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RESEARCH ARTICLE

The human carbonic anhydrase isoenzymes I and II inhibitory effects of some hydroperoxides, alcohols, and acetates

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Abstract

The 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 to bicarbonate and protons. Human CA isoenzymes I and II (hCA I and hCA II) are ubiquitous cytosolic isoforms. In this study, a series of hydroperoxides, alcohols, and acetates were tested for the inhibition of the cytosolic hCA I and II isoenzymes. These compounds inhibited both hCA isozymes in the low nanomolar ranges. These compounds were good hCA I inhibitors (K_is in the range of 24.93–97.99 nM) and hCA II inhibitors (K_is in the range of 26.04–68.56 nM) compared to acetazolamide as CA inhibitor (K_i: 34.50 nM for hCA I and K_i: 28.93 nM for hCA II).

Keywords

Acetate, alcohol, carbonic anhydrase, enzyme inhibition, enzyme purification, hydroperoxide

History

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Introduction

The carbonic anhydrases (CAs; EC 4.2.1.1) are a superfamily of metalloenzymes, which catalyze the interconversion between carbon dioxide (CO_2) and water (H_2O) to bicarbonate (HCO_3^-) and a proton (H^+) by using a metal hydroxide nucleophilic mechanism[1,2].

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow HCO_3^- + H^+$$

They are virtually ubiquitous in all living systems and participate in a variety of physiological and pathological processes such as pH regulation, fluid balance, bone resorption, glaucoma, calcification, cancer, neurological disorders, osteoporosis, tumorigenicity, and biosynthetic reactions (e.g. carboxylations, in which the produced HCO_3^- is the real substrate[3–9]). The CAs are present in either eukaryotes and prokaryotes, being encoded by six genetically distinct, non-related gene families: the α, β, γ, δ, ζ, and η-CAs. Of them, the η-CA, a novel family of CA, was discovered quite recently[10–13].

All human CAs (hCAs) belong to the α-CAs. Until now, 16 isozymes have been recognized in mammals where they play crucial physiological roles. Among these, only 13 isoforms are catalytically active (CAs I-IV, CAs VA, VB, CAs VI, VII, CA IX, and CAs XII-XV). Some of them are cytosolic ones (CA I, II, III, VII, and XIII), others are membrane associated (CA IV, IX, XII, XIV, and XV), mitochondrial (CA VA and VB), and there is a secreted one (CA VI) too. On the other hand CA VIII, X and XI, the CA-related proteins (CARPs), are devoid of any catalytic activity[14–18].

The different CA isoforms possess a widely variable kinetic properties, their pattern of expression in various cellular compartments and tissues is diverse, as it is their inhibition profile with various classes of compounds[13,19]. An inhibitor is a molecule that binds to an enzyme and diminish its activity. Also, it can hinder a substrate from entering the active site of the enzyme preventing catalyzing. The inhibition of CA enzymes is very important for living organism[11,16]. CA inhibitors (CAIs) were clinically used primarily as anti-glaucoma drugs, diuretics[6], anticonvulsant agents[19], and as anti-epileptics, while the novel generation compounds are undergoing clinical investigation as anti-obesity[20–22] or anti-tumor drugs and diagnostic tools[7,11,23]. In recent years, CAIs started to be used in the management of hypoxic tumors[22].

Many classes of CAIs bind to the catalytic zinc ion (Zn^{2+}) within the enzyme active site and prevent its activity. Acetazolamide (AZA) is the first clinically used sulfonamides as CAI[23].

The catalytic mechanism involves the presence of a hydroxide ion coordinated to the zinc within the CA active site (i.e. the basic form of the enzyme). During the catalytic cycle, the acidic form is generated, with water coordinated to zinc. For regeneration of the basic form of CA, a proton is transferred from the zinc coordinated water molecule to the solvent. This H^+ transfer may be assisted by active site residues or by buffers present in the medium[14,24,25].

Molecular oxygen is the most plentiful and accessible oxidant. Oxidations of many organic compounds (e.g. alkenes) are important in the synthesis of many widely used chemicals. Singlet oxygen is an electronically excited state of molecular

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oxygen, which is generated by reaction of triplet oxygen ($^3O_2$) with photoexcited sensitizer. Singlet oxygen is produced by irradiation of ground state triplet oxygen with light in the presence of triplet sensitizers such as *meso*-tetraphenyl porphyrin (TPP), Rose Bengal or Methylene Blue. The olefins with allylic hydrogen atoms undergo ene-type reactions with singlet oxygen to form allylic hydroperoxides, which are useful intermediates in many synthetic reactions[26–29]. Allylic hydroperoxides are involved in the development of rancidity in fat, the disruption of lipid membranes, but also in the biosynthesis of prostaglandins[30].

