Bioorganic & Medicinal Chemistry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Oxidation of cyanobenzocycloheptatrienes: Synthesis, photooxygenation reaction and carbonic anhydrase isoenzymes inhibition properties of some new benzotropone derivatives

Murat Güney [a,b,*], Ahmet Coşkun [c,*], Fevzi Topal [b,d], Arif Daştan [b], İlhami Gülçin [b,e,*], Claudiu T. Supuran [f,*]

[a] Ağri İbrahim Çeçen University, Faculty of Science and Art, Department of Chemistry, 04100 Agri, Turkey
[b] Atatürk University, Faculty of Science, Department of Chemistry, 25240 Erzurum, Turkey
[c] Konya Necmettin Erbakan University, Ahmet Keleşoglu Education Faculty, Department of Chemistry, 42099 Konya, Turkey
[d] Gumushane University, Vocational School of Health Services, Department of Medical Services and Techniques, 29100 Gumushane, Turkey
[e] King Saud University, Department Zoology, College of Science, Saudi Arabia
[f] Università degli Studi di Firenze, Laboratorio di Chimica Bioinorganica, Rm. 188, Via della Lastruccia 3, I-50019 Sesto Fiorentino (Firenze), Italy

ARTICLE INFO

Article history:
Received 4 February 2014
Revised 2 April 2014
Accepted 6 April 2014
Available online xxxx

Keywords:
Benzocycloheptatriene
Benzotropone
Oxidation
Rearrangement
Nitrile-substituted drugs
Carbonic anhydrase inhibition

ABSTRACT

The oxidation of some cyanocycloheptatrienes with CrO_3 and pyridine was investigated and a few new nitrile functionalised benzotropone derivatives were obtained. Photooxygenation reaction of these products was also studied. The structures of the formed products were determined on the basis of NMR spectroscopy and the formation mechanism of unusual products was discussed. Human carbonic anhydrase isoenzymes I, and II (hCA I and hCA II) inhibition properties of nitrile functionalized new benzotropone derivatives were also studied. Both CA isozymes were inhibited in the low micromolar range by these nitrile functionalized benzotropone analogues. The newly synthesized benzotropone derivatives showed inhibition constants in the sub-micromolar range (2.51–4.06 μM). The best hCA I inhibition was observed in 5*H*-benzocycloheptene-7-carbonitrile (K_i: 2.88 ± 0.86 μM). On the other hand, 5-oxo-5*H*-benzocycloheptatriene-7-carbonitrile showed the powerful inhibitory effect against hCA II (K_i: 2.51 ± 0.34 μM).

1. Introduction

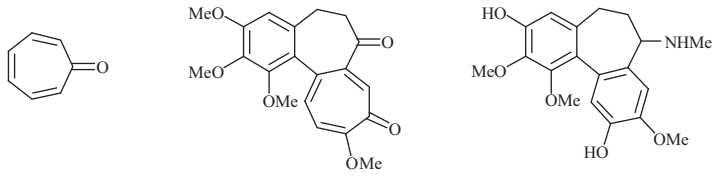
Tropone is a class of compounds of increasing interest because of its aromaticity.[1,2] Another reason for this interest is that tropone derivatives are both pivotal structural elements in biologically active natural products and useful tools in synthetic chemistry.[3–9]

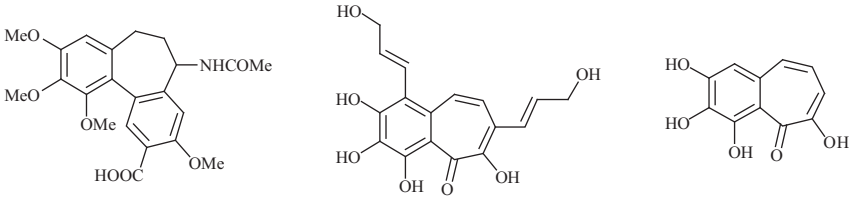
Benzotropones in which the benzo component is annulated to the tropone ring are present in a variety of pharmacologically relevant natural products including colchicone, colchicine, allocolchicine and purpurogalline.[10,11] Colchicine is an alkaloid from *Colchicum autumnale* and *Gloriosa superba* and is used to treat gout and familial Mediterranean fever.[12] Colchicine[13] and its analogues, such as thiocolchicine, thiocolchicone,[14,15] and allocolchicine[16] have been shown to be potent inhibitors of both tubulin polymerization and the growth of human cancer cell lines and they show anti-mitotic activity. Salimine and jerusalemine are natural alkaloids with allocolchicine substructure.[17] Fomentariol, which is a pupurogalline derivative, have been isolated from the tree sponge *Fomes Fomentarius*.[18,19] Purpurogalline represents a biologically relevant natural pigment that is biogenetically produced by oxidation of pyrogallol.[20]

Nitrile (C≡N or CN) functional group has very important role in bioactive molecules. Over 30 nitrile-containing pharmaceuticals are reported as having a diverse variety of medicinal indications. The nitrile functional group often plays a key role as hydrogen bond acceptors. In addition to this, the powerful electron-withdrawing nature of the CN group enables *non*-specific dipole interactions with amino acids and metal ions. In cyanoguanidines, and related structures, CN substitution authorize tuning of the guanidine basicity and hydrogen bonding properties. In addition to different electronic behavior and hydrogen bond capacity, by comparison, the C≡N unit is essentially eight times smaller than a methyl group. Several

* Corresponding authors. Fax: +90 4422360948 (M.G.), +90 3323238225 (A.C.), +90 4422360948 (İ.G.), +39 554573385 (C.T.S.).
E-mail addresses: mguney@atauni.edu.tr (M. Güney), acoskun@konya.edu.tr (A. Coşkun), igulcin@atauni.edu.tr (İ. Gülçin), claudiu.supuran@unifi.it (C.T. Supuran).

http://dx.doi.org/10.1016/j.bmc.2014.04.007
0968-0896/





crystal structures show that the nitrile projecting into narrow clefts to make polar interactions or hydrogen bonds in sterically crowded environments.[21]

Different approaches for the synthesis of benzotropones are present in the literature. These methods are commonly based on ring expansion,[22–25] cyclization,[10,26,27] chemo-enzymatic approach[19,27] and direct oxidation of cycloheptatrienes with metal oxides.[28–30] Bicyclic endoperoxides are also important intermediates for the synthesis of (benzo)troponoid systems.[31–33] For example, in our previous studies,[34] we showed that photooxygenation of benzotropone **1** results in the formation of endoperoxide **2**. Reaction of **2** with thiourea then gave an effective synthesis of benzotropolone **3** in high yields (Scheme 1).

