Journal of Enzyme Inhibition and Medicinal Chemistry

ISSN: 1475-6366 (Print) 1475-6374 (Online) Journal homepage: http://www.tandfonline.com/loi/ienz20

The synthesis of (Z)-4-oxo-4-(arylamino)but-2-enoic acids derivatives and determination of their inhibition properties against human carbonic anhydrase I and II isoenzymes

Koray Oktay, Leyla Polat Köse, Kıvılcım Şendil, Mehmet Serdar Gültekin, İlhami Gülçin & Claudiu T. Supuran

To cite this article: Koray Oktay, Leyla Polat Köse, Kıvılcım Şendil, Mehmet Serdar Gültekin, İlhami Gülçin & Claudiu T. Supuran (2015): The synthesis of (Z)-4-oxo-4-(arylamino)but-2-enoic acids derivatives and determination of their inhibition properties against human carbonic anhydrase I and II isoenzymes, Journal of Enzyme Inhibition and Medicinal Chemistry

To link to this article: http://dx.doi.org/10.3109/14756366.2015.1071808

Journal of Enzyme Inhibition and Medicinal Chemistry

http://informahealthcare.com/enz
ISSN: 1475-6366 (print), 1475-6374 (electronic)

J Enzyme Inhib Med Chem, Early Online: 1–7
 DOI: 10.3109/14756366.2015.1071808

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

The synthesis of (*Z*)-4-oxo-4-(arylamino)but-2-enoic acids derivatives and determination of their inhibition properties against human carbonic anhydrase I and II isoenzymes

Koray Oktay[1], Leyla Polat Köse[1], Kıvılcım Şendil[2], Mehmet Serdar Gültekin[1], İlhami Gülçin[1,3], and Claudiu T. Supuran[4,5]

[1]Faculty of Science, Department of Chemistry, Ataturk University, Erzurum, Turkey, [2]Faculty of Science and Arts, Department of Chemistry, Kafkas University, Kars, Turkey, [3]Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia, [4]Dipartimento di Chimica Ugo Schiff, Università degli Studi di Firenze, Sesto Fiorentino (Firenze), Italy, and [5]Neurofarba Department, Section of Pharmaceutical and Nutriceutical Sciences, Università degli Studi di Firenze, Sesto Fiorentino (Florence), Italy

Abstract

The synthesis of (*Z*)-4-oxo-4-(arylamino)but-2-enoic acid (**4**) derivatives containing structural characteristics that can be used for the synthesis of several active molecules, is presented. Some of the butenoic acid derivatives (**4a**, **4c**, **4e**, **4i**, **4j**, **4k**) are synthesized following literature procedures and at the end of the reaction. In addition, structures of all synthesized derivatives (**4a**–**4m**) were determined by ^{1}H-NMR, ^{13}C-NMR and IR spectroscopy. Carbonic anhydrase is a metalloenzyme involved in many crucial physiologic processes as it catalyzes a simple but fundamental reaction, the reversible hydration of carbon dioxide to bicarbonate and protons. Significant results were obtained by evaluating the enzyme inhibitory activities of these derivatives against human carbonic anhydrase hCA I and II isoenzymes (hCA I and II). Butenoic acid derivatives (**4a**–**4m**) strongly inhibited hCA I and II with K_is in the low nanomolar range of 1.85 ± 0.58 to 5.04 ± 1.46 nM against hCA I and in the range of 2.01 ± 0.52 to 2.94 ± 1.31 nM against hCA II.

Keywords

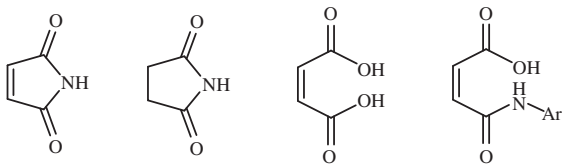
Butenoic acid, carbonic anhydrase, enzyme inhibition, enzyme purification, isoenzymes

History

Received 25 June 2015
Revised 7 July 2015
Accepted 8 July 2015
Published online 24 August 2015

Introduction

The name maleimide (**1**) was derived from maleic acid (**3**) and imide functional group[1]. Maleimides are important structural blocks with unsaturated structures in organic synthesis. In polymer chemistry, such structure blocks are used as monomers. When double bonds in maleimide molecules are saturated with hydrogenation reaction, the succinimide structural blocks are obtained (**2**).



Address for correspondence: Mehmet Serdar Gültekin, Faculty of Science, Department of Chemistry, Ataturk University, 25240 Erzurum, Turkey. Tel: +90-442 231 4428. Fax: +90 442 231 4109. E-mail: gultekin@atauni.edu.tr

İlhami Gülçin, Faculty of Science, Department of Chemistry, Ataturk University, 25240 Erzurum, Turkey. Tel: +90-442 231 4375. Fax: +90 442 231 4109. E-mail: igulcin@atauni.edu.tr

Literature offers several methods for synthesizing maleimide structures; among these methods, the main strategy is to obtain amino carboxylic acid (**4**) through the reaction of maleic anhydride with primary amines. But-2-enoic acid (**4**) derivatives are synthesized as the result of reaction of amine group with lactone carbonyl through acid catalysis[2,3]. Opening of lactone ring is a critical step that can affect the reaction time and the cost of maleimides[4–6].