As hydroperoxides were not yet investigated for their interaction with the CAs, in this study, we studied the potential inhibition effect of some allylic hydroperoxides (**1–5**), alcohols (**5–10**), and acetates (**10–15**) against hCA I and II.

Experimental

The hCA I and II isoenzymes were purified by Sepharose-4B-L tyrosine-sulphanilamide affinity chromatography[31–36] as published in previous studies[37]. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used for checking enzymes purity[37–42], and a single band was observed for each isoenzyme[43,44]. For this purpose acrylamide in the running (10%) and the stacking gel (3%), with SDS (0.1%) were employees[45–48].

CA isoenzyme activities were determined according to Verpoorte et al.[49–51] One unit of CA activity was expressed as 1 mmol/L of released *p*-nitrophenol (NP) per minute at 25 °C[52]. The quantity of protein during enzyme purification procedure was spectrophotometrically determined at 595 nm according to the Bradford method[53]. Bovine serum albumin was used as the standard protein[54–56].

The inhibition effects of allylic hydroperoxides, alcohols, and acetates (**1–15**) on both CA isoenzymes was measured by using *p*-nitrophenyl acetate (NPA) hydrolysis to NP[57,58]. The CA-catalysed reaction of CO_2 hydration was first observed in the absence of allylic hydroperoxides, alcohols, and acetates (**1–15**) and used as a control for both CA isoenzymes.

Activity (%)-[allylic hydroperoxide, alcohol, or acetate] graphs were drawn and the half maximal inhibitory concentration (IC_{50}) values of each allylic hydroperoxides, alcohols, and acetates (**1–15**) exhibiting more than 50% inhibition of CA were calculated. In addition, the K_i values were also determined. Five different concentrations of substrate were used and Lineweaver–Burk curves were drawn[59] in order to determine the K_is[60–63].

Results and discussion

Clinical use of the CAIs proved to be a reliable therapeutic method for a number of human diseases and for several decades such compounds were a major component of the therapy for high blood pressure, glaucoma, etc.[64,65] It was well known that CAs are involved in crucial physiological processes connected with CO_2/HCO_3^- transport and homeostasis, electrolyte secretion in a variety of tissues and organs, biosynthetic reactions including ureagenesis, gluconeogenesis, and lipogenesis, respiration, tumorigenicity, bone resorption, and calcification[32,66,67].

In this study, we report the inhibition profiles of allylic hydroperoxides, alcohols, and acetates (**1–15**) against the ubiquitous cytosolic isoform (hCA I) and the more rapid cytosolic isoenzyme (hCA II). Hydroperoxides are strong oxidants like ozone and have some toxic effects on living organisms by inactivation of enzymes and impairing metabolic processes. They occur as end products of polyunsaturated fatty acids biosynthetic pathways/degradation, including linoleic acid[68–78]. They can give rise to secondary oxidative damage, which can happen in two ways. The first is via one electron (free radical) reaction to get secondary radicals, whereas the second via two electron (molecular) reactions with suitable nucleophiles[79].

The allylic hydroperoxides (**1–5**) were prepared by the Schenk method. The chemical structures of allylic hydroperoxides, alcohols and acetates (**1–15**) are shown in Figure 1. The reduction of the hydroperoxides with dimetilsulfide in the presence of catalytic amount of $Ti(O\text{-}iPr)_4$ produced the corresponding allylic alcohols (**6–10**) in high yield. Allylic alcohols represent an interesting building block in organic synthesis. All allylic alcohols (**6–10**) were converted into the corresponding monoacetates with $NaOAc/Ac_2O$ at room temperature in excellent yields[30]. The synthesis of these allylic hydroperoxides, alcohols, and acetates (**1–15**) was performed as described previously[30]. The synthesis route of some allylic hydroperoxides, alcohols, and acetates (**1–15**) is shown in Figure 2.

