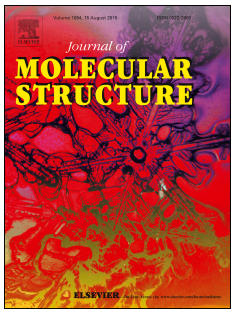

Synthesis of novel organohalogen chalcone derivatives and screening of their molecular docking study and some enzymes inhibition effects

Serdar Burmaoglu, Elif Akin Kazancioglu, Ruya Kaya, Mustafa Kazancioglu, Muhammet Karaman, Oztekin Algul, Ilhami Gulcin

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Synthesis of Novel Organohalogen Chalcone Derivatives and Screening of Their Molecular Docking Study and Some Enzymes Inhibition Effects

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ABSTRACT

Chalcones and their derivatives are increasing attention due to numerous biochemical and pharmacological applications. In this study, a series of novel organohalogen chalcone derivatives (**5**-**12**) were tested towards α-glycosidase (α-Gly), acetylcholinesterase (AChE) human carbonic anhydrase I (hCA I), and carbonic anhydrase II (hCA II) enzymes. These compounds (**5**-**12**) showed K_is in ranging of 16.24-40.96 nM on hCA I, 29.61-67.15 nM on hCA II, 1.21-4.39 nM on AChE and 12.54-35.22 nM on α-glycosidase. The novel organohalogen chalcone derivatives (**5**-**12**) had effective inhibition profiles against all tested metabolic enzymes. Also, because of the enzyme inhibitory effects of the compounds (**5**-**12**), they have the potential of drug candidates to treat of some diseases including epilepsy, glaucoma, type-2 diabetes mellitus (T2DM), Alzheimer's disease (AD), and leukemia. Also, the chalcone derivatives with best inhibition score docked into the active site of indicated metabolic enzymes receptors. Bromobenzyle and chlorophenyl moieties of chalcone derivatives contribute to their inhibitor properties on the enzymes.

Keywords: Chalcone; enzyme inhibition; carbonic anhydrase; acetylcholinesterase; α-glycosidase

1. INTRODUCTION

Chalcones are a prominent class of compounds, which are frequently encountered in the literature. The main reason is that they can be synthesized easily and show a wide range of biological activities including antidiabetic, anticancer, antifungal, antitoxic etc [1-3]. Most of chalcones have already been used as a drug in traditional medicine, and is also well known in the industry by their fluorescence properties and ability to react with metal ions [4]. Recently, it was reported that many organohalogen contains chlorine, bromine and fluorine have been recorded to have potential anticancer, anti-inflammatory, cytotoxicity, antioxidant and antimicrobial activity also protein tyrosine phosphatase, aldose reductase and hCA inhibitory actions [5].

Metabolic enzymes have a key and crucial role for regulation and persistence of the metabolism in living organisms [6,7]. Carbonic anhydrase enzymes (CAs) are an important metabolic enzyme family and catalyze reversibly transform of carbon dioxide (CO_2) and water (H_2O) to bicarbonate ion (HCO_3^-) and proton (H^+) [8-10]. They encoded by seven distinct gene families (α-θ CAs) [11-13]. Until today, sixteen distinctive α-CA isoenzymes have been discovered and characterized [14-16]. Furthermore, because of these crucial physiological and inhibition properties, many researchers have done research on CAs. Among them CA I, and II isoenzymes are the most studied and examined CA isoenzymes [17,18]. CA inhibitors (CAIs) are mainly used as therapeutic agents in treatment of antiglaucoma, diuretics, antitumor, anticonvulsant and antiobesity [19,20]. When viewed from this point, improving of novel CAIs had a great importance. CAIs have various therapeutic applications including glaucoma [21]. Chalcone derivatives are one of the best-studied CA inhibitor classes and their inhibition profiles were well established [2,3,22,23].

Alzheimer's disease (AD) is a one of the significant problems for elderly people worldwide and being characterized as a multifactorial disease [24-26]. AD is the most common progressive neurodegenerative disorder that always related to a decline of memory and cognitive functions [27-29]. So far, AChE inhibition is one of the most common and successful methods to treat AD [30-32]. For this reason, drug therapies have been developed focusing on AChE inhibitors, which increase cholinergic transmission through the inhibition of AChE responsible for breaking down the neurotransmitter acetylcholine. In addition, the effectiveness of these therapies has been investigated by several randomized tests [33,34]. Also, it is well known that the chalcone-based derivatives could modulate different pathways involved in disease progression, due to the peculiar trans-α, β-unsaturated ketone in the

chalcone framework. The simpler and more flexible framework of the new chalcone-based derivatives is known to allow less restricted molecules to be obtained. This could interact better with the narrow passage of cholinesterases [35]. In a recent study, a series of chalcone-*O*-carbamate derivatives was designed, synthesized and evaluated based on the multitarget-directed ligands strategy. Another suitable strategy for achieving better therapeutic efficacy for AD is proposed by the development of multitarget-directed ligands, which can simultaneously modulate different targets or mechanisms included in the neurodegenerative AD cascade [36]. At the same time, ethyl acetohydroxamate and chalcone epoxide incorporated chalcones were recently used as a novel class of chalcones for multitarget inhibitors against AD [37,38]. They are considered a powerful organic scaffold with extensive applications in the drug discovery and medicinal chemistry. In this perspective, chalcones were designed for the management of central nervous system disorders, which include acetylcholinesterase and butyrylcholinesterase inhibitions [39].

Glycosidases are the specific glycosidic bond hydrolases directly involved in the glycosylation on cell surface. They release from small intestines and hydrolyze oligosaccharides and polysaccharides to monosaccharides including fructose and glucose [40,41]. The occurrence and development of many diseases including diabetes, cancers, autoimmune diseases and viral infections are closely related to the abnormal expression of the glycosidases [42-44]. α-Glycosidase inhibitors (α-GIs) had a quite important to control hyperglycemia and T2DM in human [45]. They can reduce the dietary carbohydrates uptake, suppression of postprandial diabetes and hyperglycemia [46].

