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MEDICINAL CHEMISTRY RESEARCH

REVIEW ARTICLE

The potential of chalcone derivatives as human carbonic anhydrase inhibitors in the therapy of glaucoma

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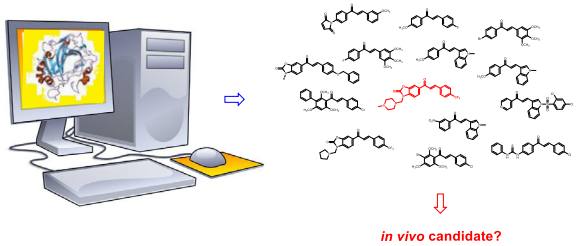
Abstract

Despite the significant development of diagnostic procedures and therapeutic options in past few decades, glaucoma is still highly prevalent and represents one of the leading causes of blindness in the world due to progressive and irreversible changes in optic nerve. Detection of carbonic anhydrase as a suitable target for the control of intraocular pressure indicated the beginning of carbonic anhydrase inhibitors application for the antiglaucoma treatment. Considering the multitude of proven and potential therapeutic applications of carbonic anhydrase inhibitors, the discovery of new chemotypes with carbonic anhydrase inhibitory activity will continue to be a significant aim. In this article we review the literature on synthetic chalcones as human carbonic anhydrase inhibitors, discussing their possible application focusing on chemical structure and Ki experimental values. From currently available experimental data and from the results we have obtained performing in silico calculations, we generated data collection on the basis of which we proposed 14 compounds of particular interest for further lead optimization and drug CAI development. Having previously experimentally determined excellent hCA II selectivity and strong inhibition effect and in our study predicted favorable physicochemical, pharmacokinetic, and toxicological profiles, a benzoxazolone chalcone derivative (**139**) stands out among the selected compounds. To examine potential therapeutic application, this candidate may be taken for further evaluation in in vivo studies.

Graphical abstract



Keywords Glaucoma · Carbonic anhydrase inhibitors · Chalcones · In silico studies

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Introduction

Glaucoma, the second most common eye disease after cataracts [1], is defined as a group of chronic ocular disorders which, if left untreated, lead to blindness as a result of irreversible damage of retinal ganglion cells and optic nerve. The most important risk factor for glaucoma onset and progression is elevated intraocular pressure (IOP) however, the disorder is associated with broader spectrum of conditions and etiologies (age, myopia, first-degree relatives diagnosed with glaucoma, thinner central corneal thickness, racial background, systemic hypertension, pseudoexfoliation etc.) [2–4]. This long-term neurodegeneration process usually remains asymptomatic, as a consequence of which glaucoma is diagnosed usually late [5] (Fig. 1).

The main principle of antiglaucoma therapy is based on lowering IOP, currently the only known modifiable risk factor, either by use of medicaments, laser therapy or surgically [6]. IOP-lowering medications function by either preventing the formation or facilitating the swelling of aqueous humor [7]. Among most commonly prescribed agents that prevent the formation of aqueous humor are carbonic anhydrase (carbonate hydro-lyases, EC 4.2.1.1 or CA) inhibitors (CAIs) that are also used as diuretics and anti-epileptics, while novel generation compounds are undergoing clinical investigation as antiobesity and antitumor drugs [8].

Carbonic anhydrases are a superfamily of metalloenzymes that catalyze the reversible hydration of carbon dioxide to bicarbonate ion and proton [9] playing a crucial role in pH regulation and in several metabolic pathways such as lipogenesis, gluconeogenesis and ureagenesis [10]. A large number of α-CA isoforms have been described in vertebrates, with Zn(II) ions at the active site and 12 catalytically active isoforms known to date, widely differing in cellular localizations (Fig. 2), tissue distribution and physiological roles, participating in many biochemical and physiological processes in which bicarbonate or carbon dioxide are substrates [11]. Modulation of CAs seems to be essential for the treatment of many diseases in which the activity of various isoforms is upregulated.

The relationship between glaucoma and CAs was established in 1950s, due to the studies on the chemistry and dynamics of aqueous humor [12–14] and to Wistrand's study [15], who demonstrated that the enzyme (forms involved in the ciliary processes), by promoting bicarbonate formation through hydration of carbon dioxide, is directly involved in production of aqueous humor. Few years after, Becker [16] showed that the sulfonamide based CAI, acetazolamide (AZA), is causing a drop of IOP in experimental animals and humans, whereas Kinsey and Reddy [17] proved that the phenomenon is due to a reduced bicarbonate production and consequent reduction of aqueous humor secretion, all conditioned by the AZA inhibition (sulfonamides tightly bind to the Zn(II) ion within the enzymatic active site, thus interrupting the carbon dioxide hydration cycle [11]). The above findings made CA a suitable target for IOP controlling, indicating the beginning of CAIs applications for the antiglaucoma treatment (Fig. 3). Many decades later, CAIs are still one of the main clinically utilized drugs that, despite the lack of isozyme selectivity (inhibiting most of the catalytically active CA isoforms, a consequence of which manifesting a wide range of side effects [18]) are used both, systemically and as topically in the treatment of glaucoma [for more details on recent advances in the medicinal chemistry of carbonic anhydrase inhibitors see an excellent review by Kumar et al. [19]].

What limits the development of effective CAIs involved in particular pathology is the lack of isoenzymes selectivity, which could lead to serious side effects [20]. Therefore, the interest is to develop not only potent CAIs, but also those with promising isoform selectivity in order to constitute a valuable therapeutic tool for the treatment of a desired pathology. For antiglaucoma (IOP-lowering hCAIs) therapy this means should be based on the selective CA targeting (i.e., on inhibition of isoforms responsible for aqueous humor secretion: CA II, IV, and XII [8]. Apart of selectivity, developing agents should possess appropriate physicochemical properties: hydrophilicity (due to drug formulation), a balanced lipophilicity (due to plasma membranes penetration ability for reaching the ciliary processes), favorable pharmacokinetics and low toxicity [18]. Not a simple task, is not it? Nevertheless, the quest for novel and more potent CA inhibitors with fewer side effects is a continuous process in context of which various scaffolds have been investigated [see three extensive reviews provided by Kumar et al. [19], Ghorai et al. [21] and Karioti et al. [22]].

Although IOP lowering with medical, laser, and surgical therapies is itself neuroprotective [23], researchers are persistently working on identifying agents that are able to provide neuroprotection independent of IOP reduction. Oxidative stress, excitotoxicity and neuroinflammation are additional factors associated with the pathophysiology of glaucoma, therefore it is assumed the use of agents with antioxidant, antiapoptotic and anti-inflammatory potential could be beneficial in protecting neuronal integrity and function [24].

Considering information presented so far, desirable scenario in quest for novel therapeutic agents for glaucoma and ocular hypertension treatment could be a structure with excellent CAIs properties accompanied by antioxidative and antiinflamatory activity, and favorable toxicological and pharmacokinetic profile.

Chalcones (*trans*-1,3-diaryl-2-propen-1-ones), the main precursors in flavonoid biosynthesis, represent a group of

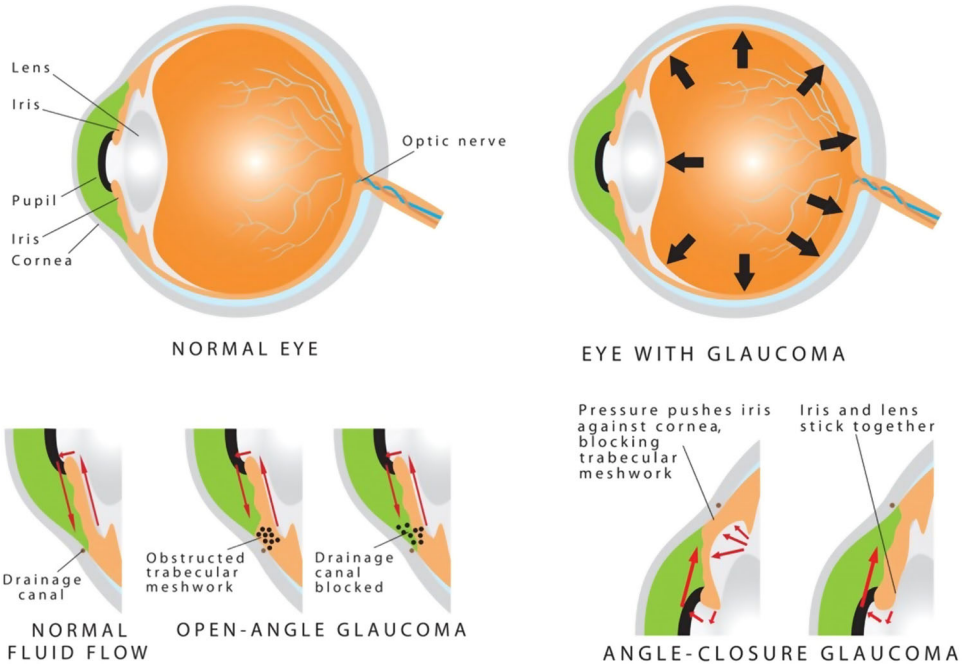

Fig. 1 Normal eye vs eye with glaucoma. In a healthy eye constant flow of aqueous humor, a transparent liquid rich in bicarbonate secreted by the ciliary body, is maintained. An increase in IOP is the result of fluid building up, caused by misfunction of trabecular meshwork cells to maintain a balanced pressure by allowing aqueous humor to flow out. Common types of glaucoma are herein presented, classified based on the appearance of the drainage system within the eye (taken from https://www.glaucomaassociates.com/glaucoma/types-of-glaucoma/ [72])

