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New natural product carbonic anhydrase inhibitors incorporating phenol moieties

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ABSTRACT

Carbonic anhydrases (CAs, EC 4.2.1.1) catalyze the fundamental reaction of CO_2 hydration in all living organisms, being actively involved in the regulation of a plethora of patho/physiological conditions. They represent a typical example of enzyme convergent evolution, as six genetically unrelated families of such enzymes were described so far. The need to find selective CA inhibitors (CAIs) triggered the investigation of natural product libraries, which proved to be a valid source of agents with such an activity, as demonstrated for the phenols, polyamines and coumarins. Herein we report an in vitro inhibition study of human (h) CA isoforms hCAs I, II, IV, VII and XII with a panel of natural polyphenols including flavones, flavonols, flavanones, flavanols, isoflavones and depsides, some of which extracted from *Quercus ilex* and *Salvia miltiorrhiza*. Several of the investigated derivatives showed interesting inhibition activity and selectivities for inhibiting some important isoforms over the off-target ones hCA I and II.

1. Introduction

CO_2, bicarbonate and protons are essential molecules and ions for many important physiologic processes occurring in all living organisms, including *Archaea*, *Bacteria*, and *Eukarya*.[1,2] The interconversion of these chemical species is shown in Eq. 1, which however is too slow to meet the physiological needs of most biochemical processes.[2,3]

$$CO_2 + H_2O \rightleftarrows HCO_3^{\ominus} + H^{\oplus} \quad (1)$$

This process thus needs catalysis, which is provided by a superfamily of metalloenzymes, that is, the carbonic anhydrases (CAs, EC 4.2.1.1). They possess in their active sites a highly nucleophilic metal hydroxide species, such as zinc(II), cadmium(II) or iron(II) hydroxide, depending on the enzyme class.[1–3] Indeed the CAs represent a typical example of convergent evolution, which resulted into the development of six genetically and distinct enzyme families: the α-, β-, γ-, δ-, ζ- and η-CAs.[1–8] Many isoforms of these enzyme classes, with the exception of the δ- and η-CAs, have been characterized (e.g., crystallized) in details.[1,8–11] The distribution of the α-CAs accounts for the vertebrates, protozoa, algae, cytoplasm of green plants and in many *Bacteria* species. Since α-CAs are the only superfamily expressed in humans, they are also the most investigated ones. Mammals (except primates) possess 16 α-CA-encoding genes, that is, CA I to CA XV, with two V-type isoforms, CA VA and CA VB. The 16 different α-CA isoforms isolated and characterized so far in mammals (where they play important physiological roles) are cytosolic (CA I, CA II, CA III, CA VII, CA XIII), membrane-bound (CA IV, CA IX, CA XII, CA XIV and CA XV), mitochondrial (CA VA and CA VB) or secreted (CA VI) proteins.[1,2] Three acatalytic forms are also reported and named as CA related proteins (CARP), CARP VIII, CARP X and CARP XI, which are found in the cytosol.[1,2] The mammalian CAs were the first such enzymes isolated and studied in detail, and many of them are established therapeutic targets for the treatment of a wide range of disorders.[1–3,5,8,12–17] Indeed, diuretics, antiglaucoma, antiepileptic, antiobesity and anticancer drugs based on CA inhibitors (CAIs) are presently known, and they target various mammalian (human) α-CA isoforms.[1,12–17] The distribution of the various isoforms differs within tissues, cellular compartments as well as in consideration of physio/pathological conditions (e.g., hypoxic tumors overexpress CA IX and/or XII; the liver seems to contain predominantly CA VA and XIV, etc.).[1,2]

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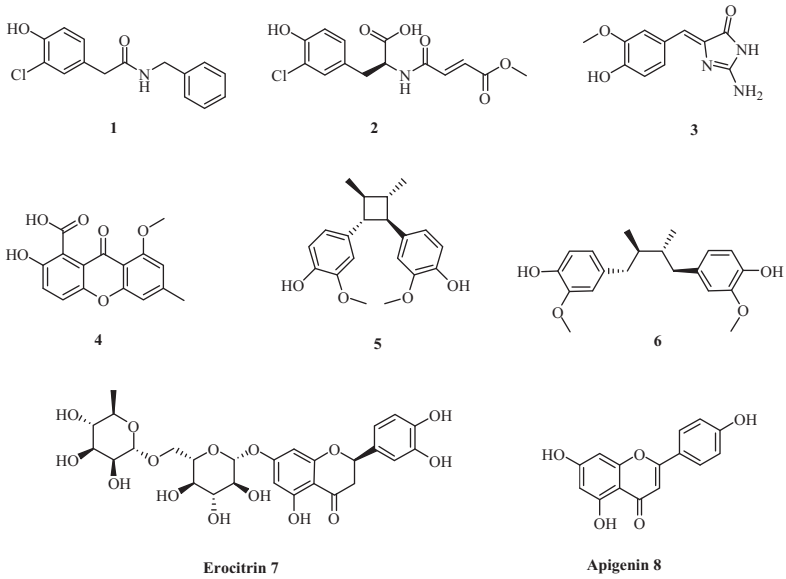