Herein, we wish to report the synthesis of new cyanobenzotropones via oxidation of some cyanobenzocycloheptatrienes and their conversions to unusual endoperoxide rearrangement products. In addition to a potential biological active troponoid unit, a nitrile functionalised target has encouraged us to investigate some biological activity test. For this purpose, firstly we aimed to test hCA I, and hCA II inhibition properties.

Enzymes occur naturally in living organisms that regulate the metabolic activities.[35] Carbonic anhydrase enzymes (CAs, EC 4.2.1.1) are from zinc-containing enzyme family involved in a variety of physiologic and pathologic processes in many types of organisms.[36] They regulate pH in most tissues including erythrocytes. They catalyze the reversible hydration of carbon dioxide (CO_2) in a two-step reaction. As a result of this reaction, bicarbonate (HCO_3^-) and protons (H^+) occur.[37]

$$CO_2 + H_2O \Longleftrightarrow HCO_3^- + H^+ \quad (1)$$

This enzyme plays an important role in different processes like respiration, biosynthetic processes, controlling of physiological pH such as acid–base regulation, gas balance, bone resorption and calcification.[38] Genetically, there are five distinct CA families. Among these, α-CAs are found in cytoplasm of green plants, algae, bacteria, and vertebrates. Another classes of CAs are β-CAs and found in chloroplasts of mono- and di-cotyledons, algae and bacteria. Conversely, the γ-CAs present in Achaea, some bacteria and especially methane-producing bacteria that grow in hot springs. The δ-CAs exists in marine diatoms and ε-CAs are in some bacteria, marine cyanobacteria and chemolithotrophs.[39–41]

α-CAs family present in sixteen isoforms, which differ by molecular features, oligomeric arrangement, cellular localization, distribution in organs and tissues, expression levels, and kinetic properties. There are five cytosolic forms (CA I, II, III, VII, and XIII), five membrane associated isozymes (CA IV, IX, XII, XIV, and XV), two mitochondrial forms (CA VA and VB), and a secreted CA isozyme (CA VI). It is a secretory enzyme that has been initially described in the ovine parotid gland and, saliva and normal human serum.[39–41] There are three additional non-catalytic CA isoforms (CA VIII, X, and XI) whose functions remain unclear.[42] Other CA isoforms are found in a variety of tissues and they participate in several important biological and physiological processes such as CO_2 and ions transport, acid–base balance, respiration, lipogenesis, bone resorption and electrolyte releasing.

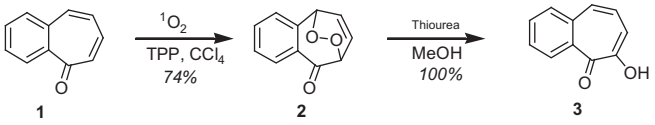
The classical CA isoenzymes inhibitors (CAIs) are the sulfonamides and their isosteres such as sulfamates and sulfamides.[43–48] CAIs are clinically used as diuretic,[49] antiglaucoma,[50] anticonvulsant,[51] and antiobesity drugs[52] and in the management of hypoxic tumors.[53–55] Therefore, the synthesis of new CA inhibitor is considerably important.

In the present study, we realized the oxidation of some cyanobenzocycloheptatrienes. Then we achieved synthesis, photooxygenation reaction of some new benzotropone derivatives. Finally, we have determined CA I, and CA II isoenzyme inhibition properties of these new benzotropone derivatives.

2. Results and discussion

2.1. Synthesis of starting materials

The starting materials 5*H*-benzocycloheptatriene-7-carbonitrile (**7**) and 9*H*-benzocycloheptatriene-6-carbonitrile (**11**) were synthesized in three to four steps starting from 1,4-dihydronapthalene, which was obtained from naphthalene selectively reduced at 1,4 positions,[56] respectively. Addition of dibromocarbene to 1,4-dihydronapthalene (**4**) under phase-transfer conditions afforded **5**[57] and the reaction of **5** with quinoline gave monobromide **6**.[58] Monobromide **6** was refluxed with cuprous cyanide in dimethylformamide and 5*H*-benzocycloheptene-7-carbonitrile (**7**)[59] was obtained. After the addition of dibromocarbene to 1,2-dihydronapthalene (**8**), which was generated by base catalyzed isomerisation of **4**, the resulting compound **9** was heated to 135 °C in the presence of quinoline, producing monobromide **10**.[60] Treatment of monobromide **10** with cuprous cyanide in the presence of DMF



Scheme 1. A new approach for synthesis of benzotropolone **3**.

resulted in the formation of 9*H*-benzocycloheptene-6-carbonitrile (**11**) (Scheme 2).

2.2. Oxidation of cyanobenzocycloheptatrienes and synthesis of benzotropones

From the oxidation of 5*H*-benzocycloheptene-7-carbonitrile (**11**) with chromium trioxide in methylene chloride and pyridine, we obtained some new benzotropone derivatives 9-oxo-9*H*-benzocycloheptene-6-carbonitrile (**12**) in 16% yield, 5-oxo-5*H*-benzocycloheptene-6-carbonitrile (**13**) in 16% yield and 7-oxo-7*H*-benzocycloheptene-6-carbonitrile (**14**) in 19% yield. Isomeric compounds were isolated by column chromatography and crystallization. Characterization of obtained benzotropones was based on the ^{1}H NMR spectral data (Scheme 3).

Similarly oxidation of **7** resulted in the formation of 5-Oxo-5*H*-benzocycloheptene-7-carbonitrile (**15**) in 37% yield (Scheme 4).

2.3. Photooxygenation of benzotroponoides

After the synthesis of benzotropones we concentrated on the synthesis of benzotropolones. For this reason, it is important to obtain endoperoxides, because endoperoxides with seven-membered rings are useful key substrates for (benzo)tropolones. The photooxygenation reaction of **15** gave expected endoperoxide **16** in 14% yield, as well as rearrangement product **17** in 14% yield (Scheme 5).

We observed that during the chromatography endoperoxide **16** decomposed to **17** on silica gel. As described for similar rearrangements in our previous paper,[34] rearrangement product **16** to **17** can be rationalized by the following mechanism; a homolitic cleavage peroxide linkage in **16** to diradical **18** followed by C–C cleavage to give diradical **19**. This diradical was then converted into **17** under the reaction conditions (Scheme 6).