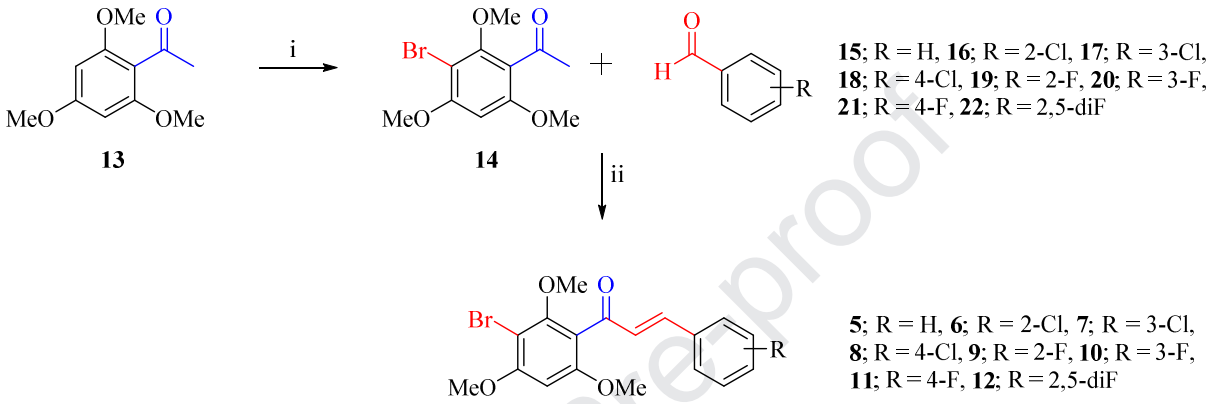
Within the scope of this information, in the present study, we searched the influence of novel chalcone derivatives (**5-12**) on hCA isoenzymes, AChE, and α-glycosidase enzymes. Furthermore, the metabolic enzyme inhibition profiles of novel chalcone derivatives (**5-12**) were compared to acetazolamide (AZA) and tacrine (TAC) as standard inhibitors for hCA isoenzymes and AChE, respectively.

2. RESULTS AND DISCUSSION

2.1. Chemistry

The main goal of the present study is to synthesize and investigate the metabolic enzyme inhibition effect of some novel organohalogen chalcone derivatives. The synthesis of the target and intermediate compounds was done accordance with the reactions presented in Scheme 1. The intermediate **14** was synthesized from compound **13**. For this, **13** was

brominated to the 3-bromo-2,4,6-trimethoxyacetophenone (**14**) in 95% yield through the employing of general bromination protocol (Scheme 1) [47,48]. The base-catalyzed reaction of compound **14** with related benzaldehydes (**15**-**22**) afforded chalcone derivatives (**5**-**12**) in good yields (Scheme 1) [10]. The formation of the chalcone skeleton was determined by measuring the interaction constants of α,β-unsaturated protons as approximately 16 Hz. Moreover, singlet 1 proton at about 6.38 ppm indicates that there is mono bromine in the A ring.



Scheme 1. Synthetic pathway of chalcone derivatives. Reagents: (i) ammonium cerium (IV) nitrate (CAN), LiBr, CH_3CN, 2 h, rt; (ii) 50% KOH, MeOH, rt.

2.2. Biochemical Studies

CAIs are chemicals or pharmaceuticals, which suppressed the CA activity o as competitive or noncompetitive. The CAIs has been clinically assigned as antiepileptics, anti-glaucoma, and diuretic agents, to manage gastric and duodenal ulcers, mountain sickness, hypertension, idiopathic intracranial, osteoporosis and neurological disorders. The inhibition of CAs has been the subject of research since the finding out the biological effectiveness of CA in living organisms and has directed researchers to these issues [49]. Recently, a large spectrum of novel organic and derivative compounds has been raised and discovered as CAIs [50,51]. In this context, novel chalcone derivatives (**5**-**12**) are found as effective and potent CA inhibitors, we synthesized some novel chalcone derivatives (**5**-**12**) to explore their possible hCA I, and hCA II, α-glycosidase and AChE inhibition effects, which summarized in Table 1 and the following results have been displayed.

The hCA I exist in erythrocytes at the high level [52]. Novel chalcone derivatives (**5**-**12**) showed low nanomolar K_is. Lower K_i value indicates strong inhibitor affinity of inhibitor to enzyme [53]. For determination of both inhibition types and K_i constants of novel chalcone

derivatives (**5-12**), Lineweaver-Burk graphs were obtained [54] as described in previous studies [55,56]. Novel chalcone derivatives (**5-12**) showed low nanomolar inhibition levels against hCA I with K_i values between 16.24±5.10-40.96±8.95 nM (Table 1). Also, compound **6** had the best inhibition effect toward hCA I (K_i: 16.24±5.10 nM). On the other hand, acetazolamide (AZA), as a sulfonamide-based reference inhibitor, had Ki value of 141.02±50.84 for same isoenzyme ($K_{i\text{-}AZA}/K_{i\text{-}hCA\text{-}I}$:8.683). AZA provides good pharmacokinetic exhibits and minimal toxicity properties. However, It exhibits several undesired side effects including increase urine volume [57-60]. All undesirable side effects form a result of its nonspecific CAs inhibition [61-63]. The results clearly exhibited that novel chalcone derivatives (**5-12**) had strong inhibition effect than that of AZA toward hCA I (Table 1). Also, selectivity indexes of novel chalcone derivatives (**5-12**) were given in Table 2. It is known that chalcone derivatives are well-studied CAIs and their CA inhibition effects well established [64,65]. Recently it was approved that tetrabromo chalcone derivatives showed excellent inhibition effects in the low nanomolar range of 11.30-21.22 nM against hCA I [2]. In a recent study, some novel chalcone-imide derivatives exhibited strong inhibition effects with K_is of 426.47-699.58 nM against hCA I, nM [3]. In our study, novel tris-chalcones derivatives were found potent cytosolic hCA I inhibitor with K_i values between 19.58-78.73 nM [23].

