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ORIGINAL ARTICLE

The effect of caffeic acid phenethyl ester (CAPE) on metabolic enzymes including acetylcholinesterase, butyrylcholinesterase, glutathione S-transferase, lactoperoxidase, and carbonic anhydrase isoenzymes I, II, IX, and XII

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Abstract

Caffeic acid phenethyl ester (CAPE) is an active component of honeybee propolis extracts. Carbonic anhydrases (CAs, EC 4.2.1.1) are widespread and intensively studied metalloenzymes present in higher vertebrates including humans as many diverse isoforms. Acetylcholinesterase (AChE) is responsible for acetyl choline (ACh) hydrolysis and plays a fundamental role in nerve impulse transmission by terminating the action of the ACh neurotransmitter at cholinergic synapses and neuromuscular junctions. Butyrylcholinesterase (BChE) is another enzyme abundantly present in the liver and released into blood in a soluble form. Lactoperoxidase (LPO) is an enzyme involved in fighting pathogenic microorganisms whereas glutathione S-transferases (GSTs) are dimeric proteins present both in prokaryotic and eukaryotic organisms and involved in cellular detoxification mechanisms. In the present study, the inhibition effect of CAPE on human carbonic anhydrase (hCA) isoforms I, II, IX, and XII, AChE, BChE, LPO, and GST was evaluated. CAPE inhibited these enzymes with K_is in the range between micromolar to picomolar. The best inhibitory effect was observed against AChE and BChE.

Keywords

Acetylcholinesterase, butyrylcholinesterase, caffeic acid phenethyl ester, carbonic anhydrase, glutathione S-transferase, lactoperoxidase

History

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Introduction

Polyphenolic compounds derived from natural products are well known to possess a range of biological activities such as antioxidant[1], antitumoral[2], anti-inflammatory, anti-viral, and immunomodulatory properties[3,4]. Anti-allergic[5], anti-carcinogenic[6], neuroprotective[7], anti-atherosclerotic, and anti-free radicals properties were also reported for these derivatives[8–11]. They incorporate one or more hydroxyl moieties bonded directly to an aromatic carbon atom[12–19] with the substitution patterns on the aromatic ring creating a large chemical variety[20–25].

Caffeic acid phenethyl ester (CAPE) is a biologically active ingredient of honeybee propolis. It has the ability to suppress lipid peroxidation[26] and is also a potent inhibitor of nuclear factor-kappa β activation[27]. This naturally bioactive and hydrophobic polyphenolic ester is found in numerous plants[28]. CAPE has important biological activities including anti-viral[29], anti-inflammatory[30], and antioxidant activities[14].

Carbonic anhydrases (CAs, EC 4.2.1.1) represent a superfamily of widespread enzymes, which catalyze a crucial biochemical reaction, the reversible hydration of carbon dioxide (CO_2) to bicarbonate (HCO_3^-) and protons (H^+)[31–37]. These enzymes are present in all organisms, from the very simple to the complex ones. This metalloenzyme superfamily includes six distinct genetic families (the α-, β-, γ-, δ-, ζ-, and η-CAs) known to date, which constitute an interesting example of convergent evolution at the molecular level[38–42]. These six CA families vary in their preference for the catalytic metal ions used within the active site[43–45], since Zn^{2+}, Cd^{2+}, or Fe^{2+} can be used within their active sites[46–52]. The α-CA isoforms differ significantly in their localization and tissue distribution. CA I, II, III, VII, and XIII are cytosolic isoforms, CA IV, IX, XII, and XIV are membrane-bound, CA VA and VB are mitochondrial, whereas CA VI is secreted. CA IX and XII are known as the membrane tumor-associated CAs, being found in a limited number of normal tissues, such as the gastrointestinal mucosa and body cavity lining[53–55]. An important role of CA IX and XII as tumor pH-regulating enzymes, involved in the survival/proliferation of the tumor cells within the hypoxic, acidic niche typical of many solid cancers[56–59].

Cholinesterases (ChE) are an enzyme family that catalyze the hydrolysis of acetyl choline (ACh) into choline and acetic acid, an essential process for the restoration of the cholinergic neurotransmission. There are two cholinesterase types:

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acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8)[11,60–64]. AChE is known to be abundant in the muscle, brain, and erythrocyte membrane, whereas BChE has a higher activity in liver, intestine, heart, kidney, and lung[65]. AChE and BChE share 65% amino acid sequence homology and have similar molecular forms and active sites despite being products of different genes on the human chromosomes[66]. Both cholinesterases participate in cholinergic neurotransmission by hydrolyzing ACh in the central and peripheral nervous system[67]. The symptomatic Alzheimer's disease (AD) treatment involves the use of cholinesterase inhibitors (ChEIs) such as Rivastigimine. ChEIs are the first-line drugs in the symptomatic treatment of AD, as by inhibiting cholinesterase they lead to an increased synaptic level of the neurotransmitter[68,69].

Lactoperoxidase (LPO, E.C.1.11.1.7) is of growing interest due to its distinctive biological activity, such as biocidal and biostatic ones[70–73]. The mechanism of the LPO antimicrobial action has been studied thoroughly regarding the conversion of thiocyanate (SCN^-) to antimicrobial products, such as hypothiocyanite ion ($OSCN^-$), hypothiocyanous acid (HOSCN), and some other highly reactive and short-lived oxidation products. These oxidations occur in the presence of hydrogen peroxide[74–77]. LPO, a member of the mammalian peroxidase family, with antibacterial properties is found in the salivary glands, in the breast secretory epithelial cells, lacrimal glands and in their secretions, such as saliva, milk, and tears[78–80]. Based on its antibacterial characteristics, currently LPO has extensive applications including preservation of raw milk during collection or transportation to processing plants in dairy industry, the extending shelf-life of pasteurized milk, and the supplementation of salivary peroxidase antimicrobial system in toothpastes and mouth rinses to reduce acid production by oral microorganisms[81].