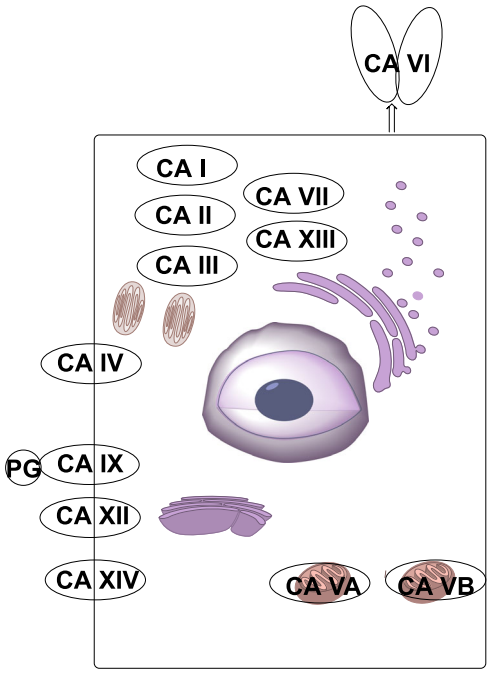

Fig. 2 Localization and multimerization of catalytically active hCA isoforms in the cell. Isoforms CA I, CA II, CA III, CA VII, and CA XIII are cytosolic, CA IV is anchored to the membrane via a covalently attached lipid moiety, CA VA and CA VB are found in mitochondria, CA VI is excreted in human saliva and milk, and CA IX (bears a proteoglycan-like (PG) domain), CA XII, and CA XIV are membrane-bound via a single transmembrane α-helix with the catalytic domain outside of the cell. Catalytically inactive isoforms CA VIII, CA X, and CA XI are not shown in the figure. In eye, the cytosolic isoforms CA I, CA II and two membrane-bound isoforms CA IV and CA XII are present [8, 19]

lipophilic plant-derived polyphenolic compounds [25] consisting of two aromatic rings (A and B) linked by α,β-unsaturated carbonyl unit (Fig. 4). α,β-Unsaturated carbonyl unit is a good Michael acceptor and participates in target selective Michael addition, a specific mechanism reported for post-translational modification (PTM), involving covalent additions to amino acid side chains and regulates protein function in cell signaling pathways and biological processes [26]. As an electrophilic moiety, α,β-unsaturated carbonyl function reacts with amino acids containing nucleophilic side chains (Cys, His, Lys, and Ser) and those covalent interactions are regarded as partial explanation for remarkable spectrum of pharmacological activities [27, 28].

Having high plasticity for chemical modifications, allowing structure building on a rigid platform, chalcones are considered as privileged scaffolds in the design of pharmacological trials [29]. In order to obtain compounds with superior cytotoxic properties, modification of the basic scaffold has been performed by modulating the aromatic residues, replacing aromatic residues with heteroaryl units, and obtaining hybrid molecules [27].

Since both, natural and synthetic chalcones are known for impressive antioxidative, antiinflamatory and neuroprotective effects [30–32], would be rational to assume that these molecules, by preventing neurodegenerative changes in retinal ganglion cells and optic nerve, could express favorable influence in glaucoma pathogenesis. In addition, as many studies shown, chalcones possess a wide variety of cytoprotective and modulatory functions which may have therapeutic potential for multiple diseases [33–36]. Because

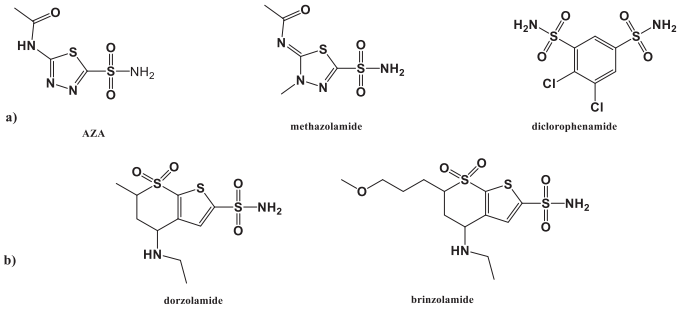

Fig. 3 Structures of CA inhibitors in clinical use: (**a**) the first generation of CAIs, sulfonamide-containing molecules AZA, methazolamide, and dichlorophenamide, agents intended for oral use. In a dose-dependent manner, oral AZA lowers IOP by about 30% and despite a non-selective strong inhibitory effect on hCAs is still considered as one among the most effective CAIs and IOP-lowering agents and (**b**) dorzolamide and brinzolamide, topically acting CAIs [7, 73]

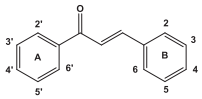

Fig. 4 Chemical structure of *trans*-chalcone

of their flexible structure, chalcones can effectively bind to many enzymes and receptors and recently, several studies involving chalcones, reported compounds of the type as potent CA I and II inhibitors [37–50].

For this reason, we aimed our current work towards evaluation of synthetic chalcone derivatives, previously assayed on hCA inhibitory potential, for in silico physicochemical, toxicological, and pharmacokinetic properties, highlighting the most suitable as ligands for the design of novel CAIs with high isoform selectivity and of particular interest for the future development as drug leads. All data related to the chalcone structures involved in CAI experiments were retrieved from the website. The corresponding experimental values were expressed as K_i and/or IC_{50}, depending on the assay type. For the analyses, only the ligands associated to a K_i and/or IC_{50} value for the hCA isoforms present in the eye/responsible for aqueous humor secretion were taken into account. Whenever was possible, in order to better process the results coming from different laboratories, selectivity ratio (SR K_i hCA I/K_i hCA II, K_i hCA I/K_i AZA and K_i hCA II/K_i AZA) calculations were made on the basis of which the results were also commented. Among the essentials, preclinical data are considered a basic requirement in the development of new drug candidates, and various physicochemical properties can be predicted by in silico approach models, all of which are available from drug evaluation tools. The structures of all ligands in SMILES notation were imported online into drug evaluation tools *SwissADME* [51] and *OSIRIS Property Explorer* [52] to predict physicochemical descriptors as well as pharmacokinetic and toxicological properties of the candidate molecules.

