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RESEARCH ARTICLE

Spirobisnaphthalenes effectively inhibit carbonic anhydrase

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Abstract

This study explores the correlation between human carbonic anhydrase (CA, EC 4.2.1.1) isoforms I and II (hCA I, II) and the inhibitory features of some spirobisnaphthalene derivatives. A group of spirobisnaphthalenes was synthesized and their hCA I and II inhibitory effects was investigated. The K_i values were similar for both CA isoenzymes, the compounds showing good inhibitory activity. K_i values ranged between 1.60 and 460.42 μM for hCA I and between 0.39 and 419.42 μM for hCA II, respectively. The spirobisnaphthalenes derivatives might be useful for designing CA inhibitors belonging to novel chemotypes compared to the highly investigated sulfonamides, sulfamates or coumarins.

Keywords

Carbonic anhydrase, enzyme inhibition, enzyme purification, spirobisnaphthalenes

History

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Introduction

The spirobisnaphthalenes exhibit a wide range of biological effects such as antifungal and antibacterial[1–4], antimitotic[5], antileishmanial[6] and antitumor[5,7] activities. The antitumor and antimitotic effect may result from the phospholipase D[8–10], DNA gyrase[11] or thioredoxin-reductase[12–14] inhibition. The palmarumycins belong to a relatively new and rare family of bioactive natural products and have a unique structural feature. They consist of a 1,8-dihydroxynaphthalene unit and a partially reduced naphthalene unit, which are connected to each other by a spiroacetal. The first representative, called bipendensin, was obtained in very small amounts from wood samples of *Afzelia bipendensis*[15]. However, it is assumed that a fungus living in this plant[16] produced this compound. Meanwhile, a large number of such spiro compounds were isolated mostly from endophytic fungi and named diepoxines[1], Sch plus number[8,17,18], CJ-plus number[11], cladosporins[19–21], palmarumycins[3,22–24], sphaerolones[25], decaspirones[4,17] and deoxypreussomerin[26].

The interesting structures, various biological activities and potential applications in agriculture, medicine and the food industry attracted the attention of many research groups with respect to the synthesis of spirobisnaphthalenes. In the last few years, many investigations have been carried out and several natural compounds of this family have been identified, analyzed and synthesized[27].

Carbonic anhydrase (CA, EC 4.2.1.1) is a metalloenzyme and is fairly ubiquitous, being found in animals, plants and in the microorganisms. CAs found in animal cells were shown to be different from CAs of plants or other oranisms[28–30]. Indeed, the CAs possess different structures, activities and isoforms, with six distinct genetic CA families (α-, β-, γ-, δ-, ζ- and η-CAs) being described nowadays[30–32].

The human CAs belongs to α-class. Until now, 16 isozymes have been determined. These 16 isozymes differ by molecular specifications, oligomeric arrangement, cellular localization, kinetic properties and tissues, expression levels, distribution. Some studies showed important roles of CAs in a diversity of physiological processes, and demonstrated that activities or unusual levels of these enzymes have been often associated with various human diseases[33–35].

Human catalytically active α-CA have different subcellular localization, where CA I, II, III, VII and XIII are in cytosol, CA IV, IX, XII and XIV are membrane-associated and CA VB and VA are in mitochondria. CA VI is secreted in milk and saliva[36–39].

CA is a well-characterized pH-regulatory metalloenzyme widely found in many tissues including red blood cells, gastrointestinal tract, kidneys, lungs, etc.[40,41] It quickly catalyzes the hydration of carbon dioxide to form bicarbonate, as well as the reversible dehydration reaction of bicarbonate which generates CO_2[42,43].

Inhibitors of carbonic anhydrases (CAIs) have been developed and used for the treatment of various conditions. Some inhibitors are used clinically as antiglaucoma agents; for the management of neurological disorders, as diuretics, in the treatment of osteoporosis or as antiepileptic agents. Ultimately a sulfonamide CA inhibitor entered Phase I clinical trials as an antitumor agent[44].