Glutathione S-transferases (GST, EC 2.5.1.18) belong to the superfamily of phase II detoxification enzymes. They are multifunctional enzymes for the cellular defense against xenobiotics and provide protection for organism. They are essential and found in all kingdoms of life[82]. The subfamily of GSTs is further distinguished into at least 14 classes (α-, β-, δ-, ε-, ζ-, θ-, κ-, λ-, μ-, π-, s-, τ-, φ-, and Ω-GST)[83]. This classification is based on the substrate specificity, sensitivity to inhibitors, N-terminal amino acid sequence and antibody cross-reactivity. Each GST contains a G-site, which is the glutathione substrate binding site and an H-site, which is hydrophobic substrate binding site[82]. The G-site is conserved in the N-terminal region among the different enzyme classes. On the other hand, the H-site is highly diverse, being characterized by a significant variation in sequence and topology and thus accounting for the variability of enzyme activity in the GST superfamily[83].

In this study, we investigated the inhibition effect of CAPE against human carbonic anhydrase (hCA) isoenzymes hCA I, II, IX, and XII, AChE, BChE, LPO, and GST.

Experimental section

Determination of hCA isoenzymes activity and inhibition

An Applied Photophysics stopped-flow instrument was used to assay the catalytic/inhibition of four CA isozymes, as reported by Khalifah[84]. Briefly, phenol red (20 mM) was used as an indicator, with an absorbance maximum of 557 nm, with HEPES (10 mM, pH 7.4) as a buffer and 0.1 M Na_2SO_4 or $NaClO_4$ for maintaining constant the ionic strength; these anions are not inhibitory at the used concentration. The CA-catalyzed CO_2 hydration was followed for a period of 10–100 s.

For the determination of the kinetic parameters and inhibition constants, the saturated CO_2 concentrations ranged from 1.7 mM to 17 mM. For CAPE, at least six traces of the initial 5–10% of the reaction were used for determining the initial velocity. The uncatalysed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (10 mM) were prepared in distilled–deionized water, and dilutions up to 0.01 μM were performed with distilled–deionized water. CAPE and enzyme solutions were preincubated together for 15 min at room temperature prior to the assay to allow for the formation of the EI complex. The inhibition constant of CAPE was obtained by non-linear least-squares methods using PRISM 3, as reported earlier, and represents the mean from at least three different determinations. All hCA isozymes were prepared in recombinant form as reported earlier by our group[85–87].

Determination of AChE/BChE activity

The inhibitory effect of CAPE on AChE/BChE activities were measured according to spectrophotometric method of Ellman et al.[88] Acetylthiocholine iodide or butyrylthiocholine iodide (AChI/BChI) were used as substrates of the reaction. 5,5′-Dithiobis(2-nitro-benzoic)acid (DTNB, D8130-1G, Sigma-Aldrich, Steinheim, Germany) was used for the measurement of the AChE/BChE activities. Briefly, 100 mL of Tris/HCl buffer (1 M, pH 8.0), 10 mL of sample solution dissolved in deionized water at different concentrations and 50 mL AChE/BChE (5.32×10^{-3} U) solution were mixed and incubated for 10 min at 25 °C. Then 50 mL of DTNB (0.5 mM) was added. The reaction was then initiated by the addition of 50 mL of AChI/BChI. The hydrolysis of these substrates was monitored spectrophotometrically by formation of the yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by enzymatic hydrolysis of AChI/BChI, with an absorption maximum at a wavelength of 412 nm[81,89].

Purification studies of lactoperoxidase

Cyanogen bromide-activated-Sepharose 4B was used for purification of LPO from bovine milk according to the method of Atasaver et al.[79] The purity of LPO was checked by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE)[90] as previously reported[91–93]. Protein concentration was determined according to the Bradford method[94]. The stacking and running gels contained 3% (w/v) and 10% (w/v) acrylamide, respectively, and 0.1% (w/v) SDS, according to a previously published procedure[95–99].

Determination of acetylcholinesterase and butyrylcholinesterase inhibition

In order to determine the effect of CAPE on AChE, different CAPE concentrations were added into the reaction medium. The enzyme activity was measured, and an experiment in the absence of drug was used as control[66]. The IC_{50} values were obtained from activity (%) versus CAPE concentration plots. To determine the K_i constant in the media with CAPE as inhibitor, different substrate (ACh/BCh) concentrations were used. Inhibitor solution was added into the reaction medium, resulting in three different fixed concentrations of inhibitor. Lineweaver–Burk graphs[100] were used to determine V_{max} and other kinetic parameters. The K_i was calculated from these graphs.

Determination of glutathione S-transferase inhibitions

The kinetic constants of GST-catalyzed reaction were determined using 2,4-dinitrochlorobenzene (25 mM, 20 μL, %95'lik), or glutathione (20 mM, 50 μL) in sodium phosphate buffer (pH 7.2, 50 mM, 200 μL) at room temperature. GST activity was measured as described previously[101]. The enzyme solution replaced by

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phosphate buffer was used as the control. The rate of reaction was used to construct a double-reciprocal Lineweaver–Burk plot of 1/V versus 1/S and the kinetic constants[100]. Also, in this experiment, varying CAPE concentrations were used to measure their ability to inhibit the GST. CAPE solution was added into the reaction medium, resulting in three different fixed concentrations of CAPE as inhibitor.