All attempts at photooxygenation of the parent compound **7** failed (Scheme 7). Initially, this observation was of great surprise to us because compound **7** is more suitable for reaction than ketone derivative **15**, which has an electronically poor tropone ring. It was assumed however that rather than the electronic factor, the conformation of the seven-membered ring determined the outcome of the reaction. Diene system of **15** is planar due to the fully conjugated π electrons in the seven-membered rings but the system of **7** is not planar due to the sp^3 hybridized carbon atom in the seven-membered rings shown below in Figure 1.

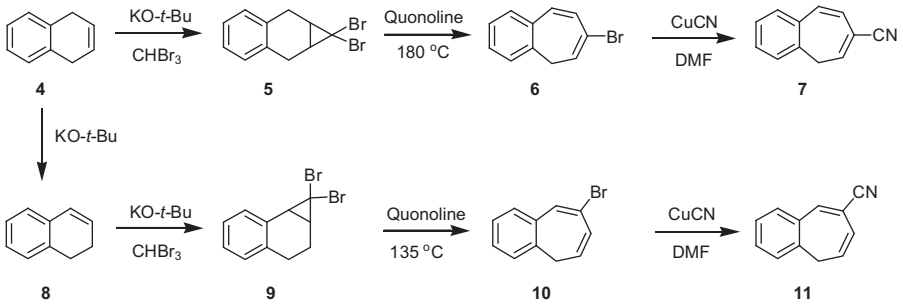
2.4. Reactions of endoperoxide 16

Endoperoxides have several applications in synthetic and mechanistic organic chemistry. Endoperoxides derived from cycloheptadiene have the potential to yield tropones and tropolones. To synthesize some benzotropone derivatives, we investigated endoperoxide **16** in more detail. Thermolysis, Co-TPP and NEt_3 reactions of endoperoxide **16** resulted in the formation of complex products but treatment of endoperoxide **16** with thiourea in MeOH at room temperature, resulted in the two isomers **21** and **22**, obtained in 14% and 12% yield, respectively. It was established that the reaction of endoperoxide generated (benzo)cycloheptatriene derivatives with thiourea results in the formation of *cis* diol or (benzo)tropone and (benzo)tropolone derivatives or unusual rearrangement products[34,61–63] As described in our previous study on other benzotropone derivatives,[34] in this reaction, thiourea catalyzed the cleavage of O—O bond, which lead to the corresponding alkoxy diketone **23**, after which the oxygen anion attacks the carbonyl group to form a cyclic ether **24**.[64–66] The benzilic acid type rearrangement of **24** gave lactone **25**. The nucleophilic addition of MeOH to the double bond in **25** yielded isomeric methoxy derivatives **21** and **22** (Scheme 8). The structures of **21** and **22** were determined based on ^{1}H NMR. The coupling constant between H_{11} and H_{12} was found to be 2.6 Hz for **21**, whereas for **22** it was found as 8.8 Hz. The large coupling constant showed that methoxy and cyanide was to be *cis*-orientation. Additionally, the *exo*-orientation of the MeO substituent and carbonyloxy branch was also confirmed by the observed small coupling between H-C8 and H-C12 ($^3J_{8,12}$: 1.8, 1.6 Hz, resp.).

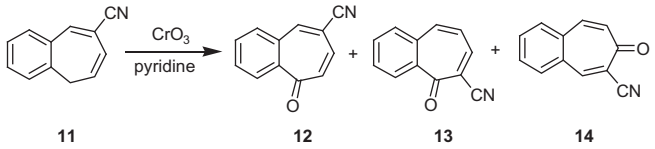
In summary, the synthesis of 2,3-benzotropone and 4,5-benzotropone derivatives by oxidation of cyanobenzocycloheptatrienes has been reported. The Photooxygenation reactions afforded only one endoperoxide, which with thiourea then formed rearrangement products instead of the expected benzotroponoid compounds.

2.5. Biochemistry

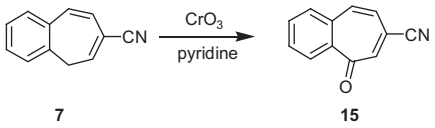
The new nitrile functionalized benzotropone analogues were reported here and the standard drug acetazolamide were assayed



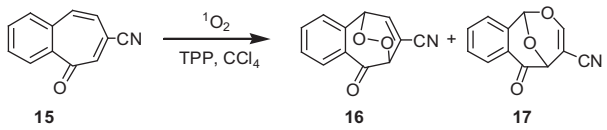
Scheme 2. Synthesis of starting material **7** and **11**.



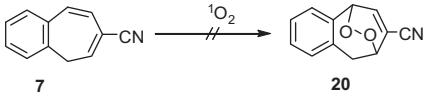
Scheme 3. Oxidation of **11** with CrO_3 and pyridine.



Scheme 4. Synthesis of benzotropone **15**.



Scheme 5. Photooxygenation of benzotropone **15**.



Scheme 7. Photooxygenation of starting material **7**.

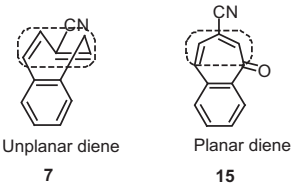
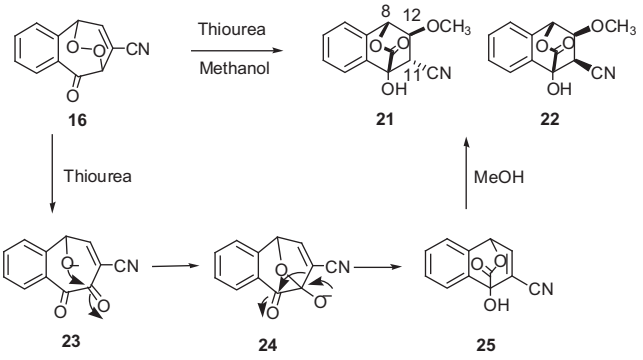


Figure 1. Planarity of dienes **7** and **15**.



Scheme 8. The reaction of endoperoxide **16** with thiourea and formation of **21** and **22**.

as inhibitors of two cytosolic human isoenzymes, hCA I, and II (Table 1). As it can be seen from the data presented in Table 1, these benzotropone derivatives demonstrated effective inhibitory activity against both tested isoforms. When examining the results, the following structure activity relationship could be easily observed.