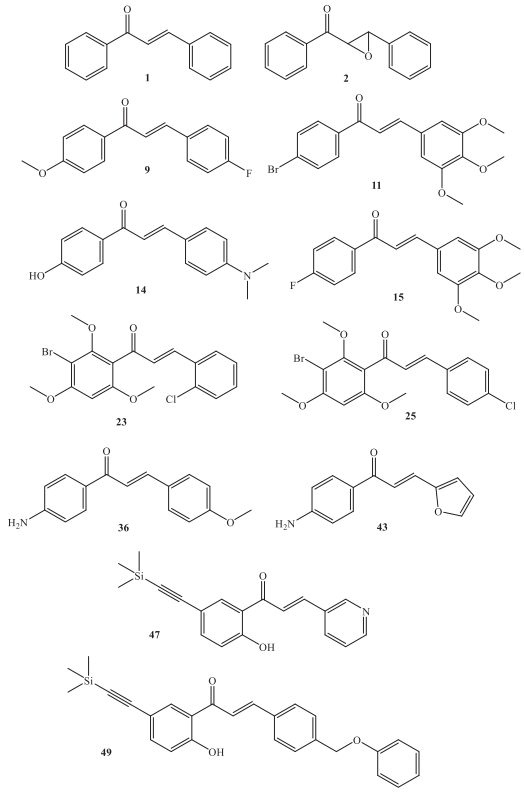

Fig. 5 Simple chalcone derivatives as CA inhibitors

Simple chalcone derivatives

The CA inhibitory potential of the simplest chalcone, (*E*)-1,3-diphenylprop-2-en-1-one or *trans*-chalcone (**1**), and its epoxide (**2**) (Fig. 5), was tested on hCA I and hCA II isoforms obtained from haemolyzed erythrocytes [53]. Compounds **1** and **2** inhibited both isoforms in a noncompetitive mode with similar K_i values (0.035 and 0.037 µM towards hCA I, respectively; 0.142 and 0.173 µM towards hCA II, respectively), but were less effective compared to the reference inhibitor AZA ($K_i = 0.016$ and 0.024 µM against

hCA I and hCA II, respectively). Compound **2** was subjected to molecular docking study which showed that epoxide oxygen might be responsible for interactions within hCA I active site (hydrogen bond interactions with Gln92). According to the authors [53], this type of interaction was not observed for **2** in the hCA II active site, but hydrogen bonding between the carbonyl oxygen with Gln92 and a water molecule was detected instead. Within hCA I of importance for the structure **2** were π-π stacking interactions between the benzene ring and His94, however inside the hCA II active site this interaction lacked. The authors concluded that aromatic rings are having more important role in enzyme inhibition than α,β-saturated system [53].

Aslan et al. [54] tested a set of seven simple chalcones (compounds **3-9**, Table S1) obtained from acetophenones and related benzaldehydes for their inhibitory activity on the isolated hCA I and hCA II. Evaluated derivatives inhibited both isoenzymes in a competitive manner, with K_i values ranging from 1.83 (compound **9**) to 7.05 µM for hCA I, and from 0.59 (compound **9**) to 5.50 µM for hCA II (Table S1, Fig. 5). The nature and the position of the groups attached to A and B aromatic rings have substantial significance for the enzyme inhibition activity; hCA I potency change in 2′-hydroxy-4-methoxychalcone (**3**) compared to 4-methoxychalcone (**6**) was observed as a result of hydroxyl group B ring addition, while by adding fluoro group to 4-fluorochalcone (**4**) at position 4 on ring B, a new compound (**8**) with increased inhibitory effects toward hCA I was obtained. Those changes induced selectivity since opposite effect on hCA II was observed (Table S1) [54].

Another group of derivatives (compounds **10–14**, Table S1), synthesized by Dizdaroglu et al. [37] were investigated for hCA I and hCA II inhibitory potential and were found to have K_i values in the low nanomolar range. All compounds had better hCA I inhibition activities (Table S1) than AZA ($K_i = 250$ nM). Among the tested chalcones, **11** (Fig. 5), bearing bromo-substituted A ring and trimethoxy-substituted B ring, had the most potent hCA I inhibition activity ($K_i = 8.03$ nM). Regarding the hCA II isoform, chalcone with hydroxy-substituted A ring and dimethylamino-substituted B ring (**14**, Fig. 5), proved to be the most potent and the only among assayed compounds with similar inhibitory activity as AZA ($K_i = 11.77$ nM and $K_i = 12$ nM, respectively) [37], based on which can be concluded that *N*,*N*-dimethylamino substituent on ring B could have large impact on the activity toward hCA II.

Several *p*-halogen/*p*-methoxy A ring-trimethoxy-substituted B ring- chalcones (compounds **15–21**, Table S1) were tested against cytosolic isoforms. CA inhibitory activities, measured in nanomolar concentrations (Table S1), were depended on the identity of A ring *p*-substitution, confirming the known fact that the halogen substituted compounds are effective as CA inhibitors [55]: A ring *p*-fluoro-substituted **15** (Fig. 5) was the best hCA I inhibitor ($K_i = 8.75$ nM), while already mentioned A ring *p*-bromo substituted **11** was the best hCA II inhibitor ($K_i = 11.47$ nM), both being more effective than reference inhibitor AZA (K_i (hCA I) = 28.75 nM; K_i (hCA II) = 31.67 nM) [38].

Burmaoglu et al. [39] synthesized eight novel halogen-containing chalcone derivatives (**22–29**, Table S1) with 3′-bromo-2′,4′,6′-trimethoxy-substituted A ring, differing only in type and position of halogen-substituted B ring. The results obtained by evaluating hCA I and hCA II inhibition effect have shown that derivatives have potent inhibitory profiles on both isozymes, with K_i values in the range of low nanomolar concentrations (16.24–40.96 nM for hCA I, 29.61–67.15 nM for hCA II) (Table S1). It could be observed that all compounds expressed slightly weaker hCA II inhibitory activities compared to AZA ($K_i = 22.17$ nM) however, found to be up to eight times more effective hCA I inhibitors than AZA ($K_i = 141.02$ nM). With the lowest K_i values, compounds **23** (with *ortho*-chloro-substituted B ring) and **25** (with *para*-chloro-substituted B ring) (Fig. 5) were the most effective against hCA I and hCA II isoforms, respectively. The study has indicated pivotal influence of methoxy groups in the A ring for the inhibition of the enzyme receptors: via metal coordination with Zn301 in the catalytic active site of the hCA I or by hydrogen bonding with Try7 and Asn67 residue of hCA II receptor [39]. The confirmation resulted from docking studies which were also part of the research.

Gürdere et al. [40] have investigated inhibition profile of different B ring substituted 4′-aminochalcones (compounds **30–44**) towards hCA I and hCA II. Synthetized compounds were more effective inhibitors than AZA (K_i (hCA I) = 83.39; K_i (hCA II) = 104.60 nM), having K_i values ranging from 2.55 to 11.75 for hCA I and from 4.31 to 17.55 nM, with 2-furyl-inserted derivative ((*E*)-1-(4-aminophenyl)-3-(furan-2-yl)prop-2-en-1-one) **43** as the most potent hCA I inhibitor ($K_i = 2.55$ nM), and 4′-amino 4-methoxy derivative **36** as the most potent hCA II inhibitor ($K_i = 4.31$ nM) (Fig. 5) [40].

In an attempt to obtain effective hCA II inhibitors, Mahar et al. [56] performed a synthesis of ten 2′-hydroxy-5′-((trimethylsilyl)ethynyl)chalcones (compounds **45–54**, Table S1). According to the results obtained by the enzyme inhibitory assay, all compounds exerted better hCA II inhibition effect than AZA ($IC_{50} = 0.998$ µM) (Table S1), with pyridine derivative **47** and compound **49** (Fig. 5) being the most potent ($IC_{50} = 0.033$ and 0.054 µM, respectively). For compound **49**, which also exhibited strong antioxidant activity and had good binding energy value in in silico study, ligand-protein binding analysis revealed three

hydrogen bonds and π–π stacking interactions at the protein active site [56].

Aryl-substituted chalcone derivatives

Burmaoglu et al. [41] designed a series of nine novel chalcone derivatives (compounds **55–63**, Table S2) and explored their inhibitory activities towards cytosolic hCA I and hCA II isozymes. Tested compounds had the same A ring (3-phenyl-2,4,6-trimethoxy-substituted) structural motif, varying B ring substitution pattern. The synthesized chalcone derivatives showed K_i values in the range of 14.71–62.95 nM against hCA I and 28.93–47.20 nM against hCA II (Table S2). The most effective among compounds was *p*-chloro substituted **58** showing good selectivity for hCA I isoform, while the most promising inhibitory effect against hCA II was for **56** (*o*-chloro-substituted B ring) compound (Fig. 6). However, no compound was more effective than the reference AZA ($K_i = 8.45$ and 4.25 nM for hCA I and hCA II, respectively) [41].

N-substituted (amino-/imino-/imido-/diazo-) chalcone derivatives

Yamali et al. [42] designed a series of nine aminoalkylated phenolic chalcones (compounds **64–72**, Table S3) via aminomethylation of the corresponding chalcones. The compounds were further screened for CA I and CA II

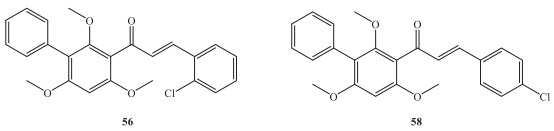

Fig. 6 Aryl-substituted chalcone derivatives

inhibition effect. According to the K_i values (Table S3), all bis-Mannich bases expressed considerable CA inhibitory activities comparable to AZA (K_i (hCA I) = 21.74 nM; K_i (hCA II) = 18.27 nM) with more than half emerging as more potent than this antiglaucoma drug. The lowest K_i values were observed for piperidine derivative **64** ($K_i = 11.76$ nM) considering hCA I isoform, and morpholine derivative **68** ($K_i = 6.08$ nM) when it comes to hCA II isoform (Fig. 7) [42].