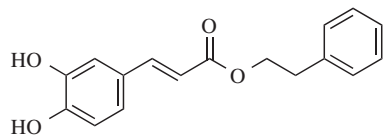
Results and discussion

Phenols are biologically active substances and possess antioxidant, anticancer, antimutagenic, anticarcinogenic, antiviral, antibacterial, and anti-inflammatory activities[102–104]. CAPE is an active phenolic component of honeybee propolis and has been used as a folk medicine for many years[14]. CAPE has a possible beneficial effect on antioxidant enzyme activity in diabetic rats. It inhibits lipid peroxidation and regulates antioxidant enzymes in the diabetic heart[105]. CAPE was able to inhibit the gene expression; production and the activity of matrix metalloproteinases induced by lipopolysaccharide and also increased the gene expression of human monocytic cell line[106]. At the molecular level, it modulates the activities of focal adhesion kinase[107], inducible human immunodeficiency virus integrase[108], nitric oxide synthase[109], lipoxygenase[110], and cyclooxygenase (COX)[111]. CAPE also inhibits proliferation of human keratinocytes and interferes with the epidermal growth factor regulation of ornithine decarboxylase[112]. It was reported that it blocks neuronal death through inhibiting inflammation and mitochondrial cytochrome c release[113]. Since nitric oxide synthesized by inducible nitric oxide synthase has been known to be involved in inflammatory and autoimmune-mediated tissue destruction. Also, CAPE inhibits nitric oxide synthase gene expression and enzyme activity[109]. With regard to the anti-inflammatory action of CAPE, it has been reported that CAPE inhibits the enzyme activities of COX. In addition, it suppresses the transcriptional expression of COX, resulting in diminished synthesis of prostaglandins, the major mediators of inflammation[111,114]. It was reported that pretreatment with intraperitoneal CAPE significantly diminished the tissue myeloperoxidase activity[115]. CAPE blocks production of reactive oxygen species in human neutrophils and xanthine/xanthine oxidase system[110]. In the present study, CAPE significantly reduced the high nitric oxide levels and adenosine deaminase activity[116]. It was reported that the neuroprotective effect of CAPE could be related to the inhibition of caspase[117].

Phenolic compounds are slightly acidic and have weak tendencies to lose the proton (H^+) ion from the hydroxyl group (–OH), resulting in the highly water-soluble phenolate anion. Phenols effectively inhibit CA isoenzymes[45]. The inhibition profile of various isozymes with this class of agents is variable, with inhibition constants ranging from the millimolar to the submicromolar range for many simple phenols[45]. Also, they inhibit the CA isozymes because of the presence of different functional groups in their scaffold, mainly the phenolic -OH and -COOH groups, which may bind to the Zn(II) ion or the water coordinated to the zinc ion from the CA active site[39]. In addition to the well-known sulfonamides, sulfamates, and sulfamides, phenolic compounds are another type of effective carbonic anhydrase inhibitors[39,118]. The classical CAIs are the primary sulfonamides, which are in clinical use as diuretics and systemically acting antiglaucoma drugs[119]. The design of CAIs as therapeutic agents is related to the large number of isoforms in humans, their rather diffuse localization in many tissues or organs. CAIs have lately emerged that CAIs could have potential as anticancer, anti-obesity, and anti-infective drugs[31,41,55,120–123].

Table 1. Inhibition constants (K_i) of caffeic acid phenethyl ester (CAPE) against four human carbonic anhydrase isoenzymes (hCA I, II, IX, and XII) acetylcholinesterase enzyme (AChE), butyrylcholinesterase enzyme (BChE), lactoperoxidase enzyme (LPO), and glutathione S-transferase (GST) enzyme.

Enzymes	K_i
Acetylcholinesterase (AChE)	517.93 pM
Butyrylcholinesterase (BChE)	322.02 pM
Lactoperoxidase (LPO)	430.03 nM
Glutathione S-transferase (GST)	0.453 nM
Human carbonic anhydrase (hCA I)	3.467 μM
Human carbonic anhydrase (hCA II)	0.797 μM
Human carbonic anhydrase (hCA IX)	4.258 μM
Human carbonic anhydrase (hCA XII)	4.467 μM



Caffeic acid phenethyl ester (**CAPE**)

Figure 1. Chemical structures of caffeic acid phenethyl ester (CAPE).

Phenolic compounds may constitute interesting lead molecules for identifying novel CAIs. Here, we report the inhibition effect of CAPE on four catalytically active isoforms, hCA I, II, IX, and XII, as well as against AChE, BChE, LPO, and GST. CAPE possesses two phenolic moieties in its scaffolds. We discovered nanomolar inhibition against some of these metabolic enzymes. The inhibition data of CAPE reported here are shown in Table 1, and the following comments can be drawn from these data:

(1) Cytosolic hCA I is expressed in the body and can be found in high concentrations in the blood and gastrointestinal tract. CAPE exhibited a weak inhibitory activity against this cytosolic isoenzyme hCA I with a K_i values 3.467 μM (Table 1). On the other hand, in another study, we found that acetazolamide (AZA), which is used as clinical CAs inhibitor and treatment of glaucoma, cystinuria, altitude sickness, epilepsy, periodic paralysis, idiopathic intracranial hypertension, dural ectasia, and central sleep apnea had been shown K_i value 0.184 μM[45]. In our previous study, we determined the effect of CAPE on hCA I (K_i: 115.0 μM), and II (K_i: 473.0 μM) purified from human erythrocyte using Sepharose-4B-L-tyrosine-sulphanilamide affinity chromatography[14]. It has been reported that phenolics are not biologically active unless substitution at either the *ortho*- or *para*-position has increased the electron density at the –OH group and lowered the oxygen–hydrogen bond energy. As can see in Figure 1, CAPE has two –OH groups at *para*-position.

(2) With regard to the profiling assay against cytosolic hCA II, CAPE was slightly more active, with a K_i value 0.797 μM. For comparison, AZA, which was used as clinical CAs inhibitor showed a K_i value 0.061 μM[43]. This result clearly showed that CAPE is a rather effective inhibitor for the cytosolic isoform hCA II. Many studies have demonstrated that the inhibition of CA II is due to the ability of an inhibitor to mimic the tetrahedral transition state when binding to the catalytic Zn^{2+} located in the active site. CA II protein fold, Zn^{2+} ion, and its coordination by histidine residues[124]. It was reported that phenols, which bind by interacting with a water molecule/hydroxide ion coordinated to Zn^{2+} through

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hydrogen bonding[85]. The physiologically dominant cytosolic isoform hCA II is ubiquitous and is being involved in several diseases, such as epilepsy, edema, glaucoma, and altitude sickness[125].