For evaluation of the biological activity of hydroperoxides, alcohols, and acetates **1–15**, the physiologically relevant human isoforms hCA I and II have been included in the study. Allylic hydroperoxides, alcohols, and acetates **1–15** demonstrated effective inhibitory effects against both CA I and II isoforms (Table 1). The following structure–activity relationship could be drawn from data of Table 1:

(i) The CA I isoenzyme is found in many tissues and is involved in retinal and cerebral edema. Its inhibition may be a valuable tool for fighting these conditions[21]. For hCA I, the

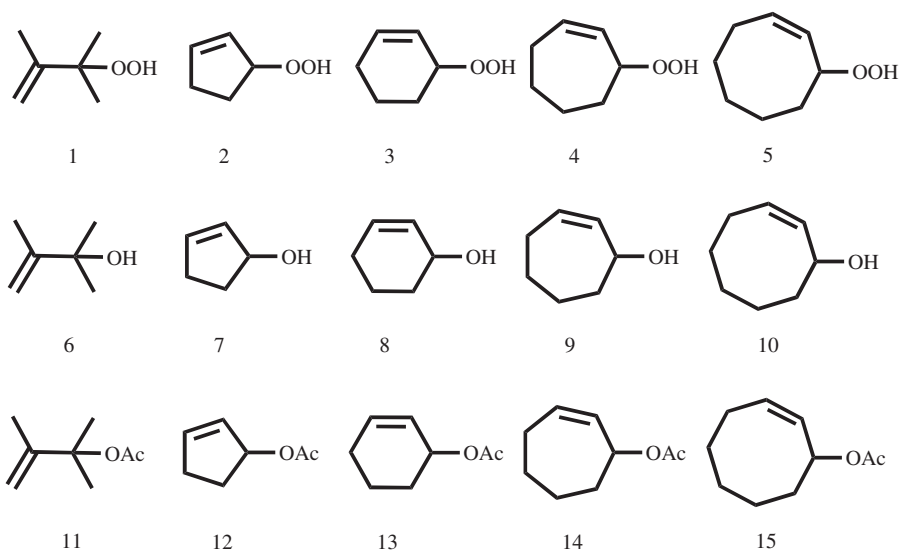

Figure 1. The chemical structures of allylic hydroperoxides, alcohols, and acetates (**1–15**) used for carbonic anhydrase isoenzymes (hCA I and II) inhibition effects.

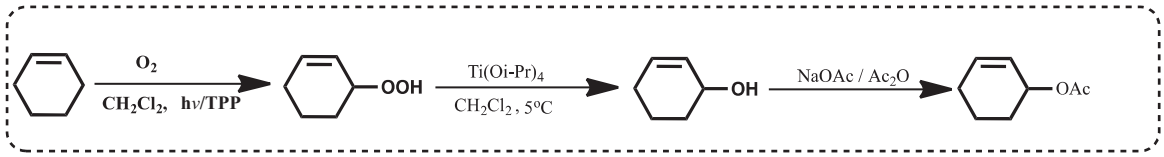

Figure 2. The synthesis route of some allylic hydroperoxides, alcohols, and acetates (**1**–**15**).

Table 1. Human carbonic anhydrase isoenzymes (hCA I and II) inhibition values with some hydroperoxides, alcohols, and acetates by an esterase assay with NPA.

	IC_{50} (nM)				K_i (nM)	
Compounds	hCA I	r^2	hCA II	r^2	hCA I	hCA II
1	74.17	0.9911	46.63	0.9901	40.46 ± 7.01	47.29 ± 5.20
2	77.02	0.9930	59.62	0.9972	71.16 ± 36.50	36.66 ± 6.69
3	91.78	0.9960	52.19	0.9926	60.12 ± 11.36	32.84 ± 1.84
4	66.71	0.9880	48.05	0.9933	24.93 ± 5.39	26.04 ± 6.51
5	59.76	0.9954	37.57	0.9877	44.62 ± 17.60	34.60 ± 7.58
6	89.49	0.9964	68.22	0.9984	82.44 ± 1.94	68.29 ± 6.65
7	97.59	0.9921	83.01	0.9990	97.99 ± 20.63	68.56 ± 11.10
8	91.23	0.9831	73.36	0.9971	85.87 ± 4.63	61.19 ± 10.36
9	67.73	0.9916	66.76	0.9955	51.40 ± 4.86	45.74 ± 9.10
10	63.38	0.9967	53.52	0.9858	64.15 ± 17.11	49.79 ± 2.01
11	64.47	0.9987	53.62	0.9990	49.58 ± 7.39	41.11 ± 9.39
12	59.72	0.9985	61.20	0.9909	80.26 ± 14.01	58.91 ± 4.99
13	59.26	0.9963	53.83	0.9983	55.78 ± 10.24	51.85 ± 4.54
14	53.85	0.9946	45.09	0.9985	45.88 ± 8.55	47.06 ± 3.58
15	52.95	0.9983	44.71	0.9966	53.93 ± 15.60	36.08 ± 8.12
AZA*	40.46	0.9996	24.16	0.9833	34.50 ± 4.09	28.93 ± 14.77