A series of 16 chalcone derivatives (compounds **73–88**, Table S3) was prepared by condensation of amino-substituted acetophenones with benzaldehydes in 1:2 molar ratio and subjected to in vitro analysis for hCA I and hCA II inhibition potencies. All of the tested compounds expressed remarkable inhibition activities (Table S3) as compared with standard AZA ($K_i = 859.07$ and 1022.20 nM for hCA I and hCA II, respectively). According to both K_i and IC_{50} values, *p*-methoxy substituted **87** was the most potent hCA I inhibitor ($K_i = 141.88$ nM; $IC_{50} = 226.10$ nM), while compound **74** with *m*-chloro substituent showed the best inhibition activity against hCA II ($K_i = 199.31$ nM; $IC_{50} = 295.28$ nM) (Fig. 7) [57].

Kocygit et al. [58] synthesized six chalcone-imide derivatives (compounds **89–94**, Table S3) and performed biochemical analysis towards both hCA I and hCA II. Cytosolic hCA I form was inhibited by these derivatives with K_i values (426.47–699.58 nM, Table S3) slightly lower than that of the reference inhibitor (977.77 nM). All derivatives were stronger inhibitors of hCA II compared to hCA I, with K_i values (214.92-532.21 nM, Table S3) up to four times lower than that of AZA (904.47 nM). Despite different electron withdrawing and electron donating properties of substituents on B ring of chalcone derivatives, a minimum difference in inhibitory potencies were observed. Among the tested compounds, **91**, with *m*-methyl-

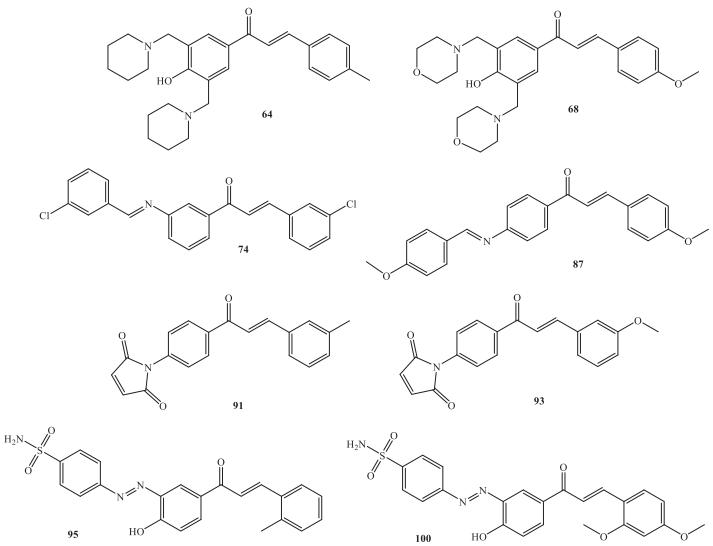

Fig. 7 *N*-substituted chalcone derivatives

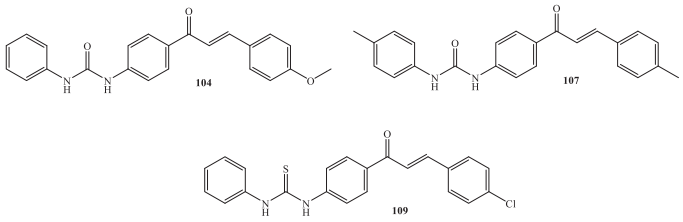

Fig. 8 Phenyl(thio)urea chalcone derivatives

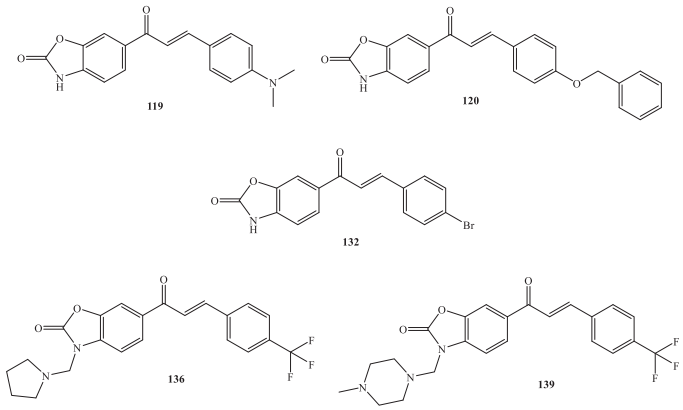

Fig. 9 Benzoxazolone chalcone derivatives

substituted B ring, was the most effective hCA I inhibitor ($K_i = 426.47$ nM), while compound **93**, with *m*-methoxy-substituted B ring was the most effective inhibitor of the hCA II isoform ($K_i = 214.92$ nM) (Fig. 7) [58].

Considering sulfonamides are among the first discovered CA inhibitors clinically used for decades (AZA), Arslan et al. [43] prepared a series of chalcone derivatives with benzensulfonamide moiety linked to the A ring via diaza bond (compounds **95–100**, Table S3). Methyl and methoxy groups were incorporated to the B ring chalcone moiety to induce a slightly lipophilic character to the molecule already contained the hydrophilic phenol and diazo moieties. The derivatives were tested towards cytosolic hCA I and hCA II isoforms, all exhibiting good inhibitory profiles. Isoform hCA I was effectively inhibited by all derivatives with K_i values (9.88–24.40 nM, Table S3) significantly lower than that of the reference AZA (250 nM) with 2,4-dimethoxy derivative **100** (Fig. 7) as the most potent inhibitor. Good inhibitory activities of new compounds towards hCA II with K_i values (18.25–55.43 nM, Table S3) higher than the reference AZA (12.00 nM) were observed. 2-Methyl derivative **95** (Fig. 7) was the most promising among the investigated hCA II, comparable to the clinically used AZA [43].

Phenyl(thio)urea chalcone derivatives

A group of eight phenylurea- (compounds **101–108**, Table S4) and four phenylthiourea- (compounds **109–112**, Table S4) substituted chalcones was synthesized and evaluated for hCA I and hCA II inhibitory activity. The results showed that all the synthesized compounds inhibited CA isoenzymes activity with IC_{50} values in micromolar range (Table S4). The most active hCA I inhibitor within phenylurea derivatives was compound **104** ($IC_{50} = 23.06$ μM) substituted with a methoxy group in *para* position of the B ring. When it comes to hCA II inhibition, the addition of a methyl group in *para* position of both phenylureinyl part and B ring (compound **107**) led to the highest inhibitory effect ($IC_{50} = 14.40$ μM). Regarding phenylthiourea derivatives, compound **109**, bearing a *p*-chloro-substituted B ring, stood out as the most potent inhibitor of both hCA I and hCA II ($IC_{50} = 36.26$ and 23.89 μM, respectively) isoforms (Fig. 8) [59].

Heteroaryl chalcone derivatives

Benzoxazolone incorporated chalcone derivatives

Bilginer et al. [60] designed and synthesized a set of eight benzoxazolone chalcone derivatives (**113–120**, Table S5) evaluating the compounds in hCA I and hCA II inhibitory activity assay. The compounds showed good to moderate CA inhibitory properties, with only compound **120**, bearing a *p*-benzyloxy substituted B ring, having a lower K_i value (28.37 μM) towards hCA I than AZA (30.18 μM), while towards hCA II all compounds had higher K_i values (10.85–37.96 μM) than AZA (4.41 μM) which qualified them as worse inhibitors than the reference (Table S5). However, as the most promising hCA II inhibitor stood out 4-dimethylamino derivative **119** ($K_i = 10.85$ μM) (Fig. 9) [60].