(3) So far, 16 isoforms of hCA have been discovered; among them the dimeric transmembrane glycoproteins hCA IX and XII are also human associated CA isoforms having extracellular active site and are found in a broad spectrum of hypoxic tumor types[125,126]. Many sulfonamide derivatives have been investigated for their CA inhibition activity in the search for selective hCA IX and hCA XII inhibitors because their lack of selectivity is the major challenge for the wide use of chemotherapeutic agents in cancer therapy[43]. Both CA IX and XII are overexpressed in many such tumors in response to the hypoxia inducible factor pathway, and research on the involvement of these isozymes in cancer has progressed significantly in recent years[125,127]. hCA IX showed moderate inhibition activity with CAPE, with an inhibition constant of 4.258 μM.

(4) hCA XII was also poorly inhibited by CAPE, with a K_i value of 4.467 μM. On the other hand, AZA, a positive standard for CA inhibition, showed an effective inhibitory activity with a K_i value 0.006 μM[43].

(5) The compounds possessing AChE inhibitory effects are used for the treatment of AD. However, these drugs have many undesired side effects. Thus, the development and utilization of new effective antioxidants as well as AChE compounds is highly desired[47]. Currently the most prescribed ChEIs are donepezil, galantamine, and rivastigmine. These drugs are used to treat patients with mild-to-moderate AD[128]. BChE has a specific role in cholinergic neurotransmission and it has been associated with AD[129]. Individual ChEIs differ from each other with respect to their pharmacologic properties. Donepezil and galantamine are short-acting reversible competitive inhibitors, whereas rivastigmine is actively metabolized by ChE. Primary target of donepezil and galantamine is AChE, however, rivastigmine shows equal affinity for both AChE and BChE enzymes[130]. These agents do not stop disease progression, but clinical studies have shown that they temporarily stabilize cognitive impairment and help to maintain global function, often delaying the need for patient placement in nursing homes by several months[131]. BChE levels in the body exceed those of AChE in all tissues except muscle and brain. The human body contains ten times more BChE than AChE[132]. It was reported that in AD, AChE is lost up to 85% in specific brain regions, whereas BChE levels rise with disease progression[129,133]. It was also shown that the main AChE inhibitory effect was primarily associated with aromatic compounds and, to a lesser degree, with aliphatic compounds[134]. AChE was very effectively inhibited by CAPE, with K_i value of 51 793 nM (Table 1). The K_i value of CAPE for AChE was calculated from Lineweaver–Burk plots[100]. On the other hand, donepezil hydrochloride, which is used for the treatment of mild-to-moderate AD and various other memory impairments, had been shown to lower AChE inhibition activity (IC_{50} of 55 nM)[135].

(6) It was reported that caffeine has therapeutic role on cholinergic system[136]. LPO is very important protein owing to the fact that LPO is found in the milk of all mammals. Our investigation showed CAPE inhibited LPO with a K_i value of 430.03 nM.

(7) Recently, many studies demonstrated that GST plays important functions in cellular defense against chemical toxicity. It was reported a link between the lack of GST enzyme activity and the susceptibility to develop different types of cancer including oral, gastric, and bladder cancers[137]. GST was effectively inhibited by CAPE, with a K_i value of 0.453 nM (Table 1). It was reported that the inhibitor of GST-bearing suitable linkers could concomitantly bind to two active sites of GST and usually possess K_i values at nanomolar levels and excellent enzyme-selectivity[138].

Conclusion

The effect of CAPE against hCA I, II, IX, XII isoenzymes, AChE, BChE, LPO, and GST was evaluated. CAPE demonstrated micromolar inhibition against four CA isoenzymes, nanomolar inhibition against LPO, and GST, picomolar inhibition against AChE, and BChE. The results showed that CAPE moderately inhibited four CA isoenzymes but effectively inhibited the other metabolic enzymes (AChE, BChE, LPO, and GST) with diverse inhibition profiles. These data may explain the beneficial health effects of some of these compounds and may lead to enzyme researchers and drug design campaigns.

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Declaration of interest

The authors have declared no conflict of interest.

References

1. Chan MM, Mattiacci JA, Hwang HS, et al. Synergy between ethanol and grape polyphenols, quercetin, and resveratrol, in the inhibition of the inducible nitric oxide synthase pathway. Biochem Pharmacol 2000;60:1539–48.
2. Suganuma M, Okabe S, Sueoka N, et al. Green tea and cancer chemoprevention. Mutat Res 1999;428:339–44.
3. Ozer MK, Parlakpinar H, Cigremis Y, et al. Ischemia-reperfusion leads to depletion of glutathione content and augmentation of malondialdehyde production in the rat heart from overproduction of oxidants: can caffeic acid phenethyl ester (CAPE) protect the heart? Mol Cell Biochem 2005;273:169–75.
4. Gokalp O, Uz E, Cicek E, et al. Ameliorating role of caffeic acid phenethyl ester (CAPE) against isoniazid-induced oxidative damage in red blood cells. Mol Cell Biochem 2006;290:55–9.
5. Jung WK, Choi I, Oh S, et al. Anti-asthmatic effect of marine red alga (*Laurencia undulata*) polyphenolic extracts in a murine model of asthma. Food Chem Toxicol 2009;47:293–7.
6. Na HK, Wilson MR, Kang KS, et al. Restoration of gap junctional intercellular communication by caffeic acid phenethyl ester (CAPE) in a ras-transformed rat liver epithelial cell line. Cancer Lett 2000; 157:31–8.
7. Fontanilla CV, Wei X, Zhao L, et al. Caffeic acid phenethyl ester extends survival of a mouse model of amyotrophic lateral sclerosis. Neuroscience 2012;205:185–93.
8. Hishikawa K, Nakaki T, Fujita T. Oral flavonoid supplementation attenuates atherosclerosis development in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol 2005;25:442–6.
9. Polat KL, Gülçin İ, Gören AC, et al. LC-MS/MS analysis, antioxidant and anticholinergic properties of galanga (*Alpinia officinarum* Hance) rhizomes. Ind Crops Prod 2015;74:712–21.
10. Kalın P, Gülçin İ, Gören AC. Antioxidant activity and polyphenol content of *Vaccinium macrocarpon.* Rec Nat Prod 2015;9:496–502.
11. Topal M, Gocer H, Topal F, et al. Antioxidant, antiradical and anticholinergic properties of cynarin purified from the illyrian thistle (*Onopordum illyricum* L.). J Enzyme Inhib Med Chem 2015. [Epub ahead of print]. doi:10.3109/14756366.2015.1018244.
12. Gulcin İ. Antioxidant activity of food constituents – an overview. Archiv Toxicol 2012;86:345–91.
13. Gulcin İ, Beydemir S, Topal F, et al. Apoptotic, antioxidant and antiradical effects of majdine and isomajdine from *Vinca herbacea* Waldst. and kit. J Enzyme Inhib Med Chem 2012;27:587–94.