Also, CA II includes the primary Na-carrying mechanism into the eyes. As a result of this transportation, it is responsible to regulate the intraocular pressure [66,67]. The high IOP is harmful for the optic nerve in eye. Thus, CA II inhibition reduces a high intraocular pressure normally accompanying glaucoma. A close look at the inhibitors of CA II is very important for glaucoma treatment [68,69]. Another pharmaceutical application of CA II inhibitors is their use in the treatment of bone resorption most commonly found during postmenopausal osteoporosis [70-72]. Sulfamates as putative CA II inhibitors are directly bound to the metal in the center of active site of hCA II [73]. As seen in Table 1, the inhibition effects of novel chalcones (**5-12**) on hCA II isoenzyme is quite similar CA I. They showed K_is between 29.61±5.65-67.15±16.21 nM. However, AZA that used to treat glaucoma and some other diseases [74] had a K_i of 22.17±0.65 nM on hCA II. As can seen in Table 2, compound **8** had greatest selectivity against hCA II isoenzyme. It is promising compound **8** had a drug candidate potential and can be used for the treatment of glaucoma ($K_{i\text{-}AZA}/K_{i\text{-}hCA\text{-}II}$: 0.748) (Tables 1 and 2). In our recent studies, it was found that tetrabromo chalcone derivatives inhibited hCA II with K_is between 8.21 and 12.86 nM. Recently, it was proven that some novel chalcone-imide derivatives had powerful inhibition effects with K_is of

214.92-532.21 nM for hCA II [3]. Also, in our recent study, novel Tris-chalcones derivatives were found potent cytosolic dominant hCA II inhibitor with Ki values between 12.23-41.70 nM [23].

AChE and BChE inhibition kinetics novel chalcones (**5-12**) were determined according to the Ellman's procedure [75] as previously described [76-78]. Novel chalcones (**5-12**) had K_is in ranging from 1.21±0.20 to 4.39±0.25 nM for AChE (Table 1), whereas, TAC had K_i of 5.99±1.79 against AChE. All studied novel chalcones (**5-12**) demonstrated powerful inhibition against both enzymes, but compound **9** showed perfect inhibition effect against AChE (K_i: 1.21±0.20 nM; $K_{i\text{-}TAC}/K_{i\text{-}AChE}$: 4.950) (Tables 1 and 2). On the other hand, in a recent study it was found that novel chalcones had efficient inhibition impact with Ki values of 1.09-6.84 nM against AChE [24]. Our results are similar to the results obtained from this study.

Finally, for the α-glycosidase, novel chalcone derivatives (**5-12**) exhibited K_i values are between 12.54±4.16-35.22±2.10 nM (Table 1). The results showed that all novel chalcone derivatives (**5-12**) had effective *α*-glycosidase inhibition effects than acarbose (IC_{50}: 22.800 mM) as standard *α*-glycosidase inhibitor [79]. Also, highly effective K_is were calculated for compound **9** (K_i: 14.02±3.73 nM). In our previous study, novel Tris-chalcones were found as potent α-GIs with K_is between 0.93±0.20-18.53±5.06 nM [23,80].

2.3. *In Silico* Studies

In silico studies carried out for clarifying binding affinity and interaction mode of chalcone derivatives with best inhibition score against hCA I, hCA II, AChE, and α-glycosidase enzymes. Before performing docking studies, we checked drug-likeness of the chalcone derivatives. After Qikprob calculation, we considered number of reactive functional groups, molecular weight, hydrogen bond donor and acceptor, octanol/water partition coefficient, IC_{50} value for blockage of HERG K^+ channels, Caco-2 cell permeability, MDCK cell permeability, brain/blood partition coefficient, and human oral absorption rate of the chalcone derivatives (Supplementary Table 1). All parameters of the chalcone derivatives were suitable for rules of Lipinski and co-workers [81] and Schrodinger software. For this reason, the chalcone derivatives are non-toxic compound due to 1 reactive functional groups and they have good membrane permeability, partition and oral absorption value. The pharmacokinetic properties of the compounds clearly demonstrated that they may exhibit excellent drug properties.

In order to identify and clarify affinity and interactions between compounds which have good drug properties and receptors, binding site of the receptors has been identified with SiteMap module. Identified receptor's binding sites have been evaluated on the basis of SiteScore and Dscore. hCA I, hCA II, AChE, and α-glycosidase receptors have 1.063, 0.971, 1.090, and 1.016 SiteScore and 1.061, 0.945, 1.113, and 0.961 Dscore, respectively. According to the SiteScores and Dscores, the binding site of each receptor was strongly acceptable as catalytic active site and druggable site. Predicted catalytic active sites have been used evaluating of induced fit docking poses as seen Figure 2.

Induced-fit docking technic has been validated by re-docking of co-crystallized ligands into the predicted catalytic active site of the receptors. Fallowing re-docking process, RMSD value calculated between re-docked ligands and co-crystallized. The RMSD values have been determined as 1.134, 2.858, 0.330, and 1.184 for hCA I, hCA II, AChE, and α-glycosidase receptors, respectively. The RMSD values have been shown that induced-fit docking technique was a reliable docking technique. The best-pose of co-crystallized and re-docked ligands have been shown in Figure 3.

After determining the accuracy of the docking method, the chalcone derivatives have been docked into the predicted active site of the receptors with the same docking technique. Their binding affinity calculated as docking score and docking result have been analyzed in the perspective of docking score. Pose with highest docking score in the negative direction has been selected as best-posed ligand. Docking score of best-posed ligands has been presented in Table 3. According to the Docking scores, the chalcone derivatives posses well binding affinity against their receptors. The docking scores of the chalcone derivatives were similar to the docking scores of reference inhibitors. In order to explain the inhibition mechanism of the chalcone derivatives, 2D and 3D binding modes of best-posed ligands have been analysed. Interacted residues with ligands into active site of receptors was shown in Table 4 and 3D interaction diagram have been shown in Figure 3.