Several derivatives involving the imide group have considerably high biological activities; however, the synthesis of imide groups in high yields and the development of low-cost synthesis methods for these groups are restricted to applications in synthetic and polymer chemistry. Cyclic structure involving the imide group occupy an important place in synthetic organic and drug chemistry. Especially, phthalimides are commonly used for the protecting amino acids in addition to their remarkable use in the field of medicine. Maleimides have easily usable characteristics in Michael Addition and Diels–Alder reactions in synthetic organic chemistry since 5-membered ring maleimides are more reactive compared to those with 6-membered and 7-memeberd rings[2,3]. As maleimides can fit in the chemical structure of some proteins, they make up an important category of biological substrates[3,7]. They can frequently be used as photon-initiator for the polymerization of free radicals in polymer chemistry[3–8], as well as

monomer in the synthesis of polymaleimides and their copolymers. They are among the most commonly mentioned chemical structures in the literature with their recently found functions, such as the use of their derivatives in the form of pharmaceutical mid-products because of their antibacterial property[5,9] and their characteristics of bonding with natural rubbers[9,10]. In addition, they are used vastly in the space industry for resin encapsulation of IC-dyes and as structural adhesives for fiber-empowered composites[9,11]. In recent times, some research groups[12,13] achieved a successful synthesis of 3-nitryl- and 3-acetoxy aryl maleimides. In the mentioned synthesis, antifungal activities of derivatives were investigated and were found to have biological activities also.

N-aryl maleimide molecules were obtained from aryl aromatic compounds and maleic anhydrate using the methods mentioned in literature. During the reaction, N-aryl maleimides associated with dehydration produced high yields (Scheme 1).

Enzyme activities of the synthesized derivatives were investigated, as well as significant results obtained by these syntheses were introduced in the literature. Carbonic anhydrases (CA, E.C.4.2.1.1) are ubiquitous metalloenzymes that catalyze a simple reaction, the interconversion between carbon dioxide (CO_2) and bicarbonate (HCO_3) generate H^+ ions in the hydration reaction; thus, being one of the main players of pH regulation in many tissues, organs and organisms[14–18].

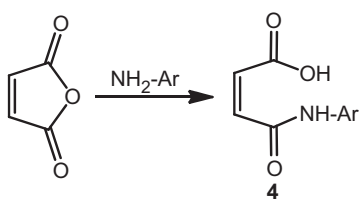
$$CO_2 + H_2O \longleftrightarrow HCO_3^- + H^+$$

In humans, CAs are present in a large variety of tissues, such as the gastrointestinal tract, the nervous system, the reproductive tract, lungs, kidneys, skin and eyes[19–24]. This regulatory reaction supports many biochemical and physiological processes associated with pH control, fluid secretion and ion transport.

Six distinct genetic CA families, the α-, β-, γ-, δ-, ζ- and η-CAs, are known to date, constituting an interesting example of convergent evolution at the molecular level[25–31]. Also, up to now, 16 different x-CA isoenzymes have been described in various organisms[32–35]. These enzymes differ in their subcellular localization, catalytic activity and susceptibility to different classes of inhibitors. Some of them are cytosolic (CA I, CA II, CA III, CA VII and CA XIII), others are membrane bound (CA IV, CA IX, CA XII and CA XIV), two are mitochondrial (CA VA and CA VB) and one is secreted in saliva (CA VI)[36–41]. The disregulated activity of some of these isoenzymes leads to a large spectrum of diseases, including retinal or cerebral edema (in which hCA I is involved); epilepsy, glaucoma, edema, high altitude sickness (hCA II seems to be the main, but not the only isoenzyme involved in these conditions); oxidative stress (hCA III); retinitis pigmentosa (hCA IV); obesity (hCA VA/VB); carcinogenesis (hCA VI); epilepsy (hCA VII); tumorigenesis (hCA IX and XII; but hCA XII is also implicated in glaucoma); sterility (hCA XIII) and various retinopathies (in which hCA XIV is the main isoform involved)[33,42–47].

Inhibitors of carbonic anhydrase enzymes (CAIs) have a large number of applications in therapy, including anticancer, antiglaucoma and anti-osteoporosis agents. They are used as diuretics, anti-obesity and anti-infective drugs. These CAIs have also been used for managing Alzheimer's disease and a variety of neurological disorders. Many types of CAI derivatives have been reported recently, together with their potential applications[39,48]. These chemical groups are used for the clinical treatment of some conditions for decades[47,49–52].

In this contest, many efforts have been made for the development of specific CAIs, and some remarkable results have been achieved in the past 15 years[53–59]. In this study, we investigated the inhibitory effects of butenoic acid derivatives (**4a**–**4m**) on hCA I and II.



Scheme 1. General synthesis (Z)-4-oxo-4-(arylamino)but-2-enoic acids derivatives from maleic acid in catalytic acidic media.

Experimental

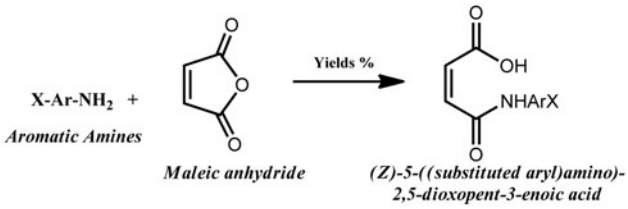
The known and unknown derivatives, having maleimide skeleton structure, were synthesized using the methods commonly known in the literature, and biological activities of such derivatives were investigated. Derivatives with amino carboxylic acid (**4**) skeleton structure were synthesized through the reaction of maleic anhydride with the related aromatic amine molecule. Due to their structural features, activities of the synthesized derivatives were investigated using enzymatic reactions. This work is the first to report the syntheses of **4a**, **4c**, **4e**, **4i**, **4j**, **4k** derivatives, and their structures were determinate by NMR spectroscopy (Supplementary material). Melting points for all these new derivatives are listed in Supplementary material, whereas that of other derivatives are provided in the literature (Table 1)[60–68].

