature of the groups in *ortho*-, *para*-, and *meta*-positions to the phenolic OH and OMe moiety strongly influences hCA I and hCA II inhibitory activity. The inhibition effect of halogenated sulfonamide derivatives has been investigated previously [31, 32]. It was indicated that the phenols, biphenyl, or diphenols bear bulky *ortho*-moieties in their molecules [32]. It was previously reported that the inhibition effect of halogenated sulfonamide derivatives has been investigated [33]. In another study, it was demonstrated that halogenated derivatives of sulfonamide such as bromosulfonamide are more effective as compared with the corresponding sulfonamides [31, 32]. Although we do not know in depth the mechanism of interaction between the synthesized compounds and both isoenzymes, we can speculate that the stable conformations of **5** and **10** are more appropriate for interaction with CA enzymes than those of the others. Considering all this information, the compounds **5** and **10** showing the highest inhibitory effects are inevitable. Also, the physiologically dominant isoenzyme hCA I was effectively inhibited by a range of compounds **5–18**, with K_i ranging from 2.19 to 293.13 μM, and isoenzyme hCA II by compounds **5–18**, with K_i ranging from 2.09 to 197.36 μM. Reconnaissance of new CA inhibitors is of great importance for pharmacological and medicinal approaches. Many inhibitors have been designed and synthesized in the literature. However, it is critically important to explore further classes of potent CA I and CA II in order to detect compounds with a different inhibition profile when compared to methanone derivatives, and to find novel applications for the inhibitors of these widespread enzymes [34, 35].

The inhibition effect of bromophenol derivatives on cytosolic isoenzymes hCA I and II was previously repoted [15, 32]. Recently, the interaction of CA I and CA II isoenzymes with several types of phenols, such as hydroxyl benzoic acids, methoxy benzoic acids, natural and unnatural bromophenols, and their substituted derivatives, was investigated. These derivatives demonstrated low micromolar or submicromolar inhibition as well as the possibility of designing isozyme selective CAIs [32, 36–38].

Conclusion

According to literature [11], known compounds **5–14** were synthesized. From novel compounds, **15** and **16** were obtained in the reactions of **5** with $NaBH_4$ in MeOH while **17** and **18** were obtained in the reactions of **6** and **11** with $LiAlH_4$ in THF. In addition, CA inhibitory properties of **5–18** have also been evaluated. These known novel compounds may be important synthons for further synthetic and biological purposes. Our data suggest that the brominated diphenylmethanone derivatives can be used as potential compounds in generating convenient CA isoenzyme inhibitors. Specifically, brominated diphenylmethanone derivatives **5–18** may be used in generating potent hCA I and hCA II inhibitors, which eventually target other isoforms that have not been assayed yet for their interactions with such agents. Also, a net distinction in the inhibition profile of the two cytosolic (hCA I and II) has not been found. Phenolic and bromophenolic compounds influence the activity of both hCA isozymes due to the presence of different functional groups such as OH, OMe, and Br in their aromatic scaffold.

Experimental section

General information

All chemicals and solvents are commercially available. All solvents were distilled and dried according to standard procedures. Silica gel (SiO_2, 60 mesh; Merck, Darmstadt, Germany) was used for column chromatography (CC). Melting point of all compounds was determined by cap. melting-point apparatus (BUCHI 530; Flawil, Switzerland) and are uncorrected. IR spectra were recorded as solns. in 0.1 mm cells with a Mattson 1000 FT-IR spectrophotometer (Cambridge, England). ^1H and ^{13}C NMR spectra were recorded by 400 (100)-MHz Varian spectrometer (Danbury, CT) in deuterated solvents ($CDCl_3$ and D_2O) with tetramethylsilane (TMS, $SiMe_4$) as an internal standard for protons, and with solvent signals as an internal standard for carbon spectra. Chemical shift values were mentioned as δ in ppm. Elemental analyses were recorded by Leco CHNS-932 apparatus (MI). CA inhibitory properties of samples were determined by a spectrophotometer (UV-1208, Shimadzu, Japan).