According to Table 4, the chalcone derivatives formed important interactions with active site residues of receptors. 4-Methoxy group of bromobenzyle moiety of compound **6** only formed methal coordination with Zn301 in the catalytic active site of the hCA I (Sup. Figures 1a and 4a). The interaction diagrams shown that Zn atom of hCA I plays an crucial role their activity. 4,6-Methoxy groups of bromobenzyle moiety of compound **8** formed hydrogen bond with Try7 and Asn67 residue of hCA II receptor. Furthermore, bromobenzyle moiety of compound **8** formed halogen bond with Trp5 residue in active site of the hCA II (Sup. Figures 1b and 4b). Moreover, aromatic ring of Trp5 residue formed aromatic hydrogen

bond with 4-methoxy group of bromobenzyle moiety of compound **8** as seen in Figure 4b. 4-Methoxy groups of bromobenzyle moiety and ketone group of compound **9** formed H-bond with Ser293 and Tyr124 residues, respectively. Aromatic hydrogen bond also existed between bromobenzyle moiety and Ser203 residues of AChE receptor as seen in Figure 4c. Furthermore, bromobenzyle moiety of compound **9** formed halogen bond with Phe295 residue in active site of the AChE (Sup. Figures 1c and 6c). Compound **9** only formed a H-bond with Gol3002 (glycerole which is solvent in protein structure) in active site of the α-glycosidase receptor, through 2-methoxy groups of bromobenzyle moiety. Compound **9** not only formed a H-bond but also aromatic H-bond with Asp327 and Asp542 residues of the receptor as seen in Figure 4d. Furthermore, bromobenzyle moiety of compound **9** formed halogen bond with Thr205 residue of the receptor (Sup. Figures 1d and 6d). All compounds closely interacted with hydrophobic residues. However, compounds **6**, **8**, and **9** exhibited quite close hydrophobic interaction with hCA I, hCA II, and α-glycosidase receptors as seen in Figure 2, respectively. Chlorophenyl moiety of compounds responsible for the hydrophobic interactions. Also, it was observed the correlations between inhibition mechanisms of the most active compounds and the positive controls with regard to surrounding and interacting residues [49].

3. CONCLUSIONS

In conclusion, we designed and synthesized novel organohalogen chalcone derivatives (**5-12**). The inhibition effects of all compounds on hCA I, and hCA II isoenzymes and AChE and *α*-glycosidase enzymes were evaluated together. The novel chalcones demonstrated inhibition effects against hCA I, and hCA II isoenzymes and AChE and α-glycosidase enzymes activities at low concentrations. Especially compound **9** for hCA I and hCA II, **8** for AChE, and **5** for α-glycosidase. Methoxy groups of bromobenzyle moiety of the compounds are crucial importance for inhibition for indicated enzyme receptors. In other respects, chlorophenyl moiety of compounds contributes to inhibition of the receptors. We also think that if the compounds have hydrogen donor groups for hydrogen bond, their inhibitory activity against the receptors could be increased. Chalcone-based derivatives could modulate different pathways involved in disease progression, due to the peculiar trans-α,β-unsaturated ketone in the chalcone framework. The simpler and more flexible framework of the new chalcone-based derivatives is known to allow less restricted molecules to be obtained. The results may be useful for designing and synthesing of new metabolic enzyme inhibitors and in

the improvement of drugs to treat some diseases including epilepsy, glaucoma, leukemia, AD and T2DM in the future.

4. EXPERIMENTALS

4.1.Organic Synthesis

4.1.1.General. All reagents used in this study were commercially available unless otherwise specified and all solvents were distilled before use. Melting points were measured with Stuart SMP20 melting point apparatus. IR Spectra: PerkinElmer Spectrum One FT-IR spectrometer. ^{1}H- and ^{13}C-NMR Spectra: Bruker 400 spectrometers. Elemental analysis results were taken in a Leco CHNS-932 instrument.

4.1.2.Synthesis of 3-bromo-2,4,6-trimethoxyacetophenone (**14**):

To a solution of ammonium cerium (IV) nitrate (1 eq.) and LiBr (1 eq.) in CH_3CN (2.5 mL/1 mmol of substrate) was added 2,4,6-trimethoxyacetophenone (**13**) (1 eq.) under N_2. The mixture was stirred for 2 h at rt. After 2 h, reaction was monitored by TLC and the solvent was removed under reduced pressure. The crude product was dissolved in EtOAc (50 mL), washed with H_2O (2 × 25 mL) and a solution of saturated aqueous $NaHCO_3$ (50 mL), and the organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure to afford 3-bromo-2,4,6-trimethoxyacetophenone (**14**) as a light brown solid (80%). The ^{1}H NMR and ^{13}C NMR spectra are in agreement with reported data [47].

4.1.3.General procedure for preparation of Compounds **5-12**:

To a solution of 3-bromo-2,4,6-trimethoxyacetophenone (**14**) (1 eq.) in MeOH (2.5 mL/mmol of substrate) was added benzaldehyde derivatives (**15-22**) (1.6 eq.) and 50% KOH solution (1.5 mL/mmol of substrate) sequentially and stirred for 15 h at 25^{o}C. After 15 h solvent was evaporated. Crude material washed with 2 M HCl solution (2 mL/mmol of substrate) and extracted with DCM (2 mL/mmol of substrate x 3). The combined extracts were dried over Na_2SO_4. The solvent was removed in vacuo and the remaining residue purified via column chromatography over silica gel using gradient elution with EtOAc and hexanes to yield compounds **5-12**.