First, for the synthesis of (*Z*)-4-oxo-4-(aryl)but-2-enoic acid skeleton structure, the derivatives **5**–**17** were treated with maleic anhydrate at room temperature. High-yield derivatives, having (*Z*)-4-oxo-4-(aryl)but-2-enoic acid (**4**) skeleton structures, were obtained via ring-opening reaction at room temperature. Finally, these derivatives were reacted with aromatic amine compounds for the synthesis of (*Z*)-4-oxo-4-(aryl)but-2-enoic acid (**4**). The yields of all the derivatives are showed in Table 1.

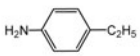
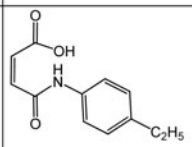
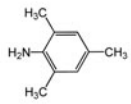
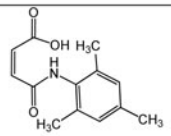
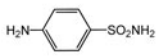
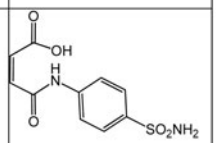
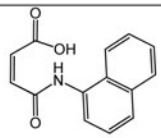
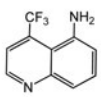
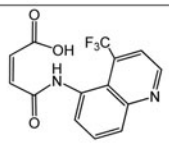
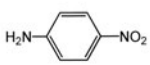
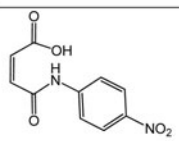
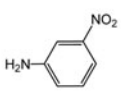
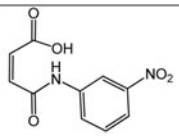
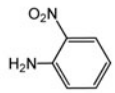
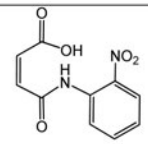
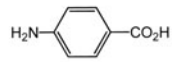
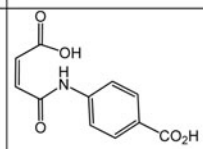
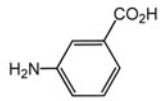
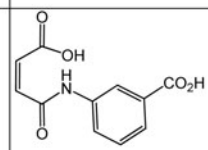
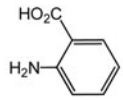
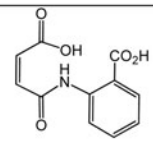
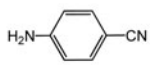
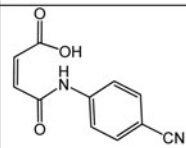
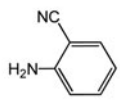
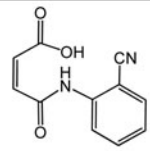
Affinity chromatography is the science of separation of biomolecules based on their specific interactions, such as that between enzyme and substrate to solid phase-coupled ligands. Due to its ability to enrich selective targets, affinity chromatography has remained a mainstay technique in separation chemistry[69–72]. In this study, both hCA I and II isoenzymes were purified by Sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography[73–76]. The affinity chromatography consists Sepharose-4B-L-tyrosine-sulfanilamide that acts as an affinity matrix for selective retention of CA isoenzymes[77–79]. The column material was prepared according to a previous method[80,81]. Thus, homogenate solution acidity was adjusted and supernatant was transferred to the previously prepared column[82]. The protein flow in the column eluates was spectrophotometrically determined at 280 nm. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was applied for the detection of both the isoenzymes' purity[83–86]. This technique is widely used in biochemistry, genetics, forensics, molecular biology and biotechnology for the separation of biological macromolecules including proteins, according to their electrophoretic mobility[87–89]. After visualizing the SDS-PAGE process, a single band was observed for hCA I and II isoenzymes. This protein-imaging method was previously described[90–93]. In this application, the imaging method was performed using 10 and 3% acrylamide for the running and the stacking gel, respectively, with 0.1% SDS[94].

hCA I and II isoenzyme activities were determined according to the method of Verpoorte et al.[95] The protein quantity was spectrophotometrically measured at 595 nm according to the Bradford method[96]. Bovine serum albumin was used as the standard protein[90]. For determining the inhibition effect of each

Table 1. The synthesis of (*Z*)-4-oxo-4-(aryl) but-2-enoic acid (4) and 3-chloro-aryl maleimide (**5**) skeleton structures.



X:H, NO_2, CN, COOH,....

Entry	Aromatic Amines	Yields (%)	(Z)-4-oxo-4-(arylamino) but-2-enoic acids[a]
1	 **5**	95	 **4a**
2	 **6**	96	 **4b**[12]
3	 **7**	95	 **4c**
4	 **8**	98	 **4d**[13]
5	 **9**	95	 **4e**
6	 **10**	90	 **4f**[14]
7	 **11**	91	 **4g**[16]
8	 **12**	92	 **4h**[16]
9	 **13**	95	 **4i**
10	 **14**	92	 **4j**
11	 **15**	90	 **4k**
12	 **16**		 **4l**[18]
13	 **17**	87	 **4m**[19]

(*continued*)

Table 2. Human carbonic anhydrase isoenzymes I and II inhibition profile of some butenoic acid derivatives (**4a**–**4m**).

	IC_{50} (nM)				K_i (nM)	
Bromophenols	hCA I	r^2	hCA II	r^2	hCA I	hCA II
4a	42.77	0.9933	34.49	0.959	41.18 ± 12.17	29.05 ± 5.21
4b	35.54	0.9902	26.04	0.9956	32.47 ± 7.72	21.35 ± 3.04
4c	30.43	0.9975	23.45	0.9931	33.00 ± 4.69	24.03 ± 7.52
4d	38.82	0.9924	29.64	0.9857	37.96 ± 5.51	21.74 ± 1.64
4e	28.72	0.9979	23.04	0.9973	29.05 ± 9.23	21.34 ± 6.26
4f	32.25	0.9948	32.71	0.9945	26.32 ± 6.49	23.52 ± 3.67
4g	30.43	0.9989	27.66	0.9921	22.15 ± 7.76	22.34 ± 4.65
4h	32.88	0.9986	32.63	0.9863	33.86 ± 10.30	28.31 ± 4.93
4i	31.87	0.9958	30.19	0.9823	29.19 ± 6.94	24.92 ± 4.24
4j	32.59	0.9990	27.48	0.9932	32.23 ± 9.73	20.91 ± 3.14
4k	37.42	0.9958	29.61	0.9889	35.18 ± 11.50	23.21 ± 5.22
4l	40.91	0.9933	27.79	0.9934	41.79 ± 13.19	23.03 ± 4.57
4m	41.07	0.9975	28.95	0.9855	31.95 ± 3.45	28.38 ± 5.18
AZA*	6.07	0.9154	5.50	0.9636	6.76 ± 2.55	5.85 ± 2.56