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14. Gocer H, Gulcin İ. Caffeic acid phenethyl ester (CAPE): correlation of structure and antioxidant properties. Int J Food Sci Nutr 2011;62: 821–5.
15. Bursal E, Gulcin İ. Polyphenol contents and in vitro antioxidant activities of lyophilized aqueous extract of kiwifruit (*Actinidia deliciosa*). Food Res Int 2011;44:1482–9.
16. Gulcin İ. Antioxidant activity of L-Adrenaline: an activity-structure insight. Chem Biol Interact 2009;179:71–80.
17. Gulcin İ. Antioxidant activity of eugenol – a structure and activity relationship study. J Med Food 2011;14:975–85.
18. Gulcin İ, Huyut Z, Elmastas M, Aboul-Enein HY. Radical scavenging and antioxidant activity of tannic acid. Arab J Chem 2010;3:43–53.
19. Balaydın HT, Gulcin İ, Menzek A, et al. Synthesis and antioxidant properties of diphenylmethane derivative bromophenols including a natural product. J Enzyme Inhib Med Chem 2010;25:685–95.
20. Gulcin İ. Antioxidant properties of resveratrol: a structure-activity insight. Innov Food Sci Emerg 2010;11:210–18.
21. Koksal E, Gulcin İ, Ozturk Sarikaya SB, et al. in vitro antioxidant activity of silymarine. J Enzyme Inhib Med Chem 2009;24: 395–405.
22. Ak T, Gulcin İ. Antioxidant and radical scavenging properties of curcumin. Chem Biol Interact 2008;174:27–37.
23. Gulcin İ. Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). Toxicology 2006;217:213–20.
24. Gulcin İ. Antioxidant and antiradical activities of L-Carnitine. Life Sci 2006;78:803–11.
25. Gulcin İ. Comparison of in vitro antioxidant and antiradical activities of L-tyrosine and L-Dopa. Amino Acids 2007;32:431–8.
26. Fadillioglu E, Gursul C, Iraz M. Effects of caffeic acid phenethyl ester on thioacetamide-induced hepatic encephalopathy in rats. Prog Neuropsychopharmacol Biol Psychiatry 2010;34:1440–5.
27. Lee Y, Shin DH, Kim JH, et al. Caffeic acid phenethyl ester-mediated Nrf2 activation and I kappa B kinase inhibition are involved in NF kappa B inhibitory effect: structural analysis for NFkappaB inhibition. Eur J Pharmacol 2010;43:21–8.
28. Murtaza G, Sajjad A, Mehmood Z, et al. Possible molecular targets for therapeutic applications of caffeic acid phenethyl ester in inflammation and cancer. J Food Drug Anal 2015;23:11–18.
29. Banskota AH, Tezuka Y, Kadota S. Recent progress in pharmacological research of propolis. Phytother Res 2001;15:561–71.
30. Murtaza G, Karim S, Akram MR, et al. Caffeic acid phenethyl ester and therapeutic potentials. BioMed Res Int 2014;2014:145342.
31. Topal M, Gulcin İ. Rosmarinic acid: a potent carbonic anhydrase isoenzymes inhibitor. Turk J Chem 2014;38:894–902.
32. Beydemir S, Gulcin İ. Effects of melatonin on carbonic anhydrase from human erythrocytes in vitro and from rat erythrocytes in vivo. J Enzyme Inhib Med Chem 2004;19:193–7.
33. Gulcin İ, Beydemir S, Buyukokuroglu ME. In vitro and in vivo effects of dantrolene on carbonic anhydrase enzyme activities. Biol Pharm Bull 2004;27:613–16.
34. 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.
35. Ceruso M, Vullo D, Scozzafava A, Supuran CT. Sulfonamides incorporating fluorine and 1,3,5-triazine moieties are effective inhibitors of three b-class carbonic anhydrases from Mycobacterium tuberculosis. J Enzyme Inhib Med Chem 2014;29:686–9.
36. Supuran CT, Casini A, Mastrolorenzo A, Scozzafava A. COX-2 selective inhibitors, carbonic anhydrase inhibition and anticancer properties of sulfonamides belonging to this class of pharmacological agents. Mini Rev Med Chem 2004;4:625–32.
37. Capasso C, Supuran CT. Sulfa and trimethoprim-like drugs-antimetabolites acting as carbonic anhydrase, dihydropteroate synthase and dihydrofolate reductase inhibitors. J Enzyme Inhib Med Chem 2014;29:379–87.