The cytosolic isoenzyme hCA I was inhibited with inhibition constants in the micromolar range by the all of the newly synthesized benzotropone derivatives (**7**, **11**–**15**), which demonstrated IC_{50} values of 3.22–7.07 μM and K_i values of 2.68 ± 0.38–4.06 ± 0.61 μM for both CA isoenzymes (Table 1). Compound **11**, possessing no carbonyl group at the part of the molecule, was the best hCA I inhibitor (K_i: 0.16 μM). However the average K_i values of new synthesized six benzotropone derivatives (**7**, **11**–**15**) was found as 4.06 ± 0.61 μM for hCA I. These results showed that the position of the nitrile group in cycloheptatriene ring is no altering of the consequences of inhibition.

Conversely, the best hCA II inhibitor in this series was compound **15** and it showed the highest inhibition activity on physiologically dominant CA II with K_i values of 2.51 ± 0.34 μM. Many studies performed on sulfonamides revealed that inhibition of CA II is brought about by their ability to mimic the tetrahedral transition state when binding to catalytic zinc ions located at the active site of the enzymes.[40,67–70] Based on these results, as it can be seen in Figure 2, we recommended a binding model between compound **15**, which is the most powerful new benzotropone derivative, and the enzyme's active site. It was reported that the compounds possessing a group in the *meta*-position have a good inhibitory activity.[71]

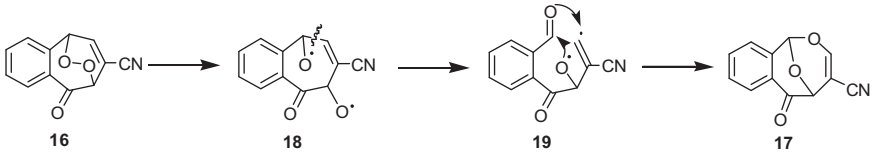
The new synthesized benzotropone **15** had carbonyl and nitrile groups in the *meta*-position. However, there are no important differences of inhibition activity between these compounds possessing anyhow K_i values ranging of 2.51 ± 0.34–3.91 ± 0.74 μM (Table 1). Indeed, K_i values of new synthesized six benzotropone derivatives (**7**, **11**–**15**) were much more effective compared to acetazolamide (K_i values are 36.20 μM for hCA I and 3.70 μM for hCA II), which is a clinically used drug.[44,72]

3. Experimental

General: Melting points were uncorrected. Infrared spectra were obtained from solution in 0.1 mm cells or KBr pellets on a regular instrument. The ^1H and ^{13}C NMR spectra were recorded on 400 (100) and 200 (50) MHz spectrometers. Apparent splitting was given in all cases. Column chromatography was performed on silica gel (60-mesh, Merck). TLC was carried out on Merck 0.2 mm silica gel 60 F254 analytical aluminum plates. All substances reported in this paper are *meso*-compounds or racemates.

3.1. Synthesis of 9*H*-Benzocycloheptene-6-carbonitrile (11)

A mixture of 1.50 g (6.79 mmol) 8-bromo-5*H*-benzosikloheptene (**10**), 7.2 g (80.40 mmol) CuCN and DMF that is freshly distilled (40 ml) was heated at 160 °C for 16 h, cooled, and filtered to remove copper salts. The residue was extracted with benzene. The organic layer was washed with 200 ml of 10% $FeCl_3$ and NaOH solutions and dried over $MgSO_4$. After the solvent was removed, the residue was purified on silica gel column eluted with EtOAc/*n*-hexane to give 350 mg (31%) of 9*H*-benzocycloheptene-6-carbonitrile (**11**). White crystals from CH_2Cl_2/*n*-hexane (2:1), mp: 48–51 °C.^1H NMR (400 MHz, $CDCl_3$): δ 7.70 (s, 1H), 7.49 (t, J = 7.1 Hz, 1H), 7.37 (d, J = 7.1 Hz, 1H), 7.32 (t, J = 7.1 Hz, 1H), 7.22 (d, J = 7.1 Hz, 1H), 6.08 (d, J = 11.9 Hz, 1H), 5.93 (dt, J = 11.9 Hz, J = 5.3 Hz, 1H), 3.07 (d, J = 5.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 145.40, 137.43, 133.90, 132.12, 130.89, 129.47, 128.42, 126.60, 123.71, 120.14, 112.89, 34.12. IR (KBr, cm^{-1}): 3037, 2964, 2886, 2835, 2214,



Scheme 6. The formation of rearrangement product **17**.

Table 1
Human carbonic anhydrase isoenzymes (hCA I and hCA II) inhibition value of some new benzotropone derivatives by an esterase bioassay with NPA as substrate

Compounds	IC_{50} (μM)		K_i (μM)	
	hCA I	hCA II	hCA I	hCA II
7	4.52	4.38	4.06 ± 0.61	3.23 ± 0.23
11	5.77	4.07	2.88 ± 0.86	3.06 ± 0.15
12	5.96	3.82	3.93 ± 0.81	3.91 ± 0.74
13	7.07	3.64	4.01 ± 0.62	2.68 ± 0.38
14	5.25	3.22	3.66 ± 0.61	3.31 ± 0.72
15	5.13	3.74	3.62 ± 0.95	2.51 ± 0.34

1597, 1556, 1445, 1324, 1206, 1133, 1010, 902. Anal. Calcd for $C_{12}H_9N$: C, 86.20; H, 5.43. Found: C, 86.10; H, 5.61.