*AZA (Acetazolamide) was used as a standard inhibitor for both hCA enzymes.

butenoic acid derivative, an activity (%)-[butenoic acid derivatives] graph was drawn. To determine K_i values, three different butenoic acid derivative concentrations were tested. Also, different substrate concentrations were used and Lineweaver–Burk curves were drawn[97], as previously described[52].

Results and discussion

Carbonic anhydrase enzyme inhibitors (CAIs) have a large spectrum of applications in therapy, including blood pressure-lowering, anticancer, vasodilator effects, antiglaucoma and anti-osteoporosis agents. They are used as diuretics, anti-obesity and anti-infective drugs. It has been shown that the CA inhibition in smooth muscle cells results in a rise in pH, leading to KCa channel activation and vasorelaxation[98,99]. Also, these CAIs have been used for managing Alzheimer's disease and a variety of neurological disorders. Many types of CAIs and new derivatives have been reported recently, together with their potential applications. In our study, physiologically relevant hCA I and II isomers are studied. A dozen of butenoic acid derivatives (**4a**–**4m**) were examined for their hCA I and II isoenzymes inhibition properties. All butenoic acid derivatives (**4a**–**4m**) have shown efficient inhibition against both isoforms. The chemical structures of butenoic acid derivatives (**4a**–**4m**) are given in Table 1. Also, CA I and II inhibiting effects of a dozen of butenoic acid derivatives (**4a**–**4m**) are summarized in Table 2. It is well known that developing isoenzyme-specific CAIs is highly beneficial in obtaining novel classes of drugs devoid of various undesired side effects.

Cytosolic isoenzyme hCA I is found in many tissues; however, it was demonstrated that this isoenzyme is involved in retinal and cerebral edema, and its inhibition may be a valuable tool for fighting these conditions. Also, it was reported that the K_i value of a dozen of butenoic acid derivatives (**4a**–**4m**) was less than 50 nM (K_is < 50 nM). The results obtained from this study clearly indicate that a dozen of butenoic acid derivatives (**4a**–**4m**) had potent inhibition profile against slow hCA I, and cytosolic dominant rapid isozymes hCA II with low nanomolar range (K_is < 30 nM). These compounds derived from butenoic acid (**4a**–**4m**) bind to hCA I in the low nanomolar range and were found as stronger inhibitors of this isoform, with K_is ranging between 22.15 ± 7.76 and 41.79 ± 13.19 nM. However, the most powerful inhibition effect was found in butenoic acid derivative **4g** derived from 3-nitroaniline (**11**), with K_i value of 22.15 ± 7.76 nM. Butenoic acid derivative **4g** possess two carbonyl groups (–C=O), an amine group ($–NH_2$), a hydroxyl group (–OH) and a nitro group ($–NO_2$). It is well known that these groups are biologically very reactive and the molecules in these groups demonstrated effective CA isoenzyme inhibition properties. On the other hand, acetazolamide (**AZA**) is considered a broad-specificity CA inhibitor due to its widespread inhibition of CAs, showing K_i value of 6.76 ± 2.55 nM against hCA I isoenzyme. The inhibition effects of all butenoic acid derivatives (**4a**–**4m**) are close to acetazolamide. **AZA** is considered a good CA inhibitor and approved for the treatment of a range of conditions, including glaucoma, epilepsy and altitude sickness[5].

CA II is involved in several diseases, including glaucoma, epilepsy, altitude sickness and edema. Compared to the physiologically dominant isoform hCA II, all butenoic acid derivatives (**4a**–**4m**) showed K_i values ranging from 20.91 ± 3.14 to 29.05 ± 5.21 nM (Table 2). The butenoic acid derivative **4j**, derived from 3-aminobenzoic acid, had two carbonyl groups (–C=O), an amine group ($–NH_2$), a hydroxyl group (–OH) and an acetate group (–COO), being the best hCA II inhibitor (K_i: 20.91 ± 3.14 nM). The molecules with acetate, azide, cyanate, sulfide and cyanide groups bind the active site of CA[100]. In another study, the binding of acetate ions to bovine CA was investigated by NMR and inhibition measurements. It was found that two acetate ions were found to interact with the protein, but only one is linked with the inhibition of the esterase activity of the bovine CA[101]. However, all butenoic acid derivatives (**4a**–**4m**) demonstrated similar hCA II inhibition properties. Also, the results obtained from this study showed that butenoic acid derivatives (**4a**–**4m**) had generally higher affinity toward hCA II than that of hCA I isoform. Also, AZA, which may interact with the distinct hydrophobic and hydrophilic halves of the CA II active site, showed K_i value of 5.85 ± 2.56 nM.