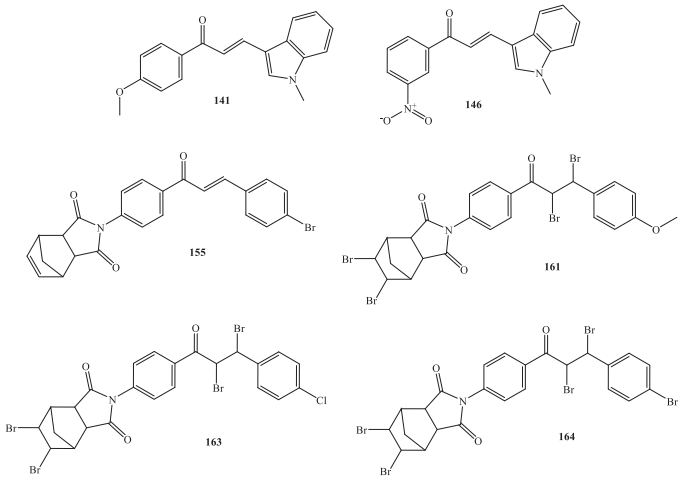

Fig. 10 Simple indole and methanoisoindole chalcone derivatives

Under the same experimental conditions, Bilginer et al. [61] conducted another study concerning inhibitory properties of benzoxazolone chalcone derivatives towards hCA I and hCA II, this time including halogen-bearing compounds. The tested derivatives (**121–132**, Table S5) differed in number and position of halogen substituents in the B ring. Similar to the abovementioned series, this group of chalcones also showed moderate inhibition profile, with compound **132** (Fig. 9) being the most active towards both hCA I and hCA II ($K_i = 33.5$ and 7.3 μM, respectively). However, no compound was more effective CA inhibitor than AZA ($K_i = 30.2$ and 4.4 μM for hCA I and hCA II, respectively) [61].

Based on the results of both studies, Bilginer et al. [44] designed a series of *N*-substituted-benzoxazolone chalcone derivatives (compounds **133–139**, Table S5) starting from compound **116** which in previous experiments already proved good inhibitory activity on both isoenzymes. Structural modifications were made by aminomethylation of the benzoxazolone ring (Mannich bases). The in vitro inhibition assay results, expressed as K_i values, revealed that aminomethylation significantly improved the inhibition potential of the tested derivatives on cytosolic hCA I and hCA II. Pyrrolidine derivative **136** was found to be the most potent hCA I inhibitor among the tested compounds, with K_i value (0.0123 μM) nearly seven times lower than that of AZA (0.0844 μM). For hCA II the most potent inhibitor was piperazine derivative **139** with a K_i value (0.0086 μM), approximately seven times lower than that of AZA (0.0592 μM) (Fig. 9). Molecular docking emphasized the importance of carbonyl group for the formation of hydrogen bonds with the amino acids present in CAI enzyme active site (thus the prevention of tautomerisation between carbonyl group and nitrogen atom in the benzoxazolone ring by inserting an aminomethyl moiety was crucial for increased CAI potency) and according to the binding pattern of the most potent CAI inhibitor, important interactions were the π-cation interaction (exhibited between the benzoxazolone ring of the compound and the side chain of the amino acid His200), seven hydrophobic interactions (between the compound **136** and the amino acids Phe91, His119, Leu131, Val143, Leu198, and Trp209) and a strong halogen bond-hCA I isoenzyme active site (trifluoromethyl group-Phe91) interaction [44]. Molecular docking study of **139** and hCA II active sites emphasized hydrogen bond interaction between carbonyl group in benzoxazolone with Gln92 once again demonstrating positive contribution of tautomerization prevention on hCA II inhibitory activity. Two additional hydrogen bond interactions were observed between the oxygen atom of the benzoxazolone ring and the amino acids Asn62 and Asn67. The carbonyl group of chalcone participated in a hydrogen bond interaction with Trp5. Indicated also were π-stacking interactions between **139** benzoxazolone ring and the amino acid His94. Four hydrophobic interactions between the molecule **139** and Trp5, Phe20, and Pro202 in the CA II active site were also predicted [44].

(Iso)indole incorporated chalcone derivatives

Indole and methanoisoindole heterochalcone derivatives *Simple indole incorporated chalcone derivatives:* Kuday et al. [62] synthesized a group of seven simple indole chalcone derivatives (compounds **140–146**, Table S5) in order to evaluate their hCA I and hCA II inhibitory properties. From the obtained results (Table S5), the authors have drawn SAR observations indicating that a presence of one methoxy group at *para* position of the A ring (compound **141**) increased the inhibitory potential of indolyl chalcones towards hCA I ($IC_{50} = 7.42$ μM). However, the incorporation of a strong electron-withdrawing group at the same position rendered strong hCA II inhibitory effect, as observed with the *p*-nitro-substituted derivative **146** ($IC_{50} = 7.22$ μM) (Fig. 10) [62].

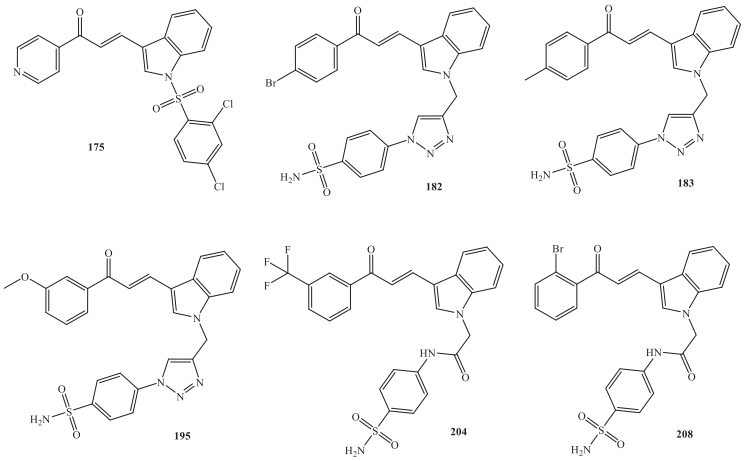

Fig. 11 Indole-sulfonamide chalcone derivatives

Methanoisondole incorporated chalcone derivatives: Kocyigit et al. [45] provided a series of 14 novel methanoisoindole chalcone derivatives (compounds **147–160**, Table S5) and evaluated their hCA I and hCA II inhibitory activity. All synthesized compounds elicited remarkable inhibitory activity against both hCA I and hCA II with IC_{50} (466.4–710.8 pM and 352.5–555.3 pM, respectively) lower or similar to those of AZA (995.7 and 485.0 pM for hCA I and hCA II, respectively). Regarding K_i values, the inhibitory effect of the tested compounds was in the range of 405.3–635.7 pM for hCA I, and 245.4–489.6 pM for hCA II. Taken together, compound **155** with bromo substituent at *para* position of the B ring stood out as the most promising inhibitor of both CA isoforms (Fig. 10) [45].

By investigating in vitro hCA I and hCA II inhibitory potential, Kocyigit et al. [46] expanded their research to tetrabromo-substituted methanoisoindole chalcone derivatives. Compounds **161–169** expressed superior inhibitory effect towards both isoforms in comparison to AZA (IC_{50} (hCA I) = 40.45 nM; IC_{50} (hCA II) = 24.16 nM; K_i (hCA I) = 34.50 nM; K_i (hCA II) = 28.92 nM) in nanomolar concentrations for both IC_{50} and K_i (Table S5) having the best hCA I inhibitory activity/potency for *p*-methoxy- and *p*-substituted B ring linked to 5,6-dibromosubstituted-4,7-methanoisoindole-1,3-dione chalcone derivatives **161** (IC_{50} = 14.14 and K_i = 11.30 nM) and **164** (IC_{50} = 13.58 nM and K_i = 13.09 nM). *p*-Chloro substituted B ring derivative **163** was the most effective inhibitor of hCA II isoform (IC_{50} = 9.62 nM and K_i = 8.20 nM) (Fig. 10) [46].

Molecular docking studies were performed on both isoenzymes, for compounds **161** and **163** that had the most effective inhibition/binding to the ligand binding site of hCA I and hCA II proteins. The hCA I polar interactions were described with residues His67, His64, His200, Gln92, His94, and Thr199, while hydrophobic interactions were formed by Trp5, Pro202, Pro201, Val207, Trp209, Leu198, Val143, Ala121, Phe91, and Val62 residues. Two different bond type interactions were formed by the residues in the ligand-binding site in hCA I: the hydrogen bond formed by Trp5 and the π–π bond formed by His94. At the hCA II isoenzyme binding site, polar interactions with His64, His94, Asn62, Gln92, and Thr200 and hydrophobic interactions with Trp5, Pro202, Leu198, Val121, and Phe131 residues were observed. A hydrogen bond was formed with Trp5 [46].

Indole incorporated chalcone-sulfonamide hybride derivatives Compounds belonging to this group were designed as ligands interacting with the middle and outer parts of the active site cavity, known as the most variable regions among the hCA isoforms. This concept was supposed to provide isoform-selective CAIs.