*Acetazolamide (AZA) was used as a standard inhibitor for both CA isoenzymes.

compounds **1**–**15** showed K_i values ranging between 24.93 ± 5.39 and 97.99 ± 20.63 nM (Table 1). The best inhibition was observed with hydroperoxide **4** (3-hydroperoxycyclohept-1-ene) with a K_i value of 24.93 ± 5.39 nM. On the other hand, AZA, used a CAI for the medical treatment of idiopathic intracranial hypertension, glaucoma, epileptic seizure, altitude sickness, cystinuria, periodic paralysis, central sleep apnea, and dural ectasia, showed a K_i value of 34.50 ± 4.09 nM. hCA I is highly abundant in red blood cells and is found in many tissues but its precise physiological function is unknown[22,67].

(ii) CA II is involved in several diseases, such as glaucoma, edema, epilepsy, and altitude sickness[22,80]. For hCA II, compounds **1**–**15** showed K_i values ranging between 26.04 ± 6.51 and 68.56 ± 11.10 nM. As for hCA I, the best hCA II inhibitor was hydroperoxide **4** (3-hydroperoxycyclohept-1-ene) with a K_i value of 26.04 ± 6.51 nM. On the other hand, AZA demonstrated a K_i value of 28.93 ± 14.77 nM. These results clearly shown that all allylic hydroperoxides, alcohols, and acetates (**1**–**15**) have effective enzyme inhibitory properties. AZA is a well-known example of a clinically established CA inhibitor[81–83] and in recent years we have reported its strong inhibition of both human cytosolic CA isoenzymes[6,14,24]. There are important differences in inhibition between the both isoenzymes. The main difference in the active site architectures of the two isoenzymes is due to the presence of more histidine residues in the hCA I isoform[5,16].

The recent extensive studies showed the importance of CA I and II isoenzyme inhibitors. In our laboratory, CA inhibitory effects were studied for a large number of compounds, including *N*-alkyl (aril)-tetra pyrimidine thiones[15], sulfamide analogs of dopamine[3], sulfonamides derived from dopamine[4], sulfamides and sulfonamides incorporating a tetralin scaffold[5], phenolic benzylamine derivatives[7], melatonin[8], sulfonamide derivatives of aminoindanes and aminotetralins[9], dimethoxy-bromophenol derivatives incorporating cyclopropane moieties[10] (3,4-dihydroxyphenyl) (2,3,4-trihydroxyphenyl), methanone and its derivatives[12], new ureido-substituted sulfonamides incorporating a GABA moiety[13], new benzotropone derivatives[14], guaiacol and catechol derivatives[1], pyrimidines[15], capsaicin[16], hydroquinone[17], novel sulfamides derived from 1-aminoindanes and anilines[30], benzylsulfamides[32], rosmarinic acid[32], dantrolene[34], morphine[34], tocopherol[35], phenolic sulfonamides[36], N-acylsulfonamides[43], novel phenolic sulfamides[43], antioxidant phenols[49], brominated diphenylmethanone and its derivatives[56], natural phenolic compounds[61,62,84], phenolic acids[85], antioxidant polyphenol products[81,86], natural product polyphenols and phenolic acids[86], caffeic acid phenethyl ester[82], carbamates and sulfamoylcarbamates[87], natural and synthetic bromophenols[88], norbornene-fused pyridazines[89], (Z)-4-Oxo-4-(arylamino)but-2-enoic acids derivatives[90], avermectins[91], spirobisnaphthalenes[92], 4-(2-substitutedhydrazinyl)benzenesulfonamides[93], and taxifolin[94]. All of these studies demonstrate the importance of CA isoenzymes in biochemical and pharmaceutical application[95–100].

Conclusions

The allylic hydroperoxides, the corresponding alcohols and acetates **1**–**15** demonstrated effective inhibition profiles against hCA I and II. The similar inhibition profiles of these compounds for the two CA isoforms can be due to the high homology between hCA I and II. Allylic hydroperoxides, alcohols, and acetates (**1**–**15**) were identified as potent low nanomolar CAIs.

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Declaration of interest

The authors declare no conflict of interest.

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