4.1.4. (E)-1-(3-bromo-2,4,6-trimethoxyphenyl)-3-phenylprop-2-en-1-one (**5**):

The above procedure was followed with benzaldehyde (**15**) to yield **5** as a light yellow solid (95% yield). $\mathbf{R_f}$ (EtOAc/Hexanes 40:60) = 0.53; **mp** = 165-166 °C; **IR** (KBr, cm^{-1}) Vmax 2941, 1606, 1457, 1269, 1206, 1129; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.56 – 7.54 (m, 2H), 7.40 – 7.36 (m, 4H), 7.01 (d, 1H, A part of AB system, *J* = 16.1 Hz), 6.38 (s, 1H), 3.98 (s, 3H), 3.83 (s, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 193.2, 158.4, 157.6, 156.2, 145.4, 134.6, 130.6, 128.9, 128.5, 128.4, 117.9, 98.3, 92.6, 62.5, 56.6, 56.2; **Anal. calcd** for $C_{18}H_{17}BrO_4$: C, 57.31; H, 4.54; Found: C, 57.80; H, 4.47.

4.1.5. (E)-1-(3-bromo-2,4,6-trimethoxyphenyl)-3-(2-chlorophenyl)prop-2-en-1-one (**6**):

The above procedure was followed with 2-chlorobenzaldehyde (**16**) to yield **6** as a white solid (96% yield). $\mathbf{R_f}$ (EtOAc/Hexanes 40:60) = 0.56; **mp** = 144-145 °C; **IR** (KBr, cm^{-1}) Vmax 2941, 1606, 1457, 1269, 1206, 1129; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.81 (d, 1H, B part of AB system, *J* = 16.1 Hz), 7.70 (dd, 1H, *J* = 7.1, 2.1 Hz), 7.44 – 7.38 (m, 1H), 7.35 – 7.28 (m, 2H), 6.97 (d, 1H, A part of AB system, *J* = 16.1 Hz), 6.39 (s, 1H), 3.99 (s, 3H), 3.85 (s, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 192.8, 158.6, 157.7, 156.4, 140.9, 135.3, 133.0, 131.2, 130.5, 130.2, 127.9, 127.1, 117.7, 98.4, 92.5, 62.6, 56.6, 56.2; **Anal. calcd** for $C_{18}H_{16}BrClO_4$: C, 52.52; H, 3.92; Found: C, 52.84; H, 3.94.

4.1.6. (E)-1-(3-bromo-2,4,6-trimethoxyphenyl)-3-(3-chlorophenyl)prop-2-en-1-one (**7**):

The above procedure was followed with 3-chlorobenzaldehyde (**17**) to yield 7 as a light yellow solid (91% yield). $\mathbf{R_f}$ (EtOAc/Hexanes 40:60) = 0.50; **mp** = 156-158 °C; **IR** (KBr, cm^{-1}) Vmax 2941, 1606, 1457, 1269, 1206, 1129; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.51-7.49 (m, 1H), 7.43 – 7.38 (m, 1H), 7.35 – 7.30 (m, 2H), 7.27 (d, 1H, *J* = 5.3 Hz), 6.97 (d, 1H, A part of AB system, *J* = 16.1 Hz), 6.36 (s, 1H), 3.96 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 192.7, 158.6, 157.6, 156.3, 143.3, 136.5, 134.9, 130.3, 130.1, 129.5, 128.2, 126.6, 117.8, 98.4, 92.6, 62.6, 56.6, 56.2; **Anal. calcd** for $C_{18}H_{16}BrClO_4$: C, 52.52; H, 3.92; Found: C, 52.49; H, 3.99.

4.1.7. (E)-1-(3-bromo-2,4,6-trimethoxyphenyl)-3-(4-chlorophenyl)prop-2-en-1-one (**8**):

The above procedure was followed with 4-chlorobenzaldehyde (**18**) to yield **8** as a light yellow solid (95% yield). $\mathbf{R_f}$ (EtOAc/Hexanes 40:60) = 0.53; **mp** = 189-190 °C; **IR** (KBr, cm^{-1}) Vmax 2941, 1606, 1457, 1269, 1206, 1129; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.48 (d, 2H, *J* = 8.5 Hz), 7.36 (d, 2H, *J* = 8.7 Hz), 7.30 (d, 1H, *J* = 9.4 Hz), 6.97 (d, 1H, A part of AB system, *J* = 16.0 Hz), 6.38 (s, 1H), 3.98 (s, 3H), 3.83 (s, 3H), 3.82 (s, 2H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 192.8, 158.5, 157.6, 156.2, 143.6, 136.4, 133.2, 129.7, 129.2, 128.7, 117.8, 98.3,

92.6, 62.6, 56.6, 56.2; **Anal. calcd** for $C_{18}H_{16}BrClO_4$: C, 52.52; H, 3.92; Found: C, 52.36; H, 3.84.

4.1.8. (E)-1-(3-bromo-2,4,6-trimethoxyphenyl)-3-(2-fluorophenyl)prop-2-en-1-one (**9**):

The above procedure was followed with 2-fluorobenzaldehyde (**19**) to yield **9** as a white solid (92% yield). $\mathbf{R_f}$ (EtOAc/Hexanes 40:60) = 0.50; **mp** = 188-189 °C; **IR** (KBr, cm^{-1}) Vmax 2941, 1606, 1457, 1269, 1206, 1129; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.61 – 7.52 (m, 2H), 7.38 – 7.37 (m, 1H), 7.19-7.16 (m, 1H), 7.12-7.06 (m, 2H), 6.38 (s, 1H), 3.98 (s, 3H), 3.84 (s, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 193.0, 162.7, 160.2, 158.5, 157.6, 156.3, 137.4, 132.0, 131.9, 130.5, 130.4, 129.0, 124.4, 122.73, 117.8, 116.3, 116.0, 98.4, 92.6, 62.5, 56.6, 56.2; **Anal. calcd** for $C_{18}H_{16}BrFO_4$: C, 54.70; H, 4.08; Found: C, 54.65; H, 3.95.