38. Supuran CT. Structure-based drug discovery of carbonic anhydrase inhibitors. J Enzyme Inhib Med Chem 2012;27:759–72.
39. Guney M, Coskun A, Topal F, et al. Oxidation of cyanobenzocy cloheptatrienes: synthesis, photooxygenation reaction and carbonic anhydrase isoenzymes inhibition properties of some new benzotropone derivatives. Bioorg Med Chem 2014;22:3537–43.
40. Alafeefy AM, Ceruso M, Al-Jaber NA, et al. A new class of quinazoline-sulfonamides acting as efficient inhibitors against the α-carbonic anhydrase from *Trypanosoma cruzi.* J Enzyme Inhib Med Chem 2015;30:581–5.
41. Cetinkaya Y, Gocer H, Gulcin I, Menzek A. Synthesis and carbonic anhydrase isoenzymes inhibitory effects of brominated diphenylmethanone and its derivatives. Arch Pharm (Weinheim) 2014;347: 354–9.
42. 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.
43. 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]. doi: 10.3109/14756366.2014.999236.
44. Ceruso M, Bragagni M, AlOthman Z, et al. New series of sulfonamides containing amino acid moiety act as effective and selective inhibitors of tumor-associated carbonic anhydrase XII. J Enzyme Inhib Med Chem 2015;30:430–4.
45. 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.
46. Scozzafava A, Menabuoni L, Mincione F, et al. Carbonic anhydrase inhibitors. Synthesis of sulfonamides incorporating dtpa tails and of their zinc complexes with powerful topical antiglaucoma properties. Bioorg Med Chem Lett 2001;11:575–82.
47. Mastrolorenzo A, Rusconi S, Scozzafava A, et al. Inhibitors of HIV-1 protease: current state of the art 10 years after their introduction. From antiretroviral drugs to antifungal, antibacterial and antitumor agents based on aspartic protease inhibitors. Curr Med Chem 2007;14:2734–48.
48. 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.
49. Durdagi S, Scozzafava G, Vullo D, et al. Inhibition of mammalian carbonic anhydrases I-XIV with grayanotoxin III: solution and in silico studies. J Enzyme Inhib Med Chem 2014;29:469–75.
50. Hisar O, Beydemir S, Gulcin I, et al. The effect of melatonin$_{54.}$ 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.
51. Aras Hisar S, Hisar O, Beydemir S, 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.
52. Hisar O, Beydemir S, Gulcin I, et al. Effects of low molecular weight plasma inhibitors of rainbow trout (Oncorhynchus mykiss) on human erythrocyte carbonic anhydrase-II isozyme activity in vitro and rat erythrocytes in vivo. J Enzyme Inhib Med Chem 2005; 20:35–9.
53. Supuran CT, Scozzafava A, Casini A. Carbonic anhydrase inhibitors. Med Res Rev 2003;23:146–89.
54. Bilginer S, Unluer E, Gul HI, et al. Carbonic anhydrase inhibitors. Phenols incorporating 2- or 3-pyridyl-ethenylcarbonyl and tertiary amine moieties strongly inhibit *Saccharomyces cerevisiae* β-carbonic anhydrase. J Enzyme Inhib Med Chem 2014;29:495–9.
55. 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.
56. Çoban TA, Beydemir S, Gülçin İ, Ekinci D. Morphine inhibits erythrocyte carbonic anhydrase in vitro and in vivo. Biol Pharm Bull 2007;30:2257–61.
57. Coban TA, Beydemir S, Gülçin İ, Ekinci D. The effect of ethanol on erythrocyte carbonic anhydrase isoenzymes activity: an in vitro and in vivo study. J Enzyme Inhib Med Chem 2008;23:266–70.
58. Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 2008;7:168–81.
59. Göçer H, Akıncıoğlu A, Öztaşkın N, et al. Synthesis, antioxidant, and antiacetylcholinesterase activities of sulfonamide derivatives of dopamine-related compounds. Arch Pharm (Weinheim) 2013;346: 783–92.
60. 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.
61. Akıncıoğlu A, Topal M, Gülçin İ, Göksu S. Novel sulfamides and sulfonamides incorporating tetralin scaffold as carbonic anhydrase