3.2. Oxidation of 9*H*-benzocycloheptene-6-carbonitrile (11) with CrO_3

A solution of 2.06 g (20.6 mmol) CrO_3 in pyridine/CH_2Cl_2 1:1 (48 ml) was cooled to 0 °C. Then 575 mg (3.44 mmol) 9*H*-benzocycloheptene-6-carbonitrile (**11**) in CH_2Cl_2 (15 ml) was added dropwise. The mixture was stirred at 0 °C for 2 h and rt for 24 h, and the solvent was evaporated. The residue was filtered over silica gel (50 g) with ethylacetate/*n*-hexane (5:95 and 50:50). The firs fraction was 9-oxo-9*H*-benzocycloheptene-6-carbonitrile (**12**) (100 mg, 16%). White crystals from CH_2Cl_2/*n*-hexane (2:1), mp: 175–176 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.49–8.46 (m, 1H, H_{aryl}), 7.85–7.73 (m, 4H, H_{aryl} and H_5), 7.05 (dd, A part of AB system, $J_{7,8}$ = 12.4 Hz, $J_{5,7}$: 1.1 Hz, 1H, H_7), 6.96 (d, B part of AB system, $J_{7,8}$ = 12.4 Hz, 1H, H_8). ^{13}C NMR (100 MHz, $CDCl_3$): δ 186.81, 148.43, 140.03, 137.33, 135.34, 133.83, 133.39, 133.19, 132.61, 131.60, 119.56, 111.94, 96.35. IR (KBr, cm^{-1}): 3436, 2215, 1639, 1616, 1584, 1549, 1450, 1402, 1332, 1246, 1195, 1092, 933. Anal. Calcd for $C_{12}H_7NO$: C, 79.55; H, 3.89. Found: C, 79.78; H, 3.92. In the second fraction, 5-oxo-5*H*-benzocycloheptene-6-carbonitrile (**13**) and 9-oxo-9*H*-benzocycloheptene-6-carbonitrile (**12**) (in a 90:10 ratio) were obtained as a mixture (100 mg, 16%). Compound **13** was not isolated as pure state, although NMR signals were chosen from the mixture. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58–8.55 (m, 1H), 7.84–7.59 (m, 5H), 6.81–6.75 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 183.56, 148.49, 145.70, 144.38, 135.38, 135.13, 133.87, 133.43, 132.78, 131.85, 124.70, 117.71. The third fraction was 7-oxo-7*H*-benzocycloheptene-6-carbonitrile (**14**) (120 mg, 19%). White crystals from CH_2Cl_2/*n*-hexane (2:1), mp: 213–214 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (s, 1H), 7.81–7.69 (m, 4H), 7.52 (d, A part of AB system, $J_{8,9}$ = 12.4 Hz, 1H), 6.89 (d, B part of AB system, $J_{8,9}$ = 12.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.70, 150.63, 142.01, 137.15, 136.22, 134.79, 134.34, 133.87, 133.64, 131.61, 120.61, 117.39. IR (KBr, cm^{-1}): 3436, 2220, 1632, 1591, 1553, 1402, 1341, 1305, 1212, 1092, 882. Anal. Calcd for $C_{12}H_7NO$: C, 79.55; H, 3.89. Found: C, 79.57; H, 3.98.

3.3. Oxidation of 5*H*-benzocycloheptene-7-carbonitrile (7) with CrO_3

The reaction was carried out according to the above-mentioned procedure using 4.86 g (48.6 mmol) CrO_3, 1.25 g (7.5 mmol) of **7** and pyridine/CH_2Cl_2 1:1 (36 ml). The product was purified by a column on silica gel (30 g) with ethylacetate/*n*-hexane (5:95). 5-Oxo-5*H*-benzocycloheptene-7-carbonitrile (**15**) was obtained as a sole product (500 mg, 37%). Yellowish crystals from CH_2Cl_2/*n*-hexane (2:1), mp: 92–93 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.39 (m, 1H), 7,74 (m, 3H), 7.39 (d, A part of AB system, $J_{8,9}$ = 12.0 Hz), 7.27 (m, 1H), 6.68 (dd, B part of AB system, $J_{8,9}$ = 12.0, $J_{6,8}$ = 1.6 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 188.07, 144.27, 142.79, 141.06, 136.59, 136.39, 135.49, 134.05, 133.00, 126.41, 122.54, 120.56. IR (KBr, cm^{-1}): 2919, 2217, 1726, 1639, 1586, 1450, 1333, 1195, 844, 772. Anal. Calcd for $C_{12}H_7NO$: C, 79.55; H, 3.89. Found: C, 79.42; H, 3.75.

3.4. Photooxygenation of 15

Tropone derivative **15** 0.88 g (4.84 mmol) and tetraphenylporphyrin (5 mg, 0.008 mmol) were dissolved in 40 mL of CCl_4. The solution was then irradiated with a projection lamp (500 W) while a slow stream of dry oxygen was passed through the solution at rt. After 20 h, the solvent was evaporated at 20 °C and the crude product was crystallized from CH_2Cl_2/*n*-hexane (3:1) to give endoperoxide **16** (125 mg, 14%). Yellow crystals from CH_2Cl_2/*n*-hexane (2:1), mp 130–131 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.15 (d, *J* = 7.2 Hz, 1H, H_{aryl}), 7.84 (d, *J* = 7.3, A part of AX system), 7.65–7.58 (m, 2H), 7.36 (d, *J* = 7.2 Hz, 1H), 5.66 (d, *J* = 7.3 Hz, X part of AX system), 5.34 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 189.70, 151.32, 137.60, 134.88, 131.55, 131.37, 131.13, 128.98, 114.01, 110.78, 84.85, 81.99. IR (KBr, cm^{-1}): 3409, 2938, 2248, 1769, 1465, 1359, 1295, 1267, 1213, 1141, 991, 939. Anal. Calcd for $C_{12}H_7NO_3$: C, 67.61; H, 3.31. Found: C, 68.15; H, 3.39. Afterwards, the residue chromographed on silica gel (30 g) using EtOAc/*n*-hexane (97:3). Pure 8-oxo-12,13-dioxa-tricyclo[7.3.1.0^{2,7}]trideca-2,4,6,10-tetraene-10-carbonitrile (**17**) (125 mg, 14%) which was crystallized from CH_2Cl_2/*n*-hexane (1:1) was obtained. White crystals mp 164–165 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (d, *J* = 7.7, 1H), 7.74 (m, 1H), 7.62 (m, 1H), 7.47 (d, *J* = 7.7 Hz, 1H), 7.22 (s, 1H), 6.43 (s, 1H), 4.89 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 189.00, 154.35, 135.73, 135.56, 131.35, 127.13, 126.88, 126.11, 114.53,

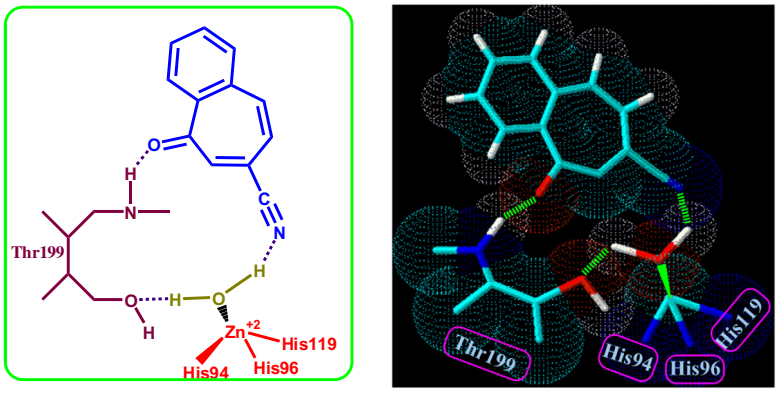


Figure 2. The purposed interaction between new benzotropone derivative (**15**) and active site region in the hCA II.