4.1.9. (E)-1-(3-bromo-2,4,6-trimethoxyphenyl)-3-(3-fluorophenyl)prop-2-en-1-one (**10**):

The above procedure was followed with 3-fluorobenzaldehyde (**20**) to yield **10** as a light yellow solid (98% yield). $\mathbf{R_f}$ (EtOAc/Hexanes 40:60) = 0.53; **mp** = 171-172 °C; **IR** (KBr, cm^{-1}) Vmax 2941, 1606, 1457, 1269, 1206, 1129; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 3H), 7.24-7.21 (m, 1H), 7.11 – 7.03 (m, 1H), 6.96 (d, 1H, A part of AB system, J = 16.1 Hz), 6.36 (s, 1H), 3.96 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 192.7, 164.2, 161.8, 158.6, 157.6, 156.3, 143.5, 137.0, 130.4, 130.3, 129.4, 124.5, 117.8, 117.4, 117.2, 114.8, 114.5, 98.4, 92.6, 62.6, 56.6, 56.2; **Anal. calcd** for $C_{18}H_{16}BrFO_4$: C, 54.70; H, 4.08; Found: C, 54.76; H, 3.94.

4.1.10. (E)-1-(3-bromo-2,4,6-trimethoxyphenyl)-3-(4-fluorophenyl)prop-2-en-1-one (**11**):

The above procedure was followed with 4-fluorobenzaldehyde (**21**) to yield **11** as a light yellow solid (90% yield). $\mathbf{R_f}$ (EtOAc/Hexanes 40:60) = 0.52; **mp** = 188-190 °C; **IR** (KBr, cm^{-1}) V_{max} 2941, 1606, 1457, 1269, 1206, 1129; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.58 – 7.50 (m, 2H), 7.38 – 7.27 (m, 1H), 7.08 (t, 2H, J = 8.6 Hz), 6.93 (d, 1H, A part of AB system, J = 16.1 Hz), 6.38 (s, 1H), 3.98 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 192.9, 158.5, 157.5, 156.2, 144.0, 130.9, 130.5, 130.4, 128.2, 117.9, 116.2, 115.93, 98.3, 92.6, 62.5, 56.6, 56.2; **Anal. calcd** for $C_{18}H_{16}BrFO_4$: C, 54.70; H, 4.08; Found: C, 54.40; H, 4.00.

4.1.11.(E)-1-(3-bromo-2,4,6-trimethoxyphenyl)-3-(2,5-difluorophenyl)prop-2-en-1-one (**12**):

The above procedure was followed with 2,5-difluorobenzaldehyde (**22**) to yield **12** as a light yellow solid (97% yield). $\mathbf{R_f}$ (EtOAc/Hexanes 20:80) = 0.33; **mp** = 171-173 °C; **IR** (KBr, cm^{-1}) Vmax 2941, 1606, 1457, 1269, 1206, 1129; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.48 (d, 1H, B

part of AB system, J = 16.2 Hz), 7.32 – 7.23 (m, 1H), 7.09-7.01 (m, 3H), 6.38 (s, 1H), 3.98 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 192.5, 159.9, 158.7, 157.7, 157.4, 156.4, 156.2, 135.7, 131.2, 131.2, 124.2, 124.1, 124.0, 123.9, 118.5, 118.5, 118.3, 118.2, 117.5, 117.5, 117.4, 117.2, 117.2, 114.7, 114.7, 114.5, 114.4, 98.4, 92.5, 62.6, 56.6, 56.2; **Anal. calcd** for $C_{18}H_{15}BrF_2O_4$: C, 52.32; H, 3.66; Found: C, 52.10; H, 3.18.

4.2. Enzymes Inhibition Studies

In this work, hCA I, and II isoenzymes were purified by Sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatography [82,83], CA activity was spectrophotometrically determined according to the method of Verpoorte et al. [84] as described in our previous study in details [85]. p-Nitrophenylacetate was used as substrate for this enzymatic reaction [86]. One CA enzyme unit is adopted the amount of CA, which had absorbance difference at 348 nm over a 3 min at 25°C [87].

For determination of inhibition kinetics of novel chalcones (**5-12**), an activity (%) and [Novel chalcone] graph was drowned. From these graphs, half maximal inhibitor concentrations (IC_{50}) for novel chalcones (**5-12**) were determined [88]. Also, for K_is, three different concentrations novel chalcones (**5-12**) were used. Then, Lineweaver-Burk graphs were drawn according to these measurements. K_is of novel chalcone derivatives (**5-12**) were determined from Lineweaver-Burk graphs [54] as previously described [89].

Quantity of protein during the purification processing, Bradford's technique was utilized [90], which bovine serum albumin used as the standard protein [91]. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis was employed for visualsing of image of isoenzymes [92].

The inhibitory effect of novel chalcone derivatives (**5-12**) on AChE activity was performed according to Ellman's method [75] as described previously [93]. α-Glycosidase inhibition effect of novel chalcone derivatives (**5-12**) was evaluated according to the method of Tao et al. [79]. The absorbance o samples were recorded at 405 nm [94].

4.3. *In silico* Studies

Drug-likeness properties and inhibition mechanism of compounds, which have the best inhibition score against hCA I, hCA II, AChE and α-glycosidase receptors were calculated and specified with *in silico* methods. The Small Drug Discovery Suites package [95] was used performing *in silico* studies.

4.3.1. Ligand preparation and pharmacokinetic properties prediction

In order to use in the Qikprob and IFD studies, 2D structure of the sulfamate derivatives with best inhibition score were sketched and then their 3D structure was produced with LigPrep module of Schrodinger Maestro 12.0. Correct molecular geometries and protonation state at pH 7.0±2.0 of ligand were prepared using Epik module and OPLS-2005 force field. The pharmacokinetic properties of prepared ligands were calculated with QikProp module of Schrodinger Maestro 12.0. Briefly, prepared ligands were opened and the calculation was performed using the default parameter by selecting identify the 5 most similar drug molecule option on QikProp module [96,97].