*(3,4-Dimethoxyphenyl)(2,3,4-trimethoxyphenyl)methanol (**15**)*

Ketone **5** (500 mg, 1.5 mmol) dissolved in MeOH (40 mL) was added to $NaBH_4$ (340 mg, 8.9 mmol) at 0°C. The reaction solution was stirred for 1 day. After it was controlled with TLC, the solvent was evaporated. It was added to water (10 mL) and HCl (10 mL, 1%). The mixture was extracted with CH_2Cl_2 (3 × 50 mL). Combined organic layers were watched and dried over Na_2SO_4. Diphenylmethanol **15** (478 mg, 1.43 mmol, 95%) was recrystallized from EtOAc/hexane. White crystals m.p. 104–106°C. ^1H NMR (400 MHz, $CDCl_3$) δ 6.96 (bs, aromatic, 1H), 6.93 (d, A part of AB-system, 1H, $J = 8.6$ Hz), 6.82 (dd, A part of AB-system, $J = 1.6$ Hz, $J = 8.3$ Hz, 1H), 6.79 (d, B part of AB-system, $J = 8.3$ Hz, 1H), 6.63 (d, B part of AB-system, $J = 8.6$ Hz, 1H), 5.88 (d, $J = 5.4$ Hz, 1H), 2.98 (d, OH, $J = 5.6$ Hz, 1H), 3.844 (s, OCH_3, 3H), 3.841 (s, OCH_3, 3H), 3.836 (s, OCH_3, 6H), 3.68 (s, OCH_3, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 153.58 (C), 151.49 (C), 149.05 (C), 148.35 (C), 136.88 (C), 130.20 (C), 122.33 (CH), 118.88 (CH), 111.04 (CH), 110.06 (CH), 109.95 (C), 107.18 (CH), 72.29 (COH), 61.05 (OCH_3), 60.90 (OCH_3), 56.19 (OCH_3), 56.11 (OCH_3), 56.06 (OCH_3). IR (CH_2Cl_2, cm^{-1}): 3513, 3001, 2936, 2839, 2032, 1905, 1838, 1600, 1512, 1352, 1287, 1138, 1100, 1027, 954,

933 Anal. calcd. for ($C_{18}H_{22}O_6$): C 64.64; H 6.63. Found C 64.96; H 6.71; MS (CI) *m/z* 317.1 (M+).

*1-((3,4-Dimethoxyphenyl)(methoxy)methyl)-2,3,4-trimethoxybenzene (**16**)*

Ketone **5** (400 mg, 1,2 mmol) dissolved in MeOH (40 mL) was added to $NaBH_4$ (320 mg, 8.4 mmol) at 0°C. The reaction solution was stirred for 1 day and controlled with TLC. After the reaction was completed, HCl (20 mL, 1%) was added and stirred for 10 min. The solvent was evaporated and the residue was extracted with CH_2Cl_2 (3 × 50 mL). After drying the organic layer over Na_2SO_4 and evaporation of the solvent, chromatography of the residue on silica gel (SiO_2, 60 g) with ethyl acetate/hexane (1:9) was utilized to obtain diphenyl methoxymethyl **16** (295 mg, 0.847 mmol, 70%) as pale yellow diphenylmethanol **15** (110 mg, 0.328 mmol, 27%). M.p. 51–54°C for compound **16**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (d, A part of AB-system, J = 8.7 Hz, 1H), 6.92 (d, J = 1.9 Hz, 1H), 6.85 (dd, A part of AB-system, J = 1.9 Hz, J = 8.2 Hz, 1H), 6.79 (d, A part of AB-system, J = 8.2 Hz, 1H), 6.65 (d, A part of AB-system, J = 8.7 Hz, 1H), 5.49 (s, CH, 1H), 3.838 (s, OCH_3, 3H), 3.835 (s, OCH_3, 3H), 3.82 (s, OCH_3, 3H), 3.81 (s, OCH_3, 3H), 3.74 (s, OCH_3, 3H), 3.35 (s, OCH_3, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 153.28 (C), 151.71 (C), 149.14 (C), 148.46 (C), 142.30 (C), 135.00 (C), 128.58 (C), 121.75 (CH), 119.68 (CH), 111.20 (CH), 110.59 (CH), 107.61 (CH), 79.37 (CH), 61.02 (OCH_3), 60.85 (OCH_3), 57.13 (OCH_3), 56.16 (OCH_3), 56.08 (OCH_3), 56.05 (OCH_3). IR (CH_2Cl_2, cm^{-1}): 2935, 2835, 1598, 1514, 1494, 1463, 1416, 1286, 1261,1234, 1139, 1095, 1029. Anal. calcd. for ($C_{18}H_{24}O_6$): C 65.50; H 6.94. Found C 65.56; H 7.21; MS (CI) *m/z* 348.1 (M^+).