Conclusion

Although (*Z*)-4-oxo-4-(aryl)but-2-enoic acid molecules have very simple skeletons, they are very active biological compounds, so their monomer property is used in polymer chemistry. Therefore, the synthesis of these molecules is an important issue for researches. (*Z*)-4-Oxo-4-(aryl)but-2-enoic acid (**4**) derivatives used in this study influence the activity of CA enzymes due to the presence of different functional groups (–Et, –Me, –COOH, $–SO_2NH_2$, –CN, $–NO_2$, and $–NH_2$) in their aromatic scaffold. These findings signify that substituted but-2-enoic acid derivatives may be used as leads for generating potent CAIs. In this study, a dozen of butenoic acid derivatives (**4a**–**4m**) were evaluated against cytosolic human carbonic anhydrase isoenzyme

I and II. All the butenoic acid derivatives (**4a**–**4m**) have shown low nanomolar inhibition against cytosolic CA I and II. Both isoenzymes were potently inhibited by butenoic acid derivatives (**4a**–**4m**) with K_is in the range of 22.15 ± 7.76–41.79 ± 13.19 nM against hCA I, and 20.91 ± 3.14–29.05 ± 5.21 nM against hCA II.

Declaration of interest

The authors report there is no conflict of interests.

References

1. Boros M, Kosi1 JK, Vamos J, et al. Methods for syntheses of *N*-methyl-*DL*-aspartic acid derivatives. Amino Acids 2007;33:709–17.
2. Faturacı Y, Coskun N. Substituent effects on the regioselectivity of maleamic acid formation and hydrogen chloride addition to N-aryl maleimides. Turk J Chem 2012;36:749–58.
3. Saedi H. Solvent-free preparation of N-substituted maleanilic acid. Bull Chem Soc Eth 2013;27:137–41.
4. Khattab MK, Ragab F, Galal SA, El Diwani HI. Synthesis of 4-(1H-benzo[d]imidazol-2-yl)aniline derivatives of expected anti-HCV activity. Int J Res Pharm Chem 2012;2:937–46.
5. Kumar PP, Devi BR, Dubey PK. A facile and green synthesis of *N*-substituted imides. Ind J Chem 2013;62:1166–71.
6. Li K, Yuan C, Zhang S, Fang Q. A facile and economical procedure for the synthesis of maleimide derivatives using an acidic ionic liquid as a catalyst. Tetrahedron Lett 2012;53:4245–7.
7. El-Gaby MSA, Gaber AM, Atalla AA, Abd Al-Wahab KA. Novel synthesis and antifungal activity of pyrrole and pyrrolo[2,3-d]pyrimidine derivatives containing sulfonamido moieties. II Farmaco 2002;57:613–17.
8. Isobe Y, Onimura K, Tsutsumi H, Oishi T. Asymmetric polymerization of *n*-1-naphthylmaleimide with chiral anionic initiator: preparation of highly optically active poly(*N*-1-naphthylmaleimide). Macromolecules 2001;34:7617–23.
9. Kaur A, Singh B, Singh Jaggi AS. Synthesis and evaluation of novel 2,3,5-triaryl-4*H*,2,3,3a,5,6,6a-hexahydropyrrolo[3,4-*d*]isoxazole-4,6-diones for advanced glycation end product formation inhibitory activity. Bioorg Med Chem Lett 2013;23:797–801.
10. Ol'shevskaya A, Luzgina VN, Kurakina YA, et al. Synthesis and antitumor properties of carborane conjugates of 5-(4-aminophenyl)-10,15,20-triphenylporphyrin. Doklady Chem 2012;443:91–6.
11. Haval KP, Mhaske SB, Argade NP. Cyanuric chloride: decent dehydrating agent for an exclusive and efficient synthesis of kinetically controlled isomaleimides. Tetrahedron, 2006;62:937–42.
12. Mohammed IA, Mustapha A. Synthesis of new azo compounds based on *N*-(4-hydroxypheneyl)maleimide and *N*-(4-methylpheneyl)maleimide. Molecules 2010;15:7498–508.
13. Molla MR, Ghosh S. Exploring versatile sulfhydryl chemistry in the chain end of a synthetic polylactide. Macromolecules 2012;45:8561–70.
14. Gülçin İ, Beydemir Ş, Büyükokuroğlu ME. In vitro and in vivo effects of dantrolene on carbonic anhydrase enzyme activities. Biol Pharm Bull 2004;27:613–16.
15. Beydemir Ş, Gülçin İ. Effect of melatonin on carbonic anhydrase from human erythrocyte in vitro and from rat erythrocyte in vivo. J Enzyme Inhib Med Chem 2004;19:193–7.
16. ArasHisar Ş, Hisar O, Beydemir Ş, et al. Effect of vitamin E on carbonic anhydrase enzyme activity in rainbow trout (*Oncorhynchus mykiss*) erythrocytes in vitro and in vivo. Acta Vet Hung 2004;52:413–22.
17. Supuran CT. Carbonic anhydrases: from biomedical applications of the inhibitors and activators to biotechnological use for CO_2 capture. J Enzyme Inhib Med Chem 2013;28:229–30.
18. Carta F, Di Cesare Mannelli L, Pinard M, et al. A class of sulfonamide carbonic anhydrase inhibitors with neuropathic pain modulating effects. Bioorg Med Chem 2015;23:1828–40.