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and acetylcholine esterase inhibitors. Arch Pharm (Weinheim) 2014; 347:68–76.
62. Göçer H, Akıncıoğlu A, Göksu S, et al. Carbonic anhydrase and acetylcholinesterase inhibitory effects of carbamates and sulfamoylcarbamates. J Enzyme Inhib Med Chem 2015;30:316–20.
63. 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;20:1–7. doi.org/10.3109/14756366.2015.1036051
64. Aksu K, Topal F, Gülçin I, et al. Acetylcholinesterase inhibitory and antioxidant activities of novel symmetric sulfamides derived from phenethylamines. Arch Pharm (Weinheim) 2015;348:446–55.
65. Dave KR, Syal AR, Katyare SS. Tissue cholinesterases. A comparative study of their kinetic properties. Z Naturforsch 2000;55:100–8.
66. Allderdice PW, Garner HAR, Galutira D, et al. The cloned butyrylcholinesterase (BCHE) gene maps to a single chromosome site, 3q26. Genomics 1991;11:452–4.
67. Pohanka M. Cholinesterases, a target of pharmacology and toxicology. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2011;155:219–30.
68. Oztaşkın N, Etinkaya Taslimi ÇY, Göksu P, Gülçin SI. Antioxidant and acetylcholinesterase inhibition properties of novel bromophenol derivatives. Bioorg Chem 2015;60:49–57.
69. Heller L, Schearz S, Weber BA, Csuk R. Gypsogenin derivatives: an unexpected class of inhibitors of cholinesterases. Arch Pharm (Weinheim) 2014;347:707–16.
70. Santos MJ, Teixeira JA, Rodrigues LR. Fractionation of the major whey proteins and isolation of b-lactoglobulin variants by anion exchange chromatography. Sep Pur Technol 2012;90:133–9.
71. Ozturk Sarikaya SB, Sisecioglu M, Cankaya M, et al. Inhibition profile of a series of phenolic acids on bovine lactoperoxidase enzyme. J Enzyme Inhib Med Chem 2015;30:479–83.
72. Şişecioğlu M, Çankaya M, Gülçin İ, Özdemir M. The Inhibitory effect of propofol on lactoperoxidase. Protein Peptide Lett 2009;16: 46–9.
73. 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.
74. Boots JW, Floris R. Lactoperoxidase: from catalytic mechanism to practical applications. Int Dairy J 2006;16:1272–6.
75. 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.
76. Şişecioğlu M, Gülçin İ, Çankaya M, et al. The effects of norepinephrine on lactoperoxidase enzyme (LPO). Sci Res Essays 2010;5:1351–6.
77. 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.
78. 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.
79. 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.
80. Köksal Z, Usanmaz H, Özdemir H, et al. Inhibition effects of some phenolic and dimeric phenolic compounds on bovine lactoperoxidase (LPO) enzyme. Int J Acad Res 2014;6:27–32.
81. Pan M, Shen S, Chen L, et al. Separation of lactoperoxidase from bovine whey milk by cation exchange composite cryogel embedded macroporous cellulose beads. Sep. Pur Technol 2015;147:132–8.
82. Liu H, He J, Zhao R, et al. A novel biomarker for marine environmental pollution of pi-class glutathione S-transferase from *Mytilus coruscus*. Ecotoxicol Environ Saf 2015;118:47–54.
83. Navaneethaiyer U, Kasthuri SR, Youngdeuk L, et al. A novel molluscan sigma-like glutathione S-transferase from *Manila clam*, *Ruditapes philippinarum*: cloning, characterization and transcriptional profiling. Comp Biochem Physiol C 2012;155:539–50.
84. Khalifah RG. The carbon dioxide hydration activity of carbonic anhydrase. I. Stop-flow kinetic studies on the native human isoenzymes B and C. J Biol Chem 1971;246:2561–73.
85. Innocenti A, Ozturk Sarıkaya SB, Gulcin I, 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.
86. Innocenti A, Gulcin I, 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.
87. 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.
88. Ellman GL, Courtney KD, Andres V, Featherston RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88–95.
89. Ozturk M, Duru ME, Kivrak S, et al. In vitro antioxidant, anticholinesterase and antimicrobial activity studies on three Agaricus species with fatty acid compositions and iron contents: a comparative study on the three most edible mushrooms. Food Chem Toxicol 2011;49:1353–60.
90. Laemmli DK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–3.
91. Aydin B, Gülcin I, Alwasel SH. Purification and characterization of polyphenol oxidase from Hemşin apple (*Malus communis* L.). Int J Food Propert 2015;18:2735–45.
92. 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 (Weinheim) 2014;347: 950–7.
93. Arabaci B, Gülçin İ, Alwasel S. Capsaicin: a potent inhibitor of carbonic anhydrase isoenzymes. Molecules 2015;19: 10103–14.
94. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–51.
95. Şentürk M, Gülçin İ, Çiftci M, Küfrevioğlu Öİ. Dantrolene inhibits human erythrocyte glutathione reductase. Biol Pharm Bull 2008; 31:2036–9.
96. Köksal E, Gülçin İ. Purification and characterization of peroxidase from cauliflower (*Brassica oleracea* L.) buds. Protein Peptide Lett 2008;15:320–6.
97. 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.
98. Gülçin İ, Küfrevioğlu Öİ, Oktay M. Purification and characterization of polyphenol oxidase from nettle (*Urtica dioica* L.) and inhibitory effects of some chemicals on enzyme activity. J Enzyme Inhib Med Chem 2005;20:297–302.
99. Beydemir Ş, Gülçin İ, Küfrevioğlu Öİ, Çiftçi M. Glucose 6-phosphate dehydrogenase: in vitro and In vivo effects of dantrolene sodium. Pol J Pharmacol 2003;55:787–92.
100. Lineweaver H, Burk D. The determination of enzyme dissociation constants. J Am Chem Soc 1934;56:658–66.
101. Liu JY, Yang XQ, Zhang YL. Characterization of lambda-cyhalothrin metabolizing glutathione S-transferase CpGSTd1 from *Cydia pomonella* (L.). Appl Microbiol Biotechnol 2014;98: 8947–62.
102. Gulcin I, Dastan A. Synthesis of dimeric phenol derivatives and determination of in vitro antioxidant and radical scavenging activities. J Enzyme Inhib Med Chem 2007;22:685–95.
103. Gülçin İ, Elias R, Gepdiremen A, Boyer L. Antioxidant activity of lignans from fringe tree (*Chionanthus virginicus* L.). Eur Food Res Technol 2006;223:759–67.
104. Gülçin İ, Elias R, Gepdiremen A, et al. Antioxidant secoiridoids from fringe tree (Chionanthus virginicus L.). Wood Sci Technol 2009;43:195–212.
105. Okutan H, Ozcelik N, Yilmaz HR, Uz E. Effects of caffeic acid phenethyl ester on lipid peroxidation and antioxidant enzymes in diabetic rat heart. Clin Biochem 2005;38:191–6.
106. Vilela PD, de Oliveira JR, de Barros PP, et al. In vitro effect of caffeic acid phenethyl ester on matrix metalloproteinases (MMP-1 and MMP-9) and their inhibitor (TIMP-1) in lipopolysaccharide-activated human monocytes. Arch Oral Biol 2015;60: 1196–202.
107. Weyant MJ, Carothers AM, Bertagnolli ME, Bertagnolli MM. Colon cancer chemopreventive drugs modulate integrin-mediated signaling pathways. Clin Cancer Res 2000;6:949–56.