94.07, 87.03, 71.58. IR (KBr, cm^{-1}): 3103, 2227, 1712, 1635, 1610, 1378, 1301, 1249, 1223, 1198, 1172, 966. Anal. Calcd for $C_{12}H_7NO_3$: C, 67.61; H, 3.31. Found: C, 67.61; H, 3.25.

3.5. The reaction of endoperoxide 16 with thiourea

To a magnetically stirred solution of **16** (285 mg, 1.33 mmol) in MeOH at 0 °C, a solution of thiourea (101 mg, 1.33 mmol) in MeOH (5 ml) was added dropwise over a period 10 min. The mixture was stirred in an ace bath for 30 min and at rt for 3 h. The solids were removed by filtration and the solvent was evaporated. The residue was filtered through silica gel (30 g) with EtOAc/*n*-hexane (5:95, 40:60). The first fraction was *trans*-1-hydroxy-12-methoxy-10-oxo-9-oxa-tricyclo[6.2.2.0^{2,7}]dodeca-2,4,6-triene-11-carbonitrile (**21**) (40 mg, 12%). Yellowish crystals from CH_2Cl_2/*n*-hexane (2:1), mp: 175–176 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, J = 7.3 Hz, 1H), 7.59–7.41 (m, 3H), 5.74 (d, J = 1.8 Hz, 1H), 3.88 (dd, J = 2.6 Hz, J = 1.8 Hz, 1H), 3.54 (s, 3H, OCH_3), 3.07 (d, J = 2.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.32, 134.70, 131.16, 130.88, 129.56, 124.67, 123.86, 116.55, 82.19, 77.97, 74.21, 57.79, 40.16. IR (KBr, cm^{-1}): 3409, 2938, 2248, 1769, 1465, 1359, 1267, 1142, 1102, 991, 940. Anal. Calcd for $C_{13}H_{11}NO_4$: C, 63.67; H, 4.52. Found: C, 63.47; H, 4.41. The second fraction was *cis*-1-hydroxy-12-methoxy-10-oxo-9-oxa-tricyclo[6.2.2.0^{2,7}]dodeca-2,4,6-triene-11-carbonitrile (**22**) (40 mg, 12%). White crystals from methanol, mp: 212–213 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.58 (d, J = 7.3 Hz, 1H), 7.52–7.42 (m, 3H), 5.90 (d, J = 1.6 Hz, 1H), 3.93 (dd, J = 8.8 Hz, J = 1.6 Hz, 1H), 3.63 (d, J = 8.8 Hz, 1H), 3.59 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.59, 137.45, 132.53, 130.00, 128.81, 124.62, 121.72, 114.96, 77.54, 76.32, 74.76, 57.25, 39.55. IR (KBr, cm^{-1}): 3420, 2941, 2251, 1766, 1464, 1356, 1210, 1110, 990, 921. Anal. Calcd for $C_{13}H_{11}NO_4$: C, 63.67; H, 4.52. Found: C, 63.32; H, 4.28.

3.6. Biochemistry

The both CA isoenzymes were purified by Sepharose-4B-L tyrosine-sulfanilamide affinity chromatography.[37,38,73] Sepharose-4B-L tyrosine-sulfanilamide affinity gel was prepared in accordance with the published method.[74] For this purpose, the pH of the homogenate was adjusted to 8.7 using solid Tris. Then, an aliquot of the supernatant (50 mL) was applied to an affinity column. The protein concentrations in the column effluents were determined spectrophotometrically at 280 nm. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed after purification of the enzymes. The isoenzymes purities were controlled by SDS–PAGE and a single band was observed for each CA isoenzymes.[75] This method was described previously.[76] It was carried out in 10% and 3% acrylamide for the running and the stacking gel, respectively, containing 0.1% SDS.[77–79]

The both CA isoenzymes activities were determined in accordance with the method of Verpoorte et al.[80] described previously.[81,82] The change in absorbance at 348 nm of p-nitrophenylacetate (NPA) to *p*-nitrophenolate (NP) was recorded during 3 min at 25 °C using a spectrophotometer (Shimadzu, UV–vis Spectrophotometer, UVmini-1240). The quantity of protein was spectrophotometrically determined at 595 nm during the purification steps in accordance with the Bradford method.[83] Bovine serum albumin was used as the standard protein.[84–86] For the determination of inhibition effect of each newly-synthesised new benzotropone derivative, an Activity (%)-[Benzotropone] graph was drawn. To determine K_i values, three different inhibitor concentrations were tested. In these experiments, NPA was used as the substrate at five different concentrations and Lineweaver–Burk curves were drawn[87] in detail described previously.[88–91]

Acknowledgements

The authors are indebted to TÜBİTAK (Project number 104T401 and TUBİTAK-BAYG National Postdoctoral Fellowship Program for A.C.), and Atatürk University for financial supports. We would also like thank to Prof. Dr. Cavit Kazaz, Res. Assistant Barış Anıl, Dr. Murat Acar, Res. Assistant Ufuk Atmaca for NMR experiments, mass spectra and elemental analyses.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.04.007.