General procedure for synthesis of benzyl alcohols 17, 18

*(5-Bromo-2,3,4-trimethoxyphenyl)(3,4-dimethoxyphenyl)-methanol (**17**)*

Ketone **6** (100 mg, 0.24 mmol) dissolved in THF (25 mL) was added to $LiAlH_4$ (60 mg, 1.58 mmol) at 0°C. The reaction was stirred for 20 min and then the solvent was evaporated. The residues were added to H_2O (30 mL) and EtOAc (40 mL). The water layer was extracted with EtOAc (2 × 40 mL). Combined organic layers were dried over Na_2SO_4. Evaporation of the solvent and chromatography of the residue on silica gel (SiO_2, 70 g) with ethyl acetate/hexane (1:9) afforded benzyl alcohol **17** (66 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (s, 1H), 6.96 (s, 1H), 6.83 (s, 2H), 5.90 (s, 1H), 3.88 (s, 6H, 2OCH_3), 3.88 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.67 (s, 1H, –OH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.94 (C), 150.85 (C), 149.14 (C), 148.63 (C), 147.56 (C), 135.93 (C), 134.41 (C), 125.46 (CH), 119.00 (CH), 111.76 (C), 111.06 (CH), 109.94 (CH), 71.68 (CH), 61.23 (OCH_3), 61.16 (OCH_3), 61.09 (OCH_3), 56.16 (OCH_3), 56.11 (OCH_3). IR (CH_2Cl_2, cm^{-1}): 3496, 2931, 2852, 1734, 1515, 1461, 1415, 1404, 1263, 1234, 1139, 1083, 1027, 1004.

*4-Bromo-6-((2-bromo-4,5-dimethoxyphenyl)(hydroxy)-methyl)-2,3-dimethoxyphenol (**18**)*

General procedure described for the synthesis of **17** was applied to synthesize compound **11** (180 mg, 0.38 mmol) and $LiAlH_4$ (0.8 g, 2.11 mmol) was used to yield diphenylmethanol **18** (115 mg, 58%) as oily. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (s, 1H), 7.02 (s, 1H), 6.76 (s, 1H), 6.59 (s, 1H), 6.28 (s, 1H), 3.96 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.60 (C), 149.32 (C), 148.87 (C), 147.54 (C), 141.12 (C), 132.46, 125.88 (CH), 125.39 (C), 115.47 (CH), 113.04 (C), 111.26 (CH), 107.38 (C), 71.11 (CH), 61.44 (OCH_3), 60.98 (OCH_3), 56.40 (OCH_3), 56.30 (OCH_3). IR (CH_2Cl_2, cm^{-1}): 3433, 2930, 2852, 1736, 1631, 1603, 1548, 1505, 1462, 1432, 1415, 1343, 1265, 1206, 1158, 1074, 1041.

Biochemistry

Purification of CA isoenzymes from human erythrocytes by affinity chromatography

CA isoenzymes were purified via a simple single-step process with Sepharose-4B-L-tyrosine-sulfanilamide affinity gel chromatography, as defined previously [39, 40]. The fresh human erythrocyte samples were centrifuged at 10,000*g* for 30 min and the precipitate was removed from serum. The pH was adjusted to 8.7 with solid Tris. Sepharose-4B-tirozyne-sulfanylamide affinity column was equilibrated with 25 mM Tris-solution. The affinity gel was washed with 25 mM Tris–HCl/22 mM Na_2SO_4 (pH 8.7). The human carbonic anhydrase (hCA VI) isoenzyme was eluted with 0.25 M H_2NSO_3H/25 mM Na_2HPO_4 (pH 6.7). All procedures were performed at 4°C [18, 41, 42].

Esterase activity assay

CA activity was assayed by the change in absorbance at 348 nm of 4-nitrophenylacetate (NPA) to 4-nitrophenylate ion over a period of 3 min at 25°C using a spectrophotometer (Shimadzu, UVmini-1240 UV–VIS spectrophotometer) according to the method described by Verpoorte [43]. The enzymatic reaction contained 1.4 mL of Tris–SO_4 buffer (0.05 M; pH: 7.4), 1 mL of 4-nitrophenylacetate (3 mM), 0.5 mL H_2O, and 0.1 mL of enzyme solution (total volume, 3.0 mL). A reference measurement was obtained by preparing the mixture without the enzyme solution. All measurements were recorded in triplicate. The K_i values were determined from a series of experiments using three different brominated diphenylmethanone compounds **5**–**18** and 4-nitrophenylacetate as the substrate at five different concentrations to construct Lineweaver–Burk curves [44].

Protein determination

The yield of protein during the purification steps was determined spectrophotometrically at 595 nm according to the Bradford method [45]. Bovine serum albumin was used as standard in this study [46, 47].