Pyridyl-indole incorporated chalcone-sulfonamide hybride derivatives: Peerzada et al. [63] synthesized a series of several sulfonamide derivatives of pyridyl-indole heteroaryl based chalcones (compounds **170–178**, Table S5), screened for in vitro hCA inhibitory activity (hCA II isoenzyme was chosen as off target). Compounds had more inhibition effect on hCA II isoform, having IC_{50} values (>9.10 µM) higher than that of AZA (0.013 µM) with 2,4-dichlorophenyl-substituted **175** (Fig. 11) as the most active derivative [63].

Indolylchalcone incorporated- benzensulfonamide triazole linked hybride derivatives: Singh et al. [47] synthesized a series of 17 indolylchalcone derivatives linked to the benzenesulfonamide group via the triazole ring (compounds **179–195**, Table S5), and tested their inhibitory activity against cytosolic hCA I and hCA II. Among the assayed derivatives *p*-bromophenyl and *m*-methoxyphenyl substituted hybrids (**182** and **195**, respectively) were the most potent against hCA I isoform (K_i = 18.8 and 38.3 nM, respectively) (Fig. 11), also being more potent than AZA inhibitor (K_i = 250 nM). Molecular docking study performed on two representative (the most active **182** and

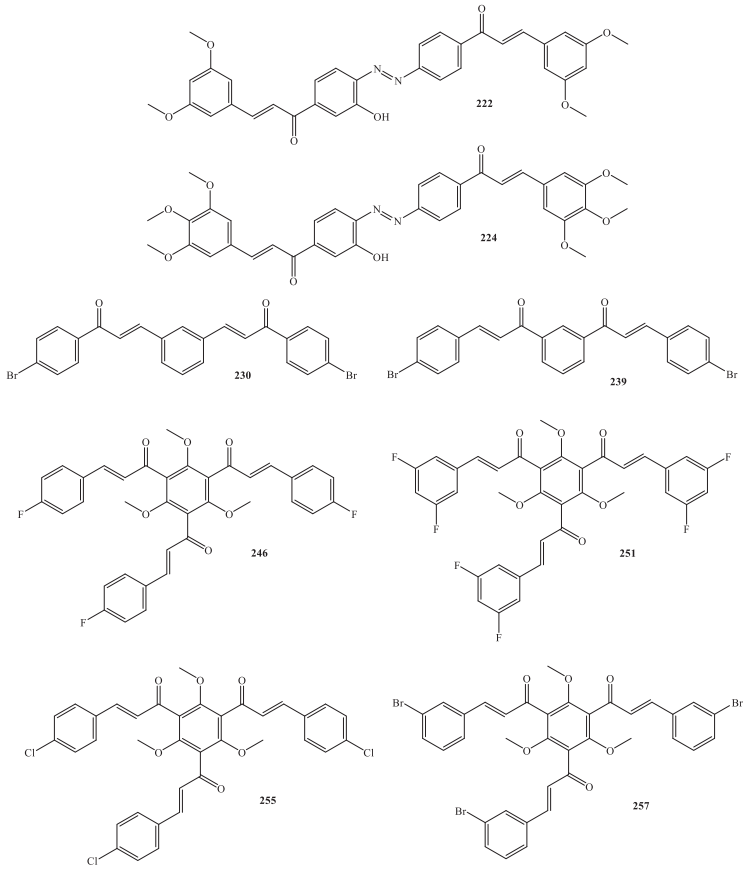

Fig. 12 Bis- and tris-chalcone derivatives as CA inhibitors

195) compounds co-crystalzing at the hCA I active site rationalized the experimental results: elicited potency is for favorable interactions with key residues of the hCA I pocket. By occupying the isoenzyme's active pocket in a fitting manner, both compounds made several positive interactions with hCA I key residues. For **182** and **195** the O atom from sulfonamide moiety, acting as hydrogen bond acceptor, was responsible for Thr199 and His200 hydrogen bond interactions, while the other O and N atom of sulfonamide moiety established metal ion interactions with Zn(II). Triazole core made an arene-arene (π–π) interaction with Phe91 and several stabilizing hydrophobic interactions were observed with Ile60, Val62, Phe91, Ala121, Leu131, Ala132, Ala135, Leu141, Val143, Leu198, Pro202, and Trp209 (the active site residues stabilizing the binding of **182** and **195** in hCA I active site). The authors speculated the importance of electronic effect of substituents attached to the core, presuming electron donating group (-CH_3 (**182**) -OCH_3 (**183**) and -Br (**195**)) A ring substituted compounds showed good inhibitory activity as compared to those containing electron withdrawing groups. Those structures, being more potent than the standard AZA, were anticipated as potential lead molecules for the design and development of selective hCA I inhibitors. As far as hCA II isoform was concerned, the most potent against was *p*-methylphenyl derivative **183** (Fig. 11) ($K_i = 36.2$ nM), although characterized with weaker inhibition properties when compared to AZA ($K_i = 12.1$ nM) [47].

Indolylchalcone incorporated -benzensulfonamide amide linked hybride derivatives: Singh et al. [48] similar to structures reported in Singh et al. [47], provided a series of hybrids, indolylchalcone-benzensulfonamide derivatives linked via amide linker (compounds **196–213**, Table S5), and tested them against hCA isoforms. Interestingly, amide linker significantly elicited inhibitory effect against hCA II and those derivatives were found to be more selective inhibitors of hCA II when compared to other hCA isoforms. Compounds **204** ($K_i = 2.3$ nM) and **208** (Fig. 11) ($K_i = 2.4$ nM) were found to be the most potent within the tested series, more potent against hCA II than AZA ($K_i = 12.1$ μM) and could be further examined in the design of novel efficient hCA II inhibitors as lead compounds [48].

Oligomeric chalcone derivatives

Bis-chalcone derivatives

Diaza linked 1,4-bis chalcone derivatives A group of 11 bis-chalcone derivatives (compounds **214–224**, Table S6) was prepared by Arslan et al. [64] via diazotization and

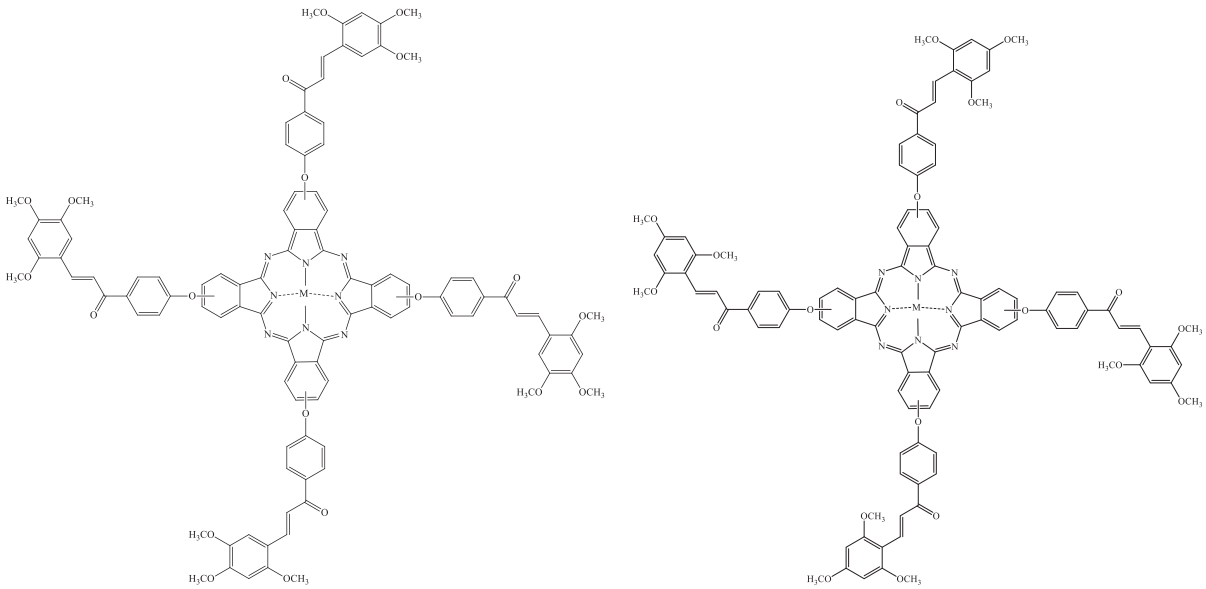

Fig. 13 Tetra-chalcone derivatives as CA inhibitors

diazocoupling reactions, and tested for cytosolic hCA I and hCA II inhibitory effect. Monomeric chalcone units were interconnected by -N=N- bond formed between A rings in *para*- position, generating bis-chalcones differing only in number and position of B ring methoxy groups. Among the investigated bis-chalcones containing single methoxy substituent in the monomeric unit, compound with *m*-methoxy group in the B ring showed better hCA I inhibitory activity compared to its nearly two times less effective *o*- and *p*- analogs (Table S6). The addition of one more methoxy group in the position 5 (compound **222**) or two more methoxy groups in positions 4 and 5 (compound **224**) of the B ring (Fig. 12) resulted in a significant enhancement of hCA I inhibitory activity, making these two compounds the most potent hCA I inhibitors among the tested derivatives, with K_i values (72.90 and 103.55 nM, respectively) lower than that of AZA ($K_i = 250$ nM). A rather similar inhibition profile was observed for hCA II, with compound **222** being the most active among the methoxy-substituted derivatives ($K_i = 166.54$ nM). However, there were no compounds as active as the reference inhibitor was ($K_i = 12$ nM) [64].