References and notes

1. Dewar, M. J. S. *Nature* **1945**, *155*, 50.
2. Ohkita, M.; Sano, K.; Suzuki, T.; Tsuji, T. *Tetrahedron Lett.* **2001**, *42*, 7295.
3. Takahashi, S.; Kamiya, T.; Saeki, K.; Nezu, T.; Takeuchi, S.; Takasawa, R.; Sunaga, S.; Yoshimori, A.; Ebizuka, S.; Abe, T.; Tanuma, S. *Bioorg. Med. Chem.* **2010**, *18*, 8112.
4. Pietra, F. *Acc. Chem. Res.* **1979**, *12*, 132.
5. Pietra, F. *Chem. Rev.* **1973**, *73*, 293.
6. Coşkun, A.; Güney, M.; Daştan, A.; Balci, M. *Tetrahedron* **2007**, *63*, 4944.
7. Banwell, M. G. *Aust. J. Chem.* **1991**, *44*, 1.
8. Najda-Bernatowicz, A.; Krawczyk, M.; Stankiewicz-Drogon, A.; Bretner, M.; Boguszewska-Chachulska, A. M. *Bioorg. Med. Chem.* **2010**, *18*, 5129.
9. Li, W.; Li, H.; Li, Z. *Tetrahedron Lett.* **2010**, *51*, 5448.
10. Albrecht, U.; Nguyen, T. H. V.; Langer, P. *J. Org. Chem.* **2004**, *69*, 3417.
11. Gill, M.; Steglich, W. *Prog. Chem. Org. Nat. Prod.* **1987**, *51*, 216.
12. Levy, M.; Spino, M.; Read, S. E. *Pharmacotherapy* **1991**, *11*, 196.
13. Yeh, H. J. C.; Chrzanowska, M.; Brossi, A. *FEBS Lett.* **1988**, *229*, 82.
14. Shi, Q.; Chen, K.; Brossi, A.; Verdier-Pinard, P.; Hamel, E.; McPhail, A. T.; Lee, K. H. *Helv. Chim. Acta* **1998**, *81*, 1023.
15. Shi, Q.; Verdier-Pinard, P.; Brossi, A.; Hamel, E.; McPhail, A. T.; Lee, K. H. *J. Med. Chem.* **1997**, *40*, 961.
16. Boye, O.; Brossi, A. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1983; Vol. 41, p 125.
17. Banwell, M. G.; Fam, M. A.; Gable, R. W.; Hamel, E. *J. Chem. Soc., Chem. Commun.* **1994**, *21*, 2647.
18. Arpin, N.; Fauvre-Bovin, J.; Steglich, W. *Phytochemistry* **1974**, *13*, 1949.
19. Baisch, G.; Wagner, B.; Öhrlein, R. *Tetrahedron* **2010**, *66*, 3742.
20. Dürckheimer, W.; Paulus, E. F. *Angew. Chem.* **1985**, *97*, 219. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 224.
21. Fleming, F. F.; Yao, L. H.; Ravikumar, P. C.; Funk, L.; Shook, B. C. *J. Med. Chem.* **2010**, *53*, 7902.
22. Ranken, P. F.; Harty, B. J.; Kapicak, L.; Battiste, M. A. *Synth. Commun.* **1973**, *3*, 311.
23. Saraf, S. D. *Synthesis* **1971**, *5*, 264.
24. Sato, M.; Tanaka, T.; Tsunetsugu, J.; Ebine, S. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2395.
25. Ohkita, M.; Nishida, S.; Tsuji, T. *J. Am. Chem. Soc.* **1999**, *121*, 4589.
26. Ewing, G. D.; Paquette, L. A. *J. Org. Chem.* **1975**, *40*, 2965.
27. Kerschensteiner, L.; Löbermann, F.; Steglich, W.; Trauner, D. *Tetrahedron* **2011**, *67*, 1536.
28. Srivastava, K. C.; Dev, S. *Tetrahedron* **1972**, *28*, 1083.
29. Dastan, A.; Yıldız, Y. K.; Kazaz, C.; Balci, M. *Turk. J. Chem.* **2002**, *26*, 143.
30. Dastan, A.; Saracoglu, N.; Balci, M. *Eur. J. Org. Chem.* **2001**, 3519.
31. Oda, M.; Kitahara, Y. *Tetrahedron Lett.* **1969**, *38*, 3295.
32. Asao, T.; Yagihara, M.; Kitahara, Y. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 2131.
33. Daştan, A.; Balci, M. *Tetrahedron* **2006**, *62*, 4003.
34. Güney, M.; Daştan, A.; Balci, M. *Helv. Chim. Acta* **2005**, *88*, 830.
35. Shahwar, D.; Raza, M. A.; Khan, T. *Turk. J. Chem.* **2012**, *36*, 257.
36. Güzel-Akdemir, O.; Akdemir, A.; Pan, P.; Vermelho, A. B.; Seppo Parkkila, S.; Scozzafava, A.; Capasso, C.; Supuran, C. T. *J. Med. Chem.* **2013**, *56*, 5773.
37. Gülçin, İ.; Beydemir, Ş.; Büyükokuroğlu, M. E. *Biol. Pharm. Bull.* **2004**, *27*, 613.
38. Beydemir, Ş.; Gülçin, İ. *J. Enzyme Inhib. Med. Chem.* **2004**, *19*, 193.
39. Supuran, C. T. *Future Med. Chem.* **2011**, *3*, 1165.
40. Supuran, C. T. *Nat. Rev. Drug Disc.* **2008**, *7*, 168.
41. Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C. T.; De Simone, G. *Chem. Rev.* **2012**, *112*, 4421.
42. Sethi, K. K.; Verma, S. M.; Tanç, M.; Carta, F.; Supuran, C. T. *Bioorg. Med. Chem.* **2013**, *21*, 5168.
43. Aggarwal, M.; McKenna, R. *Expert Opin. Ther. Pat.* **2012**, *22*, 903.
44. Akıncıoğlu, A.; Akbaba, Y.; Göçer, H.; Göksu, S.; Gülçin, İ.; Supuran, C. T. *Bioorg. Med. Chem.* **2013**, *21*, 379.
45. Akıncıoğlu, A.; Nar, M.; Gülçin, İ.; Göksu, S. *Arch. Pharm.* **2014**, *347*, 68.
46. Göçer, H.; Akıncıoğlu, A.; Öztaşkın, N.; Göksu, S.; Gülçin, İ. *Arch. Pharm.* **2013**, *346*, 783.
47. Aksu, K.; Nar, M.; Tanç, M.; Vullo, D.; Gülçin, İ.; Göksu, S.; Tümer, F.; Supuran, C. T. *Bioorg. Med. Chem.* **2013**, *21*, 2925.