SDS–polyacrylamide gel electrophoresis

The purity of the enzymes was confirmed using SDS–polyacrylamide gel electrophoresis. The running and stacking gels contained 10 and 3% acrylamide, respectively, and 0.1% SDS, according to the Laemmli procedure [48] and as described previously [49, 50]. A 20 mg sample was applied to the electrophoresis medium [50]. Gels were stained for 1.5 h in 0.1% Coomassie Brilliant Blue R-250 in 50% methanol and 10% acetic acid, then destained with several changes of the same solvent without the dye [51–53].

We thank to Ataturk University for the financial supports of this work (BAP, Project Numbers: 2009/84 and 2009/251).

The authors have declared no conflict of interest.

References

[1] G. W. Gribble, *Chem. Soc. Rev.* **1999**, *28*, 335–346.

[2] H. T. Balaydın, Y. Akbaba, A. Menzek, E. Şahin, S. Göksu, *Arkivoc* **2009**, *XIV*, 75–87.

[3] H. T. Balaydın, İ. Gülçin, A. Menzek, S. Göksu, E. Şahin, *J. Enzyme Inhib. Med. Chem.* **2010**, *25*, 685–695.

[4] Y. Çetinkaya, H. Göçer, A. Menzek, İ. Gülçin, *Arch. Pharm.* **2012**, *345*, 323–334.

[5] N. Xu, X. Fan, X. Yan, X. Li, R. Niu, C. K. Tseng, *Phytochemistry* **2003**, *62*, 1221–1224.

[6] Y. Akbaba, H. T. Balaydın, A. Menzek, S. Göksu, E. Şahin, D. Ekinci, *Arch. Pharm.* **2013**, *346*, 447–454.

[7] H. T. Balaydın, S. Durdagi, D. Ekinci, M. Senturk, S. Göksu, A. Menzek, *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 467–475.

[8] M. Nar, Y. Çetinkaya, İ. Gülçin, A. Menzek, *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 402–406.

[9] H. T. Balaydın, M. Şentürk, A. Menzek, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1352–1357.

[10] A. Akıncıoğlu, M. Topal, İ. Gülçin, S. Göksu, *Arch. Pharm.* **2014**, *347*, 68–76.

[11] D. Shahwar, M. A. Raza, T. Khan, *Turk. J. Chem.* **2012**, *36*, 257–266.

[12] A. Akıncıoğlu, Y. Akbaba, H. Göçer, S. Göksu, İ. Gülçin, C. T. Supuran, *Bioorg. Med. Chem.* **2013**, *21*, 1379–1385.

[13] C. T. Supuran, A. Scozzafava, *Exp. Opin. Ther. Pat.* **2002**, *12*, 217–242.

[14] C. T. Supuran, A. Scozzafava, *Exp. Opin. Ther. Pat.* **2000**, *10*, 575–600.

[15] İ. Gülçin, S. Beydemir, *Mini Rev. Med. Chem.* **2013**, *13*, 408–430.

[16] T. H. Maren, *Physiol. Rev.* **1967**, *47*, 595–781.

[17] A. Fersht, *Enzyme Structure and Mechanism*, 2nd edn., W.H. Freeman and Company, **1985**, pp. 436–439.

[18] İ. Gülçin, Ş. Beydemir, M. E. Büyükokuroğlu, *Biol. Pharm. Bull.* **2004**, *27*, 613–616.

[19] N. Hen, M. Bialer, B. Yagen, A. Maresca, M. Aggarwal, A. H. Robbins, R. McKenna, A. Scozzafava, C. T. Supuran, *J. Med. Chem.* **2011**, *54*, 3977–3981.

[20] F. Mincione, A. Scozzafava, C. T. Supuran, *Curr. Pharm. Des.* **2008**, *14*, 649–654.

[21] E. R. Swenson, L. J. Teppema, *J. Appl. Physiol.* **2007**, *102*, 1305–1307.

[22] A. Scozzafava, C. T. Supuran, *Med. Res. Rev.* **2003**, *23*, 146–189.

[23] Y. Çetinkaya, A. Menzek, E. Sahin, H. T. Balaydın, *Tetrahedron* **2011**, *67*, 3483–3489.

[24] J. M. Armstrong, D. V. Myers, J. A. Verpoorte, J. T. Edsall, *J. Biol. Chem.* **1966**, *214*, 5137–5149.

[25] H. Gocer, İ. Gülçin, *Int. J. Acad. Res.* **2013**, *5*, 150–155.