19. Hisar O, Beydemir Ş, Gülçin İ, et al. Effect of low molecular weight plasma inhibitors of rainbow trout (*Oncorhyncytes mykiss*) on human erythrocytes carbonic anhydrase-II isozyme activity in vitro and rat erythrocytes in vivo. J Enzyme Inhib Med Chem 2005;20:35–9.
20. Hisar O, Beydemir Ş, Gülçin İ, et al. The effect of melatonin hormone on carbonic anhydrase enzyme activity in rainbow trout (*Oncorhynchus mykiss*) erythrocytes in vitro and in vivo. Turk J Vet Anim Sci 2005;29:841–5.
21. Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 2008;7:168–81.
22. Innocenti A, Öztürk Sarıkaya SB, Gülçin İ, Supuran CT. Carbonic anhydrase inhibitors. Inhibition of mammalian isoforms I-XIV with a series of natural product polyphenols and phenolic acids. Bioorg Med Chem 2010;18:2159–64.
23. Innocenti A, Gülçin İ, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors. Antioxidant polyphenol natural products effectively inhibit mammalian isoforms I-XV. Bioorg Med Chem Lett 2010;20:5050–3.
24. Gülçin İ, Beydemir S. Phenolic compounds as antioxidants: Carbonic anhydrase isoenzymes inhibitors. Mini Rev Med Chem 2013;13:408–30.
25. Polat Kose L, Gülçin İ, Özdemir H, et al. The effects of some avermectins on bovine carbonic anhydrase enzyme. J Enzyme Inhib Med Chem 2015. [Epub ahead of print]. http://dx.doi.org/10.3109/14756366.2015.1064406.
26. Taslimi P, Gülçin İ, Öztaşkın N, et al. The effects of some bromophenol derivatives on human carbonic anhydrase isoenzymes. J Enzyme Inhib Med Chem 2015. [Epub ahead of print]. http://dx.doi.org/10.3109/14756366.2015.1054820.
27. Taslimi P, Gulcin İ, Ozgeris B, et al. The human carbonic anhydrase isoenzymes I and II (hCA I and II) inhibition effects of trimethoxyindane derivatives. J Enzyme Inhib Med Chem 2015. [Epub ahead of print]. doi:10.3109/14756366.2015.1014476.
28. Gocer H, Aslan A, Gülçin İ, Supuran CT. Spirobisnaphthalenes effectively inhibit carbonic anhydrase. J Enzyme Inhib Med Chem 2015. [Epub ahead of print]. http://dx.doi.org/10.3109/14756366.2015.1047359.
29. Gocer H, Topal F, Topal M, et al. Acetylcholinesterase and carbonic anhydrase isoenzymes I and II inhibition profiles of taxifolin. J Enzyme Inhib Med Chem 2015. [Epub ahead of print]. http://dx.doi.org/10.3109/14756366.2015.1036051.
30. Scozzafava A, Kalın P, Supuran CT, et al. The impact of hydroquinone on acetylcholine esterase and certain human carbonic anhydrase isoenzymes (hCA I, II, IX, and XII). J Enzyme Inhib Med Chem 2015. [Epub ahead of print]. http://dx.doi: 10.3109/14756366.2014.999236.
31. Scozzafava A, Passaponti M, Supuran CT, Gülçin İ. Carbonic anhydrase inhibitors: guaiacol and catechol derivatives effectively inhibit certain human carbonic anhydrase isoenzymes (hCA I, II, IX, and XII). J Enzyme Inhib Med Chem 2015;30:586–91.
32. Gül HI, Kucukoglu K, Yamali C, et al. Synthesis of 4-(2-substitutedhydrazinyl)benzenesulfonamides and their carbonic anhydrase inhibitory effects. J Enzyme Inhib Med Chem 2015. [Epub ahead of print]. http://dx.doi.org/10.3109/14756366.2015.1047359.
33. Alterio V, Di Fiore A, D'Ambrosio K, et al. Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms. Chem Rev 2012;112:4421–68.
34. Ceruso M, Vullo D, Scozzafava A, Supuran CT. Sulfonamides incorporating fluorine and 1,3,5-triazine moieties are effective inhibitors of three β-class carbonic anhydrases from *Mycobacterium tuberculosis*. J Enzyme Inhib Med Chem 2014;29:686–9.
35. Akıncıoğlu A, Akıncıoğlu H, Gülçin I, et al. Discovery of potent carbonic anhydrase and acetylcholine esterase inhibitors: novel sulfamoylcarbamates and sulfamides derived from acetophenones. Bioorg Med Chem 2015;23:3592–602.
36. Supuran CT, Scozzafava A. Applications of carbonic anhydrase inhibitors and activators in therapy. Expert Opin Ther Pat 2002;12:217–42.
37. Bozdag M, Carta F, Vullo D, et al. Synthesis of a new series of dithiocarbamates with effective human carbonic anhydrase inhibitory activity and antiglaucoma action. Bioorg Med Chem 2015;23:2368–76.
38. Yıldırım A, Atmaca U, Keskin A, et al. N-Acylsulfonamides strongly inhibit human carbonic anhydrase isoenzymes I and II. Bioorg Med Chem 2015;23:2598–605.
39. Boztaş M, Çetinkaya Y, Topal M, et al. Synthesis and carbonic anhydrase isoenzymes I, II, IX, and XII inhibitory effects of dimethoxy-bromophenol derivatives incorporating cyclopropane moieties. J Med Chem 2015;58:640–50.
40. Akbaba Y, Bastem E, Topal F, et al. Synthesis and carbonic anhydrase inhibitory effects of novel sulfamides derived from 1-aminoindanes and anilines. Arch Pharm 2014;347:950–7.