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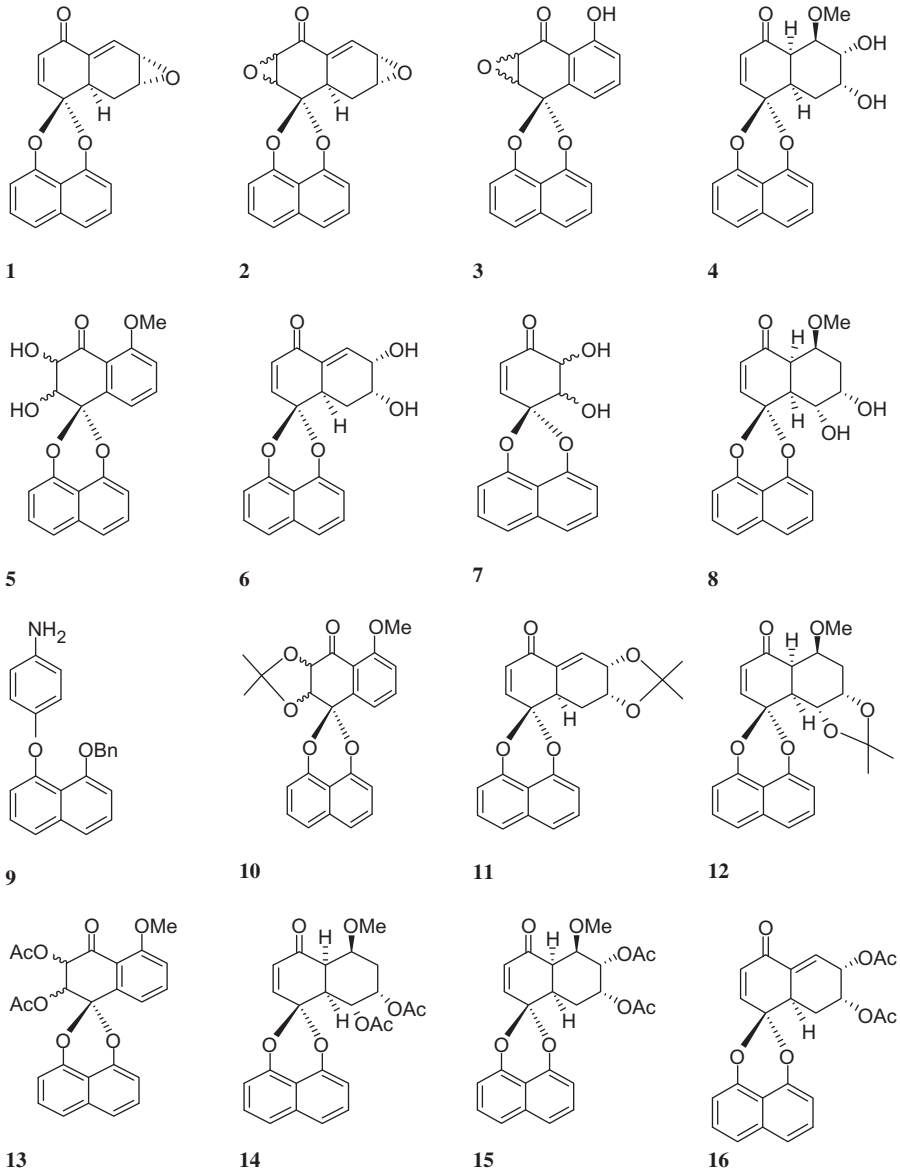

Figure 1. The chemical structures of synthesized spirobisnaphthalenes derivatives (**1–16**).

Besides on these applications of CA inhibitors, there are continued efforts in developing diverse agents for the management of cancer, obesity and other diseases in which the activity of these enzymes is disregulated[45–49].