Figure 1. Natural phenolic compounds **1**–**8** selective against the mitochondrial CAs over the offtarget isoforms hCA I and II.[28,29]

The inhibition and activation mechanisms of CAs are well understood processes at the molecular level. Usually most classes of inhibitors bind to the metal center thus causing disruption of the carbon dioxide hydration reaction.[1,2,12] CA activators bind at the rim of the active site cavity and participate through a hydrogen bonds network to the proton shuttling between the metal ion–bound water molecule and the external environment,[1] thus resulting in a faster formation of the metal hydroxide species of the enzyme, which are the catalytically active forms.[1,2,11]

The classical CA inhibitors (CAIs) are the primary sulfonamides, RSO_2NH_2, which are in clinical use for more than 70 years as diuretics and systemically acting antiglaucoma drugs.[1,12,13] However the high number of isoforms in humans, as well as their diffuse localization in many tissues/organs usually represented the main hurdle in the design of sulfonamide based CAIs as therapeutic agents.[1,12,13] Among the different strategies developed for the design of selective CAIs such as the tail and the ring approaches,[12] many novel chemotypes have been explored for such an activity among which the phenols, which possess a distinct inhibition mechanism compared to the sulfonamides: they anchor to the zinc-coordinated water molecule instead of binding directly to the metal ion.[18] Recently we reported that a similar binding mode was also exhibited by polyamines such as spermine.[19] A wide range of natural and synthetic phenols have also been investigated as CAIs[20–27] but only few natural products, such as compounds **1**–**8** (Fig. 1) showed an appropriate level of selectivity for the inhibition of the mitochondrial hCA isoforms VA and VB over the cytosolic ones hCA I and II.[28,29]

Natural products represent excellent scaffolds for the search of new drugs. Especially plant polyphenols are the subject of intense research due to their antioxidant properties as well as to their occurrence in our daily diet. They play a key role in prevention and reduction of oxidative stress which is implicated in age-related disorders, such as cardiovascular and neurodegenerative diseases, type II diabetes and cancers.[30–32] Constituents **10**, **12**, **13** are common flavonoids present in most vegetables and fruits consumed, while Galangin, **11**, is present in honey and propolis.[33,34] Chrysin (**9**) is one the main flavonoids of *Passiflora incarnata* a medicinal plant used phytotherapy as a mild sedative and anxiolytic.[35] Depsides from *Salvia miltiorrhiza*, also known as Salvianolic acids (**27**–**29**), were proved both in vitro and in vivo to have protective roles in CNS neuronal injuries and degeneration.[36–39] The main mechanism involved is such processes is the decrease of reactive oxygen species (ROS) levels. Furthermore, there is evidence of the protective role of depsides in several cardiovascular diseases, osteoporosis, liver fibrosis and hepatic failure.[36–39] Naringenin **14**, Eriodictyol **15** and Hesperitin **16** (the major flavanones of edible *Citrus* spp.) possess antioxidant, anti-inflammatory and antitumoral properties.[39] The isoflavones, such as Daidzein **18**, are abundant in legumes and show potent estrogenic activity by interfering with the estrogenic receptors.[40,41] The role of isoflavones in the chemoprevention of breast and prostate cancers as well as in osteoporosis is currently under investigation.[40,41]

2. Results and discussion

2.1. Chemistry

Previous studies on simple polyphenols carried out by some of us have shown that compounds of type **1**–**8** (Fig. 1) as well as simple phenolic acids such as caffeic and ferulic acid, are effective inhibitors of the α-CAs (in the micro–nanomolar range).[20–29,42] Based on these results, herein we investigated a wider selection of polyphenolic compounds, which were selected in order to include scaffolds bearing various substitution patterns, diverse moieties and conformations, including subgroups of flavones, flavonols, flavanones, isoflavones and flavonoid glycosides, compounds **9**–**29** (Figs. 2–4).

From the structural viewpoint flavones and flavonols (structures **9**–**13**) are planar, with a carbon–carbon σ connection at position 2 and 1′ which allows for free rotation. Steric interactions between closely placed substituents in the phenyl and benzo-γ-pyrone rings may further affect the coplanarity.[43] Flavanones **14**–**16** instead, have one stereocentre (generally *S*), whereas dihydroflavonols, like Taxifolin (**17**), have two centers of asymmetry. The flavonols **24**–**26** herein investigated, were isolated from leaves of *Quercus ilex* and belong to the group of acylated flavonoids as they bear one to two coumaric acid moieties. The presence of acyl group seems to play an important role in the bioactivity of these natural constituents: acylated flavonoid glycosides possess increased antioxidant and antibacterial effects when compared to their corresponding glycosides.[41,44,45] Tiliroside