41. Göksu S, Naderi A, Akbaba Y, et al. Carbonic anhydrase inhibitory properties of novel benzylsulfamides using molecular modeling and experimental studies. Bioorg Chem 2014;56:75–82.
42. Supuran CT. Carbonic anhydrases as drug targets. Curr Pharm Des 2008;14:601–2.
43. Supuran CT. Structure-based drug discovery of carbonic anhydrase inhibitors. J Enzyme Inhib Med Chem 2012;27:759–72.
44. Supuran CT. Carbonic anhydrase inhibitors: an editorial. Expert Opin Ther Pat 2013;23:677–9.
45. Harju AK, Bootorabi F, Kuuslahti M, et al. Carbonic anhydrase III: a neglected isozyme is stepping into the limelight. J Enzyme Inhib Med Chem 2013;28:231–9.
46. Del Prete S, De Luca V, Vullo D, et al. Biochemical characterization of the γ-carbonic anhydrase from the oral pathogen *Porphyromonas gingivalis*, PgiCA. J Enzyme Inhib Med Chem 2014;29:532–7.
47. Carta F, Osman SM, Vullo D, et al. Poly(amidoamine) dendrimers with carbonic anhydrase inhibitory activity and antiglaucoma action. J Med Chem 2015;58:4039–45.
48. Arabaci B, Gülçin İ, Alwasel S. Capsaicin: a potent inhibitor of carbonic anhydrase isoenzymes. Molecules 2015;19:10103–14.
49. Göçer H, Akıncıoğlu A, Göksu S, et al. Carbonic anhydrase and acetylcholine esterase inhibitory effects of carbamates and sulfamoylcarbamates. J Enzyme Inhib Med Chem 2015;30:316–20.
50. Güney M, Coşkun A, Topal F, et al. Oxidation of cyanobenzocycloheptatrienes: synthesis, photooxygenation reaction and carbonic anhydrase isoenzymes inhibition properties of some new benzotropone derivatives. Bioorg Med Chem 2014;22:3537–43.
51. Topal M, Gülçin İ. Rosmarinic acid: a potent carbonic anhydrase isoenzymes inhibitor. Turk J Chem 2014;38:894–902.
52. Çetinkaya Y, Göçer H, Gülçin İ, Menzek A. Synthesis and carbonic anhydrase isoenzymes inhibitory effects of brominated diphenylmethanone and its derivatives. Arch Pharm 2014;347:354–9.
53. Neri D, Supuran CT. Interfering with pH regulation in tumours as a therapeutic strategy. Nat Rev Drug Discov 2011;10:767–77.
54. Pacchiano F, Carta F, McDonald PC, et al. Ureido-substituted benzenesulfonamides potently inhibit carbonic anhydrase IX and show antimetastatic activity in a model of breast cancer metastasis. J Med Chem 2011;54:1896–902.
55. Supuran CT. Structure-based drug discovery of carbonic anhydrase inhibitors. J Enzyme Inhib Med Chem 2012;27:759–72.
56. Akıncıoğlu A, Topal M, Gülçin İ, Göksu S. Novel sulfamides and sulfonamides incorporating tetralin scaffold as carbonic anhydrase and acetylcholine esterase inhibitors. Arch Pharm 2014;347:68–76.
57. Çetinkaya Y, Göçer H, Göksu S, Gülçin İ. Synthesis and carbonic anhydrase isoenzymes inhibitory effects of novel benzylamine derivatives. J Enzyme Inhib Med Chem 2014;29:168–74.
58. Aksu K, Nar M, Tanç M, et al. The synthesis of sulfamide analogues of dopamine related compounds and their carbonic anhydrase inhibitory properties. Bioorg Med Chem 2013;21:2925–31.
59. Akbaba Y, Akıncıoğlu A, Göçer H, et al. Carbonic anhydrase inhibitory properties of novel sulfonamide derivatives of aminoindanes and aminotetralins. J Enzyme Inhib Med Chem 2014;29:35–42.
60. Patil SV, Mahale KA, Gosavi KS, et al. Solvent-mediated one-pot synthesis of cyclic N-substituted imides. Org Prep Proc Int 2013;45:314–20.
61. Samgina TY, Gorshkov VA, Vorontsov EA, et al. New cysteine-modifying reagents: efficiency of derivatization and influence on the signals of the protonated molecules of disulfide-containing peptides in matrix-assisted laser desorption/ionization mass spectrometry. J Anal Chem 2010;65:1320–7.
62. Patel MV, Balasubramaniyan V. Maleamic acids from maleic anhydride and aromatic amines. Ind J Chem 1977;15B:1142–3.
63. Deshpande SR, Maybhate SP, Likhite AP, Chaudhary PM. A facile synthesis of *N*-substituted maleimides. Ind J Chem 2010;49B:487–8.
64. Gaina C, Gaina V. Versatile preparation of ester bismaleimides by dehydrochlorination-condensation reactions. Des Monom Polym 2005;50:655–61.
65. Hiran BL, Paliwal SN, Chaudhary J, Meena S. Industrial chemistry and chemical engineering – preparation, polymerization and characterization of some new maleimides. J Ind Chem Soc 2007;84:385–8.
66. Kumar B, Verma RK, Singh H. Esterification of maleanilic acids: intramolecular esterification through imidate ester. Ind J Chem 1986;25B:692–6.
67. Ravinder V, Rani PU, Balaswamy G. Ind J Heterocyclic Chem 2004;14:73–4.
68. Lindgren AEG, Karlberg T, Ekblad T, et al. Chemical probes to study ADP-ribosylation: synthesis and biochemical evaluation of inhibitors of the human ADP-ribosyltransferase ARTD3/PARP3. J Med Chem 2013;56:9556–68.
69. Janson JC. Protein purification: principles, high resolution methods, and applications. 3rd ed. Hoboken (NJ): John Wiley & Sons, Inc.; 2011.
70. Ahirwar R, Nahar P. Development of an aptamer-affinity chromatography for efficient single step purification of Concanavalin A from *Canavalia ensiformis*. J Chromatogr B 2015;997:105–9.
71. Atasaver A, Özdemir H, Gülçin İ, Küfrevioğlu Öİ. One-step purification of lactoperoxidase from bovine milk by affinity chromatography. Food Chem 2013;136:864–70.
72. Gülçin İ. Antioxidant activity of food constituents – an overview. Arch Toxicol 2012;86:345–91.
73. Çoban TA, Beydemir Ş, Gülçin İ, Ekinci D. Morphine inhibits erythrocyte carbonic anhydrase in vitro and in vivo. Biol Pharm Bull 2007;30:2257–61.
74. Çoban TA, Beydemir Ş, Gülçin İ, Ekinci D. The inhibitory effect of ethanol on carbonic anhydrase isoenzymes: in vivo and in vitro studies. J Enzyme Inhib Med Chem 2008;23:266–70.
75. Coban TA, Beydemir S, Gücin İ, et al. Sildenafil is a strong activator of mammalian carbonic anhydrase isoforms I-XIV. Bioorg Med Chem 2009;17:5791–5.
76. Şentürk M, Gülçin İ, Daştan A, et al. Carbonic anhydrase inhibitors. Inhibition of human erythrocyte isozymes I and II with a series of antioxidant phenols. Bioorg Med Chem 2009;17:3207–11.
77. Öztürk Sarıkaya SB, Gülçin İ, Supuran CT. Carbonic anhydrase inhibitors. Inhibition of human erythrocyte isozymes I and II with a series of phenolic acids. Chem Biol Drug Des 2010;75:515–20.
78. Öztürk Sarıkaya SB, Topal F, Şentürk M, et al. In vitro inhibition of α-carbonic anhydrase isozymes by some phenolic compounds. Bioorg Med Chem Lett 2011;21:4259–62.
79. Şentürk M, Gülçin İ, Beydemir Ş, et al. In vitro inhibition of human carbonic anhydrase I and II isozymes with natural phenolic compounds. Chem Biol Drug Des 2011;77:494–9.
80. Nar M, Çetinkaya Y, Gülçin İ, Menzek A. (3,4-Dihydroxyphenyl)(2,3,4-trihydroxyphenyl)methanone and its derivatives as carbonic anhydrase isoenzymes inhibitors. J Enzyme Inhib Med Chem 2013;28:402–6.
81. Akıncıoğlu A, Akbaba Y, Göçer H, et al. Novel sulfamides as potential carbonic anhydrase isoenzymes inhibitors. Bioorg Med Chem 2013;21:1379–85.
82. Gülçin İ, Beydemir Ş, Hisar O. The effect of α-tocopherol on the antioxidant enzymes activities and lipid peroxidation of rainbow trout (*Oncorhynchus mykiss*). Acta Vet Hung 2005;53:425–33.
83. Köksal E, Gülçin İ. Purification and characterization of peroxidase from cauliflower (*Brassica oleracea* L.) buds. Protein Peptide Lett 2008;15:320–6.
84. Şentürk M, Gülçin İ, Çiftci M, Küfrevioğlu Öİ. Dantrolene inhibits human erythrocyte glutathione reductase. Biol Pharm Bull 2008;31:2036–9.
85. Gülçin İ, Beydemir Ş, Çoban TA, Ekinci D. The inhibitory effect of dantrolene sodium and propofol on 6-phosphogluconate dehydrogenase from rat erythrocyte. Fresen Environ Bull 2008;17:1283–7.
86. Şişecioğlu M, Çankaya M, Gülçin İ, Özdemir M. The inhibitory effect of propofol on lactoperoxidase. Protein Peptide Lett 2009;16:46–9.
87. Şişecioğlu M, Çankaya M, Gülçin İ, Özdemir M. Interactions of melatonin and serotonin to lactoperoxidase enzyme. J Enzyme Inhib Med Chem 2010;25:779–83.
88. Şişecioğlu M, Gülçin İ, Çankaya M, et al. Purification and characterization of peroxidase from Turkish black radish (*Raphanus sativus* L.). J Med Plants Res 2010;4:1187–96.
89. Şişecioğlu M, Kireçci E, Çankaya M, et al. The prohibitive effect of lactoperoxidase system (LPS) on some pathogen fungi and bacteria. Afr J Pharm Pharmacol 2010;4:671–7.
90. Gülçin İ, Küfrevioğlu Öİ, Oktay M. Purification and characterization of polyphenol oxidase from nettle (*Urtica dioica* L.) and inhibition effects of some chemicals on the enzyme activity. J Enzyme Inhib Med Chem 2005;20:297–302.