This group has reported some inhibition studies of several CA isozymes with sulfamides. As sulfamide exhibits important biological activities, CA inhibitory properties of some sulfamide derivatives will be useful for further biological studies. It has been shown that this simple compound behaves as a weak inhibitor against the cytosolic isoforms CA I and II. Sulfonamides have R-SO_2NH_2 general chemical structure. According to the recent studies, sulfonamides have high affinity for CA. Especially for hCA isoenzymes inhibitor studies increased in recent years. At present, strong CAIs have been discovered using modified moieties such as sulfonamide or sulfamate among others[50].

The synthesis of spirobisnaphthalenes derivatives (**1–16**) was performed as described previously[51]. In this study, we have examined the inhibition effects of spirobisnaphthalenes derivatives (**1–16**) against the both human cytosolic CA isoforms (hCA I and II). Also, the inhibition results were compared to acetazolamide (AZA) and dorzolamide (DRZ).

Materials and methods

The human erythrocytes were centrifuged at 10 000× *g* for 0.5 h. Then precipitate and the serum were separated. The serum pH was adjusted with solid Tris (pH 8.7)[52–55]. As well as sepharose-4B-tirozyne-sulfanylamide affinity column balanced with Tris–HCl/Na_2SO_4 (25 mM/0.1 M, pH 8.7). Then, this sample was applied to the Sepharose-4B-tirozyne-sulfanylamide affinity column and equilibrated with Tris–HCl/Na_2SO_4 (25 mM/22 mM, pH 8.7). Finally, HCA I and II isozymes were eluted with 1.0 M NaCl/25 mM sodium phosphate (pH 6.3) and 0.1 M sodium acetate/0.5 M $NaClO_4$ (pH 5.6), respectively. All of enzyme studies were performed at 4 °C[56–60]. The protein content during the purification steps, Bradford method was used[61]. Bovine serum albumin was used as standard[62–64].

The purity of both hCA I and II was controlled by SDS polyacrylamide gel electrophoresis (SDS–PAGE)[65]. This method had been described in previous studies[66,67]. The running and separating gels contained SDS (0.1%) and 3% and 10% acrylamide, respectively[68,69]. Before loading into the gel, samples were added 1% SDS and 10% 2-mercaptoethanol and then were waited in boiling water bath for 5 min[70]. The gel was painted in coloring solution after bands were detected. This coloring solution is

Table 1. Human carbonic anhydrase I and II isoenzymes (hCA I and II) inhibition values of spirobisnaphthalenes derivatives (**1–16**), by an esterase assay with NPA.

	IC_{50} (μM)				K_i (μM)	
Compounds	hCA I	r^2	hCA II	r^2	hCA I	hCA II
1	37.54	0.9658	22.44	0.9909	35.68 ± 7.13	33.49 ± 16.36
2	45.93	0.982	18.85	0.9796	50.63 ± 13.83	30.12 ± 6.14
3	55.32	0.9907	47.80	0.9602	97.69 ± 59.86	55.48 ± 27.74
4	297.80	0.9763	126.96	0.98	254.03 ± 109.77	1.28 ± 1.43
5	345.29	0.9553	382.45	0.991	165.18 ± 53.14	71.31 ± 5.89
6	353.21	0.9916	186.59	0.9585	460.42 ± 108.86	419.42 ± 382.91
7	202.04	0.9813	158.47	0.9755	246.98 ± 18.24	185.65 ± 79.06
8	324.13	0.9787	184.41	0.9818	145.27 ± 24.01	133.98 ± 79.09
9	0.78	0.9723	0.49	0.9959	1.60 ± 0.24	0.39 ± 0.08
10	27.82	0.989	25.39	0.9833	21.22 ± 13.84	3.85 ± 0.24
11	23.71	0.99	34.08	0.9752	19.01 ± 4.22	27.07 ± 14.61
12	24.78	0.9913	19.13	0.9892	16.23 ± 5.403	14.52 ± 8.5680
13	21.16	0.9613	17.86	0.9967	9.75 ± 2.13	3.799 ± 1.32
14	14.35	0.958	17.93	0.9929	8.13 ± 3.75	11.08 ± 4.71
15	23.56	0.9738	15.63	0.962	64.05 ± 29.94	10.68 ± 1.33
16	32.09	0.9664	15.81	0.9838	4.23 ± 3.32	1.80 ± 0.92
AZA*	0.14	0.966	0.11	0.955	0.05 ± 0.01	0.04 ± 0.02
DRZ*	1.58	0.958	0.02	0.952	1.68 ± 0.34	0.02 ± 0.01