4.3.2. Protein preparation and binding site prediction

X-ray crystal structures of hCA I, hCA II, AChE and α-glycosidase receptors (PDB code: 4WR7, 5AML, 4M0E, and 3L4Y, respectively) have been acquired from RCSB Protein Data Bank. They have been selected because of their best resolution and good percentile ranks. They have been chosen due to their better resolution and stronger percentile ranks than others. Moreover, the structures have a ligand, which can be used for docking validation test in the catalytic active site. The typical crystal structure in the PDB format is not suitable for immediate use in molecular modeling calculations. Hence, the crystal structures were repaired and prepared with Protein preparation wizard module of Schrodinger Maestro 12.0, before use binding site prediction and induced-fit docking studies. The workflow that includes a detailed description in previous studies [49,98] was described in overview. After protein preparation, the active site of the receptors has been predicted with SiteMap module of Schrodinger Maestro 12.0 for target selection and docking hits evaluation. The prepared receptor was imported into Maestro12.0 and binding site has been calculated using the default parameter of top-ranked potential protein binding sites. Whether the binding site has catalytic active site characteristics has been determined by analyzing SiteScore and Dscore [55,99].

4.3.3. Induced-fit docking

In order to determine binding affinities and inhibition mechanisms of the chalcone derivatives with best inhibition score was carried out molecular docking studies. Induced-fit docking technique is one of the best ways to calculate binding affinity between ligand and receptor because both ligand and receptor are completely flexible in this docking technique. The technique has been performed with the induced-fit docking module of Schrodinger Maestro 12.0 according to proposed previous studies [100]. Before calculating banding affinity

between ligands and the receptors, docking validation test has been carried out for understanding accuracy of induced-fit docking technique. The test has been performed with re-docking procedure by evicting inhibitor complexed in the crystal structure of the receptor. RMSD value between co-crystallized ligand and re-docked ligand have been calculated with Atom pair method in Superposition panel of Schrodinger Maestro 12.0. Following the docking process, it has been determined best-scored sulfamate derivatives by analyzing docking scores.

Conflicts of Interests

The authors declare that no conflicts of interests.

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Table 1. Inhibition parameters including IC_{50} and K_is for novel chalcone derivatives (**5-12**) of metabolic enzymes hCA I, and II isoenzymes, α-glycosidase and AChE (α-Gly: α-Glycosidase, AChE: acetylcholinesterase, CA I: carbonic anhydrase I isoenzyme, CA II: carbonic anhydrase II isoenzyme, *Acetazolamide (AZA) was used as a positive standard for CA isoenzymes, ** Tacrine (TAC) was used as a positive standard for cholinergic enzymes)

Compounds	IC_{50} (nM)								K_i (nM)			
	hCA I	**r^2**	**hCA II**	**r^2**	**AChE**	**r^2**	**α-Gly**	**r^2**	**hCA I**	**hCA II**	**AChE**	**α-Gly**
5	35.0	0.9453	37.66	0.9528	5.63	0.9908	18.28	0.9697	26.58±8.77	52.00±13.44	4.39±0.25	16.76±4.42
6	28.06	0.9473	29.74	0.9456	4.36	0.9893	22.28	0.9697	16.24±5.10	40.36±13.50	1.46±0.26	35.22±2.10
7	30.94	0.9596	31.22	0.9736	4.65	0.9705	20.69	0.9408	16.66±4.91	45.89±12.21	2.95±1.21	18.85±7.84
8	32.08	0.9885	34.48	0.9979	2.97	0.9428	26.76	0.9957	31.79±9.24	29.61±5.65	1.72±0.72	14.60±1.24
9	2.07	0.9487	28.17	0.9504	5.72	0.9887	20.56	0.9805	21.80±9.69	49.11±10.67	1.21±0.20	12.54±4.16
10	28.17	0.9777	38.08	0.9554	3.73	0.9546	23.26	0.9674	26.59±1.86	51.65±2.30	3.10±0.89	23.35±1.15
11	20.69	0.9754	33.81	0.9475	3.82	0.9895	23.02	0.9825	24.40±6.92	42.35±10.03	3.06±1.40	14.78±1.62
12	31.94	0.9685	37.26	0.9763	3.38	0.9776	18.48	0.9528	40.96±8.95	67.15±16.21	2.83±0.76	10.37±1.55
AZA*	113.79	0.9932	31.79	0.9816	-	-	-		141.02±50.84	22.17±0.65		
TAC**	-	-	-	-	5.97	0.9706		-	-	-	5.99±1.79	

Table 2. Selectivity indexes of novel chalcone derivatives (**5-12**)

Compounds	K_i-hCA II / K_i-hCA I	K_i-AZA / K_i-hCA I	K_i-AZA / K_i-hCA II	K_i-TAC / K_i-AChE
5	1.956	5.305	0.426	1.364
6	2.485	8.683	0.549	4.102
7	2.754	8.464	0.483	2.030
8	0.931	4.435	0.748	3.482
9	2.252	6.468	0.451	4.950
10	1.942	5.303	0.429	1.932
11	1.735	5.779	0.523	1.957
12	1.639	3.442	0.330	2.116

Table 3. Docking scores (kcal/mol) of the chalcone derivatives in the sites of hCA I, and II, AChE, and α-glycosidase.