1,3-Bis chalcone derivatives A group of 19 1,3-bis-chalcone derivatives (**225-243**, Table S6), with monomeric chalcone units symmetrically condensed, sharing either A (**234–242**) or B (**225–233**, and **243**) phenyl ring as structure motif, were tested in vitro against hCA I and hCA II. Both isoforms were effectively inhibited by these compounds, with K_i values in the range of 94.33 to 787.38 nM for hCA I, and of 100.37 to 801.76 nM for hCA II, respectively (Table S6) which was in contrast to the higher K_i values for clinical standard AZA (1054.38 nM against hCA I, and 983.78 nM against hCA II). Moreover, IC_{50} values for the tested derivatives (ranged from 98.63 to 855.44 nM for hCA I and from 77.33 to 717.77 nM for hCA II, Table S6) were lower than those for the reference inhibitor (997.67 and 901.36 nM for hCA I and hCA II, respectively). Among the derivatives "sharing" A ring, a remarkable activity was observed for bromine derivative **239** (Fig. 12) with nearly 11 times lower K_i and IC_{50} values than AZA for both isoforms. Regarding derivatives "sharing" B ring, the striking feature of the most promising CA inhibitor was bromo substitution (**230**, Fig. 12) [65]. For the tested 1,3-bis-chalcone derivatives, it could be observed that the presence of a halogen substituent can have a large impact on the hCA inhibitory activity compared to the presence of some other substituents such as, for example, methoxy groups.

Tris-chalcone derivatives

Burmaoglu et al. [49] synthesized via Claisen-Schmidt condensation reaction a group of tris-chalcone derivatives (**244–252**, Table S6) consisting of identical fluoro-substituted chalcone subunits sharing A ring. The tris-chalcones were further evaluated for their hCA inhibitory activity, and as for the hCA I isoform, all compounds expressed good inhibitory activity with K_i values (19.58–78.73 nM, Table S6) lower than that of the reference AZA (141.02 nM). Derivative **251** (Fig. 12) with two fluorine atoms in *m*-positions of each subunit expressed the highest potency. Quite similar K_i values were observed for hCA II and were in a range of 12.23 to 41.70 nM (Table S6) with *p*-fluoro-substituted **246** (Fig. 12) being the most potent among the investigated species and also more potent than AZA ($K_i = 22.17$ nM) [49].

The same authors [50] tested another group of substituted tris-chalcone derivatives (**253–264**, Table S6) for CA inhibitory activity. Similar, yet slightly higher activity was observed within the series, with K_i values ranging from 13.6 to 50.0 nM for hCA I and from 9.9 to 39.5 nM for hCA II. *p*-Chloro-substituted **255** and *m*-bromo-substituted **257** (Fig. 12) compounds had the best inhibition of hCA I and hCA II, respectively, being even more potent than the reference AZA ($K_i = 141.0$ nM and 22.2 nM towards hCA I and hCA II, respectively) [50].

Tetra-chalcone substituted phthalocyanines as CA inhibitors

Özen et al. [66] designed two tetra-chalcone substituted phthalocyanine complexes containing Zn(II) or Co(II) ion in their core, and evaluated their hCA I inhibition properties. Compound with Zn(II) ion inhibited hCA I with an IC_{50} value of 1.69 μM, which was also lower than that of AZA ($IC_{50} = 7.07$ μM), while the Co(II)-containing compound showed no inhibition effect, even at 100 μM. As a mechanism of action, the authors suggested that there might be a competition between Zn(II) ion in the complex with Zn(II) ion in the enzyme active site [66].

Arslan [67] synthesized one metal-free and three metallophthalocyanines bearing Co(II), Mn(II), or Cu(II) ion, coordinated with four identical chalcone subunits (Fig. 13). Obtained complexes were tested for their hCA I and hCA II inhibition effect. All derivatives had lower IC_{50} values (0.5621–0.6413 μM) when compared to that of AZA (0.9857 μM) towards hCA I, but only Mn(II)-coordinating phthalocyanine showed potency towards hCA II inhibition ($IC_{50} = 0.4823$ μM), that was comparable to that of the reference inhibitor ($IC_{50} = 0.4894$ μM). Since compounds differed only in the presence and type of incorporated metal ion, the author suggested the metal complexing effect changed the electron intensity of the phthalocyanines, resulting in dissimilar interplays via these enzymes [67].

In silico carbonic anhydrase inhibitors studies

SAR

Inhibition efficacy is a multifactorial process, which is often not easy to rationalize [68], even for compounds with simple chalcone scaffold. By handling the selected chemotype experimental data, focusing our observations primarily on K_i values extracted from the literature [37–50, 53, 54, 56–67] and on calculated selectivity ratios (SR), efforts were made for establishing structure–activity relationships. An attempt was directed toward finding useful information about structural elements important for the development of more selective and potent CA inhibitors as an alternative to classical ones.

Previous studies revealed that the modulation of CA inhibitors efficacy strongly depends on the type and position of substituents on both A and B aromatic rings. A general observation regarding the activity is that most of compounds containing electron-donating groups (-CH_3, -OH, -NH_2, -OCH_3, etc.) were having stronger inhibitory effect against hCA I and hCA II [41]. As confirmed by many docking studies, by generating strong electrostatic interactions with the amino acids present in the enzyme active site, these groups are establishing effective connections [41].

Having impact on both hCA I and hCA II isoforms, A ring substituted with -NH_2 strongly induces CAI effect [40]. The observed effect is due to aryl amine moiety, which acts as an effective H acceptor, with a tendency to form rather strong H-bonds involving the enzyme active site [37, 40]. The effect of another strong hydrophilic substituent, an -OH group, on inhibition activity was also observed, however not as pronounced as for -NH_2. In order to modify and improve the binding characteristics, the design of novel ligands often involves the replacement of functional groups. By replacing hydrophilic substituents (-NH_2, -OH) on the A ring with -Br or -CH_3 [37], a negligible effect on the activity was achieved. According to the authors [37], the phenomenon can be attributed to newly introduced hydrophobic groups that can also enhance binding affinity, only through hydrophobic interactions. Compounds containing halogen groups are also effective CA inhibitors [55]. In this context, -F group as aromatic ring substituent has particularly good ability for developing strong interactions with the amino acids of active site [41]. Adding polar substituents on B ring affects changes in the inhibitory effect. For example, addition of hydroxyl group at position 2 on ring B in 4-methoxychalcone or fluoro group to 4-fluorochalcone at position 4- on ring B had increased hCA I inhibitory effects. Induced changes however had opposite effect on hCA II [54]. A useful modification to increase the effect on hCA II by lowering the K_i values is the introduction of methoxy (-OCH_3) substituents on the B ring. The above consideration is of particular interest, as selective CA targeting is the crucial aspect for the successful pharmacological treatment of diseases in which such enzymes are involved. As can be seen form Table S1, compounds **21**, **38**, **39**, **42**, **44** (K_i hCA I/K_i hCA II > 1 and K_i hCA II/K_i AZA < 1) might constitute promising leads for the development of more selective and potent inhibitors as alternatives to the classical CA inhibitors.

What can be noticed only by analyzing numerical data from Table S1, without interpreting the electronic effects or the substitution patterns of the simple chalcones, is that larger number of structures are with pronounced effect on hCA I, with K_i values compatible or often exceeding K_i AZA (except for **19**, Table S1). The above observation may suggest the importance of α,β unsaturated carbonyl system

as the structural motif interacting with the hCA I active site, but in the absence of sufficient data to confirm this remark, it remains at the level of speculation.