[26] J. Y. Winum, A. Scozzafava, J. L. Montero, C. T. Supuran, *Mini Rev. Med. Chem.* **2006**, *6*, 921–936.

[27] C. T. Supuran, *Curr. Top. Med. Chem.* **2007**, *7*, 825–833.

[28] C. T. Supuran, *Nat. Rev. Drug Disc.* **2008**, *7*, 168–181.

[29] G. De Simone, A. Di Fiore, C. T. Supuran, *Curr. Pharm. Des.* **2008**, *14*, 655–660.

[30] C. T. Supuran, *Bioorg. Med. Chem.* **2013**, *21*, 1377–1378.

[31] D. Ekinci, M. Senturk, O. I. Kufrevioglu, *Expert Opin. Ther. Pat.* **2011**, *21*, 1831–1841.

[32] H. T. Balaydın, M. Senturk, S. Goksu, A. Menzek, *Eur. J. Med. Chem.* **2012**, *54*, 423–428.

[33] D. Ekinci, H. Cavdar, S. Durdagi, O. Talaz, M. Senturk, C. T. Supuran, *Eur. J. Med. Chem.* **2012**, *49*, 68–73.

[34] A. Innocenti, S. B. Öztürk Sarıkaya, İ. Gülçin, C. T. Supuran, *Bioorg. Med. Chem.* **2010**, *18*, 2159–2164.

[35] S. Pastorekova, D. Vullo, I. Nishimori, A. Scozzafava, J. Pastoreka, C. T. Supuran, *Bioorg. Med. Chem.* **2008**, *16*, 3530–3536.

[36] C. T. Supuran, A. Scozzafava, *Expert Opin. Ther. Pat.* **2002**, *12*, 217–242.

[37] C. T. Supuran, A. Scozzafava, *Bioorg. Med. Chem.* **2007**, *15*, 4336–4350.

[38] M. Senturk, I. Gulcin, A. Dastan, O. I. Kufrevioglu, C. T. Supuran, *Bioorg. Med. Chem.* **2009**, *17*, 3207–3211.

[39] S. ArasHisar, O. Hisar, Ş. Beydemir, İ. Gülçin, T. Yanik, *Acta Vet. Hung.* **2004**, *52*, 413–422.

[40] S. B. Öztürk Sarikaya, İ. Gülçin, C. T. Supuran, *Chem. Biol. Drug Des.* **2010**, *75*, 515–520.

[41] M. Şentürk, İ. Gülçin, Ş. Beydemir, Ö. İ. Küfrevioğlu, C. T. Supuran, *Chem. Biol. Drug Des.* **2011**, *77*, 494–499.

[42] Ş. Beydemir, İ. Gülçin, *J. Enzyme Inhib. Med. Chem.* **2004**, *19*, 193–197.

[43] J. A. Verpoorte, S. Metha, J. T. Edsall, *Biol. Chem.* **1967**, *242*, 4221–4229.

[44] H. Lineweaver, D. Burk, *Am. Chem. Soc.* **1934**, *57*, 685–693.

[45] M. Bradford, *Anal. Biochem.* **1976**, *72*, 248–254.

[46] İ. Gülçin, Ö. İ. Küfrevioğlu, M. Oktay, *J. Enzyme Inhib. Med. Chem.* **2005**, *20*, 297–302.

[47] D. P. Iga, R. Schmidt, S. Iga, C. L. Hotoleanu, F. Duica, A. Nicolescu, S. S. Gitman, *Turk. J. Chem.* **2013**, *37*, 299–307.

[48] D. K. Laemmli, *Nature* **1970**, *227*, 680–685.

[49] T. A. Çoban, Ş. Beydemir, İ. Gülçin, D. Ekinci, *Biol. Pharm. Bull.* **2007**, *30*, 2257–2261.

[50] T. A. Çoban, S. Beydemir, I. Gulcin, D. Ekinci, *J. Enzyme Inhib. Med. Chem.* **2008**, *23*, 266–270.

[51] A. Atasaver, H. Özdemir, İ. Gülçin, Ö. İ. Küfrevioğlu, *Food Chem.* **2013**, *136*, 864–870.

[52] M. Şişecioğlu, İ. Gülçin, M. Çankaya, H. Özdemir, *Int. J. Food Propert.* **2012**, *15*, 1182–1189.

[53] K. Aksu, M. Nar, M. Tanç, D. Vullo, İ. Gülçin, S. Göksu, F. Tümer, *Bioorg. Med. Chem.* **2013**, *21*, 2925–2931.