Compounds	IFD Glide Score			
	hCA I	**hCA II**	**AChE**	**α-Glycosidase**
6	-6.227	-	-	-
8	-	-7.250	-	-
9	-	-	-8.571	-6.432
AZA*	-9.016	-9.560	-	-
TAC**	-	-	-9.579	-
ACR***	-	-	-	-16.933

*Acetazolamide (AZA) was used as positive control for hCA I, and II.
** Tacrine (TAC) was used as was used as positive control for AChE.
*** Acarbose (ACR) was used as was used as positive control for α-glycosidase [70]

Table 4. Interaction between ligand and residues of receptors

Interactions	H-bonds	Close hydrophobic	Metal coordination	π-π stacking	Halogen bonds
hCA I-6	-	Phe91, Leu131, Ala132, Ala135, Leu198, Pro202, Tyr204, Val207, Trp209	ZN301	-	
hCA II-8	Trp5, Try7, Asn67	Trp5, Try7, Ala65, Leu198, Pro201, Pro202, Leu203, Leu204, Val135	-	-	Trp5
AChE-9	Try124, Ser203, Ser293,	Tyr72, Tyr 124, Ala204, Val204, Phe 295, Phe 297	-	-	Phe 295
α-Glycosidase-9	Asp327, Asp542, Gol3002	Ile328, Ile364, Trp441, Met444, Tyr605, Phe 575	-	Phe575	Thr205

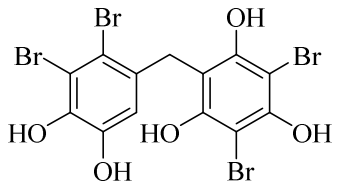
FIGURE LEGENDS

Figure 1. Biologically active some organohalogen compounds.

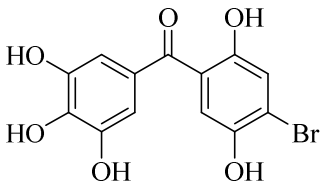
Figure 2. Predicted catalytic active sites. (**a**) hCAI, (**b**) hCAII, (**c**) AChE, and (**d**) α-Gly. The active site has been represented as pink mash surface, hydrophobic site has been represented as yellow bubble, the hydrophilic site has been represented as green bubble and metal-binding site has been represented as purple bubble.

Figure 3. Induced-fit docking validation test. The best-pose of co-crystallized and re-docked ligands. (**a)** 3TV, (**b**) 51J, (**c**) 1YL, and (**d**) NR4. Co-crystalized ligands were represented with orange thick tube and re-docked ligands were represented with grey ball-stick.

Figure 4. 3D interaction diagram of the chalcone derivatives. (**a**) hCA I-**6**, (**b**) hCA II-**8**, (**c**) AChE-**9**, and (**d**) α-Gly-**9**. Receptor structures were represented as ribbon model, amino acid residues were represented as thick tube model, and the chalcone derivatives were represented as grey ball-stick model.



Vidalol (**1**)



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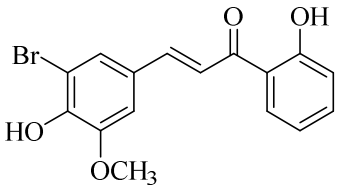

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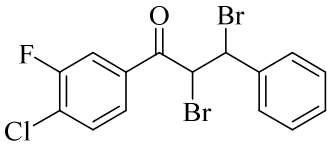

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Figure 1

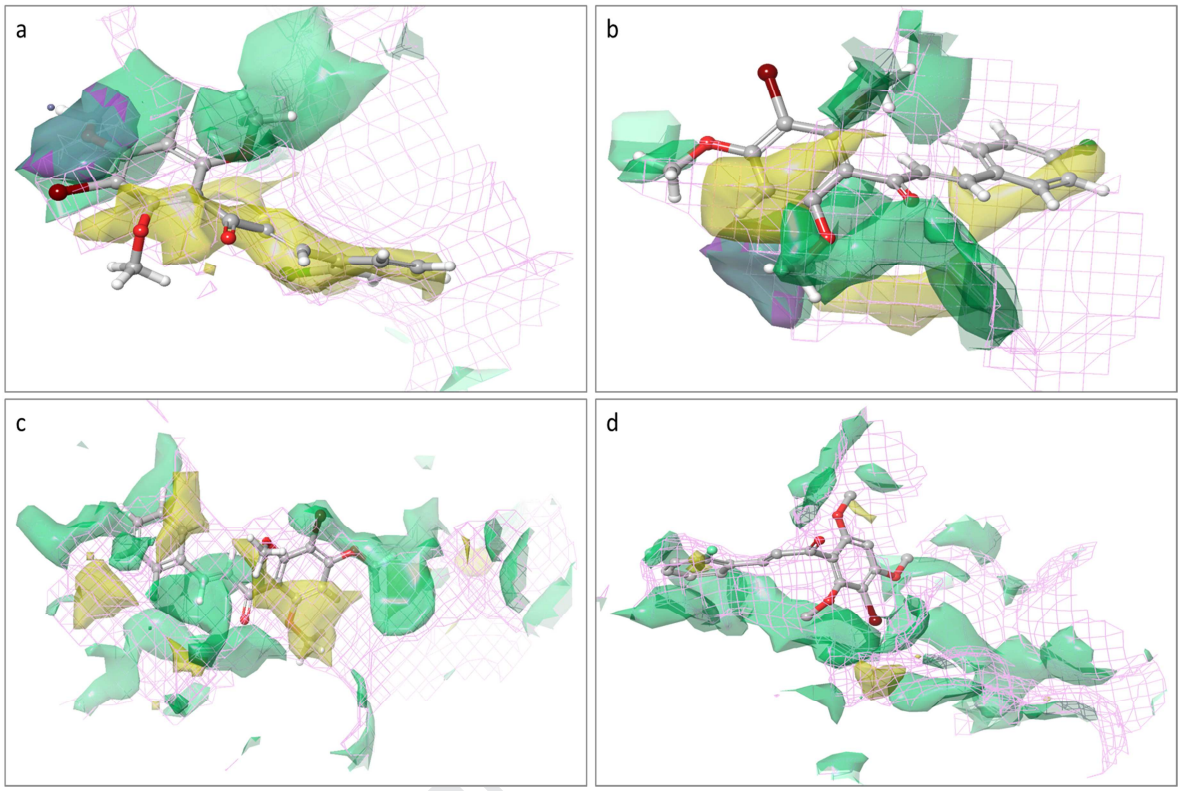

Figure 2