Structural hybridization, which covalently links two or more pharmacophores into a single structural framework, is considered a successful approach for the discovery of new scaffolds with therapeutic applications. Many strategies for the development of selective CAI have been adopted [8, 18, 69] and the entities presented in Table S3 (entries 64–100 compared to Table S1 and Table S2 compounds) perfectly illustrates the purpose of implementing the strategy for the CAI development. As can be also seen from Table S3, chalcone derivatives containing Mannich [42] and Schiff bases [57] and chalcone-imide derivatives [58] are structures with excellent selectivity for the hCA II isoform and strong inhibition effect (K_i values for **74**, **93**, **68**, and **64** are approximately five, four, three, and two times lower than that of AZA) that might be adopted as a promising candidate in drug development. *N*-aminomethylation by the Mannich reaction proved to be a useful modification in terms of CA inhibitory properties [44] with an interesting example of three 3-[4-(trifluoromethyl) phenyl]acryloyl-2(3*H*)-benzoxazolones (**133**, **137**, and **139**, (Table S5)) with **139** as the best candidate of the series that with the highest hCA II inhibition effect and the lowest K_i hCA II value (8.6 nM) can be regarded as molecule for further investigations [44], representing even a promising scaffold that can be further explored in order to generate chemotype of enhanced CA inhibitory potential and selectivity.

1,3-Bis and tris-chalcone derivatives were also effective, in particular structures (Table S6) reported by Tutar et al. [65], Burmaouglu et al. [49, 50]. Generally speaking, both isozymes were inhibited, without a particularly pronounced effect in selectivity. Only compound **257** [50], showing four times greater selectivity toward isoform hCA II than for hCA I (Table S6, SRs: K_i hCA I/K_i AZA < 1) and having two times stronger inhibition effect in comparison to AZA (Table S6, SRs: K_i hCA II/K_i AZA < 1), can be considered effective drug candidate. The majority of the investigated structures had better inhibitory profiles compared to AZA (Table S6, SR: K_i hCA I/K_i AZA < 1 and K_i hCA II/K_i AZA < 1).

In silico study of physicochemical, pharmacokinetic, and toxicological properties

Physico-chemical properties

Physico-chemical properties of the evaluated chalcone derivatives (**1**–**264**) were calculated using *SwissADME* [51] in order to predict their oral bioavailability (Table S7). According to "Lipinski's rule of five" and the "Veber's rule", for optimal oral bioavailability it is desirable that the molecule has the following characteristics: molecular weight ≤500, number of hydrogen bond donors ≤5, number of hydrogen bond acceptors ≤10, partition coefficient ≤5, number of rotatable bonds ≤10 and polar surface area ≤140 Å^2 [70, 71]. Summarizing the obtained results (Table S7), 30 out of 50 most effective CA inhibitors (Figs. 5–12), including all simple chalcones (**1**, **2**, **9**, **11**, **14**, **15**, **23**, **25**, **36**, **43**, **47**, and **49**), both aryl-substituted chalcones (**56** and **58**), 6 (out of 8) *N*-substituted chalcones (**64**, **68**, **87**, **91**, **93,** and **95**), each representative of phenyl(thio)urea (**104**, **107** and **109**), benzoxazolone (**119**, **120**, **132**, **136**, and **139**), simple indole (**141** and **146**), methanoisoindole (**155**) and pyridyl indole-incorporated (**175**) chalcones, as well as one (out of 3) indole-incorporated chalcone with a triazole-linked benzensulfonamide (**183**), fulfilled both "rules" predicting their good oral bioavailability. On the other hand, each of the most effective tetrabromo-substituted methanoisoindole chalcones (**161**, **163** and **164**), indole-incorporated chalcones with an amide-linked benzensulfonamide (**204** and **208**), and bis- and tris-chalcones (**222**, **224**, **230**, **239**, **251**, **246**, **255**, and **257**), as well as AZA, had at least one parameter outside the acceptable values.

Pharmacokinetic properties

According to the pharmacokinetic properties calculated by *SwissADME* (Table S8) [51], the ability of intestinal absorption was predicted for 35 out of 50 most effective CA inhibitors (Figs. 5–12), including all simple (**1**, **2**, **9**, **11**, **14**, **15**, **23**, **25**, **36**, **43**, **47**, and **49**), aryl-substituted (**56** and **58**), phenyl(thio) urea (**104**, **107** and **109**), benzoxazolone (**119**, **120**, **132**, **136** and **139**), simple indole (**141** and **146**), methanoisoindole (**155**, **161**, **163**, and **164**), and pyridyl indole-incorporated (**175**) chalcones, as well as 6 (out of 8) *N*-substituted chalcones (**64**, **68**, **74**, **87**, **91**, and **93**). With the exception of **49**, **74**, **109**, **161**, **163**, **164**, and **175**, these compounds were also predicted with the ability to pass through BBB. Nine most effective CA inhibitors (**49**, **56**, **58**, **64**, **68**, **74**, **107**, **230**, and **239**) are potential P-glycoprotein substrates. The evaluated chalcone derivatives differ sigificantly in their predicted metabolic properties, in terms of whether they are potential CYP inhibitors. Apart from bis-chalcone **222** and tris-chalcones **246**, **251**, **255**, and **257**, the most effective CA inhibitors were predicted as inhibitors of at least one CYP isoenzyme (Table S8).

Toxicological properties

Toxicological properties of the evaluated chalcone derivatives (**1**–**264**) were predicted using *OSIRIS Property Explorer* [52] in terms of their potential to cause mutagenic, tumorigenic, irritating and/or reproductive effects (Table S9). Nearly half of the most effective CA inhibitors (compounds **9**, **11**, **15**, **25**, **58**, **74**, **93**, **109**, **120**, **132**, **136**, **139**, **141**, **146**, **175**, **204**, **208**, **230**, **239**, **246**, **251**, **255**, and **257**) was predicted with no risk for mutagenic, tumorigenic, irritating and reproductive effects. Two compounds (**91** and **107**) were predicted with medium

risk for tumorigenic effects, but with no risk for mutagenic, irritating, and reproductive effects. The other half of the compounds, as well as AZA, was predicted with risk for at least one of the mentioned effects.

Taken together, in silico study of the most effective chalcone CA inhibitors indicates that 14 compounds (**9**, **11**, **15**, **25**, **58**, **93**, **109**, **120**, **132**, **136**, **139**, **141**, **146**, and **175**) were predicted with the most promising physicochemical, pharmacokinetic and toxicological properties, even better than those predicted for AZA.

Conclusion and future perspectives

Lowering intraocular pressure remains the main therapeutic aim in glaucoma therapy and majority of antiglaucoma drugs present on the market are based on this approach. There are evidences that even in patients with well-regulated IOP, glaucomatous changes still progress, indicating that other factors, such as oxidation and inflammation, contribute to neurodegenerative changes in optic nerve. With high plasticity for chemical modifications, impressive antioxidative, antiinflamatory and neuroprotective effects and therapeutic potential for multiple diseases, chalcones are considered privileged scaffolds and potential CA inhibitors. The in silico physico-chemical, toxicological and pharmacokinetic properties evaluation for chalcone derivatives previously assayed on hCA inhibitory activity indicated **9**, **11**, **15**, **25**, **58**, **93**, **109**, **120**, **132**, **136**, **139**, **141**, **146,** and **175** as the most promising CA inhibitors. Having better properties than those envisioned for AZA, we hypothesize that these few compounds may be of particular interest for the future development as drug leads. Having insights into K_i and by analyzing SAR, we pointed out structural elements important for the hCA II isoform activity/selectivity. For the inhibition of this isoform mainly implicated in glaucoma, several structural elements on chalcone template are of particular interest: B ring methoxy (-OCH_3) substituents, amino groups (-NH_2, -NHR, -NR_2) and halogens. Data collection related to previous experimental results: (K_i), the SAR and in silico studies, clearly distinguish one candidate from the others. Selected as a compound with an excellent hCA II selectivity and strong inhibition effect, predicted to have favorable physico-chemical, pharmacokinetic and toxicological profiles, **139** may be taken for further evaluation in in vivo studies. Suitable for predicting the active potential of the molecules, for the purpose of drug development, the combined application of in vitro hCA inhibitory potential/in silico tools could be helpful strategy in designing novel and selective CAIs.

Author contributions All authors contributed to each stage of the manuscript preparation. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest Authors know of no conflict of interest associated with this publication and there has been no financial support of this work that could have influenced its outcome.

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