91. Şişecioğlu M, Gülçin İ, Çankaya M, et al. The effects of norepinephrine on lactoperoxidase enzyme (LPO). Sci Res Essay 2010;5:1351–6.
92. Şişecioğlu M, Uguz MT, Çankaya M, et al. Effects of Ceftazidime pentahydrate, prednisolone, amikacin sulfate, ceftriaxone sodium and teicoplanin on bovine milk lactoperoxidase activity. Int J Pharmacol 2011;7:79–83.
93. Köksal E, Ağgül AG, Bursal E, Gülçin İ. Purification and characterization of peroxidase from sweet gourd (*Cucurbita moschata* Lam. Poiret). Int J Food Propert 2012;15:1110–19.
94. Şişecioğlu M, Gülçin İ, Çankaya M, Özdemir H. The inhibitory effects of L-Adrenaline on lactoperoxidase enzyme (LPO) purified from buffalo milk. Int J Food Propert 2012;15:1182–9.
95. Verpoorte JA, Mehta S, Edsall JT. Esterase activities of human 616 carbonic anhydrases B and C. J Biol Chem 1967;242:4221–9.
96. Bradford MM. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
97. Lineweaver H, Burk D. The determination of enzyme dissociation constants. J Am Chem Soc 1934;56:658–66.
98. Calder JA, Schachter M, Sever PS, Potassium channel opening properties of thiazide diuretics in isolated guinea pig resistance arteries. J Cardiovasc Pharmacol 1994;24:158–64.
99. Pickkers P, Garcha RS, Schachte M, et al. Inhibition of carbonic anhydrase accounts for the direct vascular effects of hydrochlorothiazide. Hypertension 1999;33:1043–8.
100. Coleman JE. Mechanism of action of carbonic anhydrase. J Biol Chem 1967;242:5212–19.
101. Lanir A, Navon G. Interaction of bovine carbonic anhydrase with acetate ions. Biochim Biophys Acta 1974;341:65–74.

Supplementary material available online