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108. Fesen MR, Kohn KW, Leteurtre F, Pommier Y. Inhibitors of human immunodeficiency virus integrase. Proc Natl Acad Sci USA 1993; 90:2399–403.
109. Song YS, Park EH, Hur GM, et al. Bioanalysis and biotransformation of caffeic acid phenethyl ester inhibits nitric oxide synthase gene expression and enzyme activity. Cancer Lett 2002;175:53–61.
110. Sudina GF, Mirzoeva OK, Pushkareva MA, et al. Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant properties. FEBS Lett 1993;329:21–4.
111. Michaluart P, Masferrer JL, Carothers AM, et al. Inhibitory effects of caffeic acid phenethyl ester on the activity and expression of cyclooxygenase-2 in human oral epithelial cells and in a rat model of inflammation. Cancer Res 1999;59:2347–52.
112. Zheng ZS, Xue GZ, Grunberger D, Prystowsky JH. Caffeic acid Phenethyl ester inhibits proliferation of human keratinocytes and interferes with the EGF regulation of ornithine decarboxylase. Oncol Res 1995;7:445–52.
113. Yiş U, Topçu Y, zbal ÖS, et al. Caffeic acid phenethyl ester prevents apoptotic cell death in the developing rat brain after pentylenetetrazole-induced status epilepticus. Epilepsy Behav 2013;29:275–80.
114. Mirzoeva OK, Calder PC. The effect of propolis and its components on eicosanoid production during the inflammatory response. Prostaglandins Leukot Essent Fatty Acids 1996;55:441–9.
115. Gurel A, Armutcu F, Sahin S, et al. Protective role of alpha-tocopherol and caffeic acid phenethyl ester on ischemia-reperfusion injury via nitric oxide and myeloperoxidase in rat kidneys. Clin Chim Acta 2004;339:33–41.
116. Uzar E, Sahin O, Koyuncuoglu HR, et al. The activity of adenosine deaminase and the level of nitric oxide in spinal cord of methotrexate administered rats: protective effect of caffeic acid phenethyl ester. Toxicology 2006;218:125–33.
117. Ilhan A, Iraz M, Gurel A, et al. Caffeic acid phenethyl ester exerts a neuroprotective effect on CNS against pentylenetetrazol-induced seizures in mice. Neurochem Res 2004;29:2287–92.
118. Ozturk Sarıkaya SB, Gulcin I, 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.
119. Supuran CT. Carbonic anhydrase inhibitors and activators for novel therapeutic applications. Future Med Chem 2011;3:1165–80.
120. Maresca A, Temperini C, Vu H, et al. Non-zinc mediated inhibition of carbonic anhydrases: coumarins are a new class of suicide inhibitors. J Am Chem Soc 2009;131:3057–62.
121. Davis RA, Innocenti A, Poulsen SA, Supuran CT. Carbonic anhydrase inhibitors. Identification of selective inhibitors of the human mitochondrial isozymes VA and VB over the cytosolic isozymes I and II from a natural product-based phenolic library. Bioorg Med Chem 2010;18:14–18.
122. Zhang P, Tang Y, Li NG, et al. Bioactivity and chemical synthesis of caffeic acid phenethyl ester and its derivatives. Molecules 2014; 19:16458–76.
123. Barclay LRC, Vinqvist MR, Mukai K, et al. Chain-breaking phenolic antioxidants: steric and electronic effects in polyalkylchromanols, tocopherol analogs, hydroquinones, and superior antioxidants of the polyalkylbenzochromanol and naphthofuran class. J Org Chem 1993;58:7416–20.
124. Akbaba Y, Akıncıoglu A, Gocer H, et al. Carbonic anhydrase inhibitory properties of novel sulfonamide derivatives of aminoindanes and aminotetralins. J Enzyme Inhib Med Chem 2014;29: 35–42.
125. Riafrecha LE, Rodriguez OM, Vullo D, et al. Attachment of carbohydrates to methoxyaryl moieties leads to highly selective inhibitors of the cancer associated carbonic anhydrase isoforms IX and XII. Bioorg Med Chem 2014;22:5308–14.
126. Supuran CT. Carbonic anhydrase inhibitors. Bioorg Med Chem Lett 2010;20:3467–74.
127. Alterio V, Hilvo M, Di Fiore A, et al. Crystal structure of the catalytic domain of the tumor-associated human carbonic anhydrase IX. Proc Natl Acad Sci USA 2009;106:16233–8.
128. Qaseem A, Snow V, Cross Jr JT, Current pharmacologic treatment of dementia: a clinical practice guideline from the American College of Physicians and the American Academy of Family Physicians. Ann Intern Med 2008;148:370–8.
129. Bono GF, Simão-Silva DP, Batistela MS, et al. Butyrylcholinesterase: K variant, plasma activity, molecular forms and rivastigmine treatment in Alzheimer's disease in a Southern Brazilian population. Neurochem Int 2015;81:57–62.
130. Jann MW, Shirley KL, Small GW. Clinical pharmacokinetics and pharmacodynamics of cholinesterase inhibitors. Clin Pharmacokinet 2002;41:719–39.
131. Michaelis ML. Drugs targeting Alzheimer's disease: some things old and some things new. J Pharmacol Exp Ther 2003;304: 897–904.
132. Li B, Stribley JA, Ticu A, et al. Abundant tissue butyrylcholinesterase and its possible function in the acetylcholinesterase knockout mouse. J Neurochem 2000;75:1320–31.
133. Perry E, McKeith I, Ballard C. Butyrylcholinesterase and progression of cognitive deficits in dementia with Lewy bodies. Neurology 2003;60:1852–3.
134. Holth TF, Tollefsen KE. Acetylcholine esterase inhibitors in effluents from oil production platforms in the North Sea. Aquat Toxicol 2012;112–113:92–8.
135. Sugimoto H, Yamanishi Y, Iimura Y, Kawakami Y. Donepezil hydrochloride (E2020) and other acetylcholinesterase inhibitors. Curr Med Chem 2000;7:303–33.
136. Anwar J, Spanevello RM, Thome G, et al. Effects of caffeic acid on behavioral parameters and on the activity of acetylcholinesterase in different tissues from adult rats. Pharmacol. Biochem. Behav. 2012; 103:386–94.
137. Kassogue Y, Dehbi H, Quachouh M, et al. Association of glutathione S-transferase (GSTM1 and GSTT1) genes with chronic myeloid leukemia. SpringerPlus 2015;4:210
138. Xu B, Tan D, Yang X, et al. Fluorometric titration assay of affinity of tight-binding nonfluorescent inhibitor of glutathione s-transferase. J Fluoresc 2015;25:1–8.