*Acetazolamide (AZA) and dorzolamide (DRZ) were used as a standard inhibitor for both CA isoenzymes.

prepared in (10%) acetic acid and methanol (50%), which contain Coomassie Brilliant Blue (R-250, 0.1%)[71].

During the isoenzyme purification and inhibition process, esterase activity studies were performed according to the Verpoorte procedure[72]. Both CA isoenzymes activities were determined by following the change in absorbance at 348 nm[73]. The K_i values were calculated from experiments using three different spirobisnaphthalenes derivative (**1–16**) concentrations and NPA as the substrate at five different concentrations to create Lineweaver–Burk curves reported previously[74–76].

Results and discussion

CA purification and activity assay

First, hCA I and II isoenzymes were purified by sepharose-4B-L-thyrosine-sulfanilamide chromatography. Then, the purity of the enzymes was detected by SDS–PAGE[53,54,77] and single band was observed for each isoenzyme. We studied the inhibition effects of spirobisnaphthalenes derivatives against hCA I and II. For this purpose, the esterase activity method was performed[78]. In this activity determination method, the phenyl acetate is hydrolyzed and the resulting product gives the absorbance at 348 nm[56,57,79].

In the last decades, many valuable bioactive secondary metabolites, which show a great variety of biological activities, have been successfully isolated from the endophytic fungi. The spirobisnaphthalenes are a growing group of fungal secondary metabolites, which contain two 1,8-dihydroxynaphthalene-derived units bridged through a spiroketal linkage. This group could be mainly classified as spairoxin, preussomerin, palmarumycin and urnucratin-type spirobisnaphthalenes, according to their structural features[51,80]. The chemical formula of spirobisnaphthalenes, which are used in this study, are given in Figure 1.

The CA isoenzymes have become an interesting target for the design of activators or inhibitors with biomedical applications. With this purpose, we have investigated the inhibitory effects of many compounds on the CA isoenzymes. In this circumstance, Lineweaver–Burk graphs were drawn for determination of inhibition effect of each spirobisnaphthalenes derivate. Then, the average of IC_{50} and K_i values were calculated from drawn graphs (Table 1). A first step, we report the inhibitory effects of derivatives spirobisnaphthalenes on the esterase activity of hCA I and II under the *in vitro* conditions. The results were summarized in Table 1.

Spirobisnaphthalenes were given to be effect inhibitors of hCA I, II (Table 1). Spirobisnaphthalenes derivatives (**1–16**) were all determined to inhibit hCA I, with K_i values ranging of 1.60–460.42 μM for spirobisnaphthalenes derivatives. Spirobisnaphthalenes derivatives (**1–16**) were all found to inhibit hCA II, with K_i values ranging of 0.39–419.42 μM for spirobisnaphthalenes derivatives.

These results demonstrated that hCA I and II are inhibited by spirobisnaphthalenes derivatives in the micromolar range. The mechanism by which these compounds inhibit the enzyme is not well understood at this moment and work is in progress to decipher it.

Conclusion

Carbonic anhydrase inhibitory properties of spirobisnaphthalenes derivatives (**1–16**) have been evaluated. These biologically active compounds generally demonstrated effective inhibition against both hCA I and II isoenzymes. Spirobisnaphthalenes **1–16** behaved as micromolar inhibitors of hCA I and II and may be used for generating more potent hCA I and II inhibitors for the treatment of glaucoma, as diuretics, for the management of mountain sickness, epilepsy, neurological disorders, gastric and duodenal ulcers, osteoporosis conditions.

Declaration of interest

The authors have declared no conflict of interest.

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