48. Akbaba, Y.; Akıncıoğlu, A.; Göçer, H.; Göksu, S.; Gülçin, İ.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 35.
49. Supuran, C. T. *Curr. Pharm. Des.* **2008**, *14*, 641.
50. Mincione, F.; Scozzafava, A.; Supuran, C. T. In *Drug Design of Zinc-Enzyme Inhibitors: Functional, Structural, and Disease Applications*; Supuran, C. T., Winum, J. Y., Eds.; Wiley: Hoboken, 2009; pp 139–154.
51. Thiry, A.; Dogne, J. M.; Masereel, B.; Supuran, C. T. *Curr. Top. Med. Chem.* **2007**, *7*, 855.
52. De Simone, G.; Di Fiore, A.; Supuran, C. T. *Curr. Pharm. Des.* **2008**, *14*, 655.
53. Dubois, L.; Lieuwes, N. G.; Maresca, A.; Thiry, A.; Supuran, C. T.; Scozzafava, A.; Wouters, B. G.; Lambin, P. *Radiother. Oncol.* **2009**, *92*, 423.
54. Ahlskog, J. K.; Dumelin, C. E.; Trüssel, S.; Marlind, J.; Neri, D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4851.
55. Buller, F.; Steiner, M.; Frey, K.; Mircsof, D.; Scheuermann, J.; Kalisch, M.; Bühlmann, P.; Supuran, C. T.; Neri, D. *Chem. Biol.* **2011**, *6*, 336.
56. Menzek, A.; Altundas, A.; Gultekin, D. *J. Chem. Res. Synop.* **2003**, *11*, 752.
57. Balenkenship, R. M.; Burdett, K. A.; Swenton, J. S. *J. Org. Chem.* **1974**, *39*, 2300.
58. Mayor, C.; Jones, W. M. *Tetrahedron Lett.* **1977**, *44*, 3855.
59. Swenton, J. S.; Burdett, K. A.; Madigan, D. M.; Rosso, P. D. *J. Org. Chem.* **1975**, *40*, 1280.
60. Dastan, A.; Yıldız, Y. K.; Balcı, M. *Synth. Commun.* **2001**, *31*, 3807.
61. Güney, M.; Çelik, Z. C.; Daştan, A.; Balci, M. *Can. J. Chem.* **2005**, *83*, 227.
62. Balci, M. *Chem. Rev.* **1981**, *81*, 91.
63. Çelik, M.; Akbulut, N.; Balci, M. *Helv. Chim. Acta* **2000**, *83*, 3131.
64. Balci, M.; Atasoy, B. *Tetrahedron Lett.* **1984**, *25*, 4033.
65. Atasoy, B.; Balci, M. *Tetrahedron* **1986**, *42*, 1461.
66. Şengül, M. E.; Ceylan, Z.; Balci, M. *Tetrahedron* **1997**, *53*, 10401.
67. Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3467.
68. Sly, W. S.; Hu, P. Y. *Annu. Rev. Biochem.* **1995**, *64*, 375.
69. Supuran, C. T.; Scozzafava, A.; Casini, A. *Med. Res. Rev.* **2003**, *23*, 146.
70. Supuran, C. T. Carbonic Anhydrases: Catalytic Mechanism, Distribution and Physiological Roles. In *Carbonic Anhydrase-Its Inhibitors and Activators*; Supuran, C. T., Scozzafava, A., Conway, J., Eds.; CRC: Boca Raton FL, USA, 2004; pp 1–24.
71. Ulus, R.; Yesildag, I.; Tanç, M.; Bülbül, M.; Kaya, M.; Supuran, C. T. *Bioorg. Med. Chem.* **2013**, *21*, 5799.
72. Sarikaya, S. B.; Gülçin, I.; Supuran, C. T. *Chem. Biol. Drug Des.* **2010**, *75*, 515.
73. Çoban, T. A.; Beydemir, Ş.; Gülçin, İ.; Ekinci, D. *Biol. Pharm. Bull.* **2007**, *30*, 2257.
74. ArasHisar, Ş.; Hisar, O.; Beydemir, Ş.; Gülçin, İ.; Yanık, T. *Acta Vet. Hung.* **2004**, *52*, 413.
75. Laemmli, D. K. *Nature* **1970**, *227*, 680.
76. Gülçin, İ.; Küfrevioğlu, Ö. İ.; Oktay, M. *J. Enzyme Inhib. Med. Chem.* **2005**, *20*, 297.
77. Hisar, O.; Beydemir, Ş.; Gülçin, İ.; Küfrevioğlu, Ö. İ.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2005**, *20*, 35.
78. Beydemir, Ş.; Gülçin, İ.; Hisar, O.; Küfrevioğlu, Ö. İ.; Yanık, T. *J. Appl. Anim. Res.* **2005**, *28*, 65.
79. Çoban, T. A.; Beydemir, Ş.; Gülçin, İ.; Ekinci, D. *J. Enzyme Inhib. Med. Chem.* **2008**, *23*, 266.
80. Verpoorte, J. A.; Mehta, S.; Edsall, J. T. *J. Biol. Chem.* **1967**, *242*, 4221.
81. Şentürk, M.; Gülçin, İ.; Daştan, A.; Küfrevioğlu, Ö. İ.; Supuran, C. T. *Bioorg. Med. Chem.* **2009**, *17*, 3207.
82. Coban, T. A.; Beydemir, S.; Gücin, İ.; Ekinci, D.; Innocenti, A.; Vullo, D.; Supuran, C. T. *Bioorg. Med. Chem.* **2009**, *17*, 5791.
83. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.
84. Köksal, E.; Gülçin, İ. *Protein Peptide Lett.* **2008**, *15*, 320.
85. Şentürk, M.; Gülçin, İ.; Çiftci, M.; Küfrevioğlu, Ö. İ. *Biol. Pharm. Bull.* **2008**, *31*, 2036.
86. Iga, D. P.; Schmidt, R.; Iga, S.; Hotoleanu, C. L.; Duica, F.; Nicolescu, A.; Gitman, S. S. *Turk. J. Chem.* **2013**, *37*, 299.
87. Lineweaver, H.; Burk, D. *Am. Chem. Soc.* **1934**, *56*, 658.
88. Çetinkaya, Y.; Göçer, H.; Menzek, A.; Gülçin, İ. *Arch. Pharm.* **2012**, *345*, 323.
89. Innocenti, A.; Gülçin, İ.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5050.
90. Öztürk Sarıkaya, S. B.; Topal, F.; Şentürk, M.; Gülçin, İ.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4259.
91. Balaydın, H. T.; Senturk, M.; Goksu, S.; Menzek, A. *Eur. J. Med. Chem.* **2012**, *54*, 423.

Please cite this article in press as: Güney, M.; et al. *Bioorg. Med. Chem.* (2014), http://dx.doi.org/10.1016/j.bmc.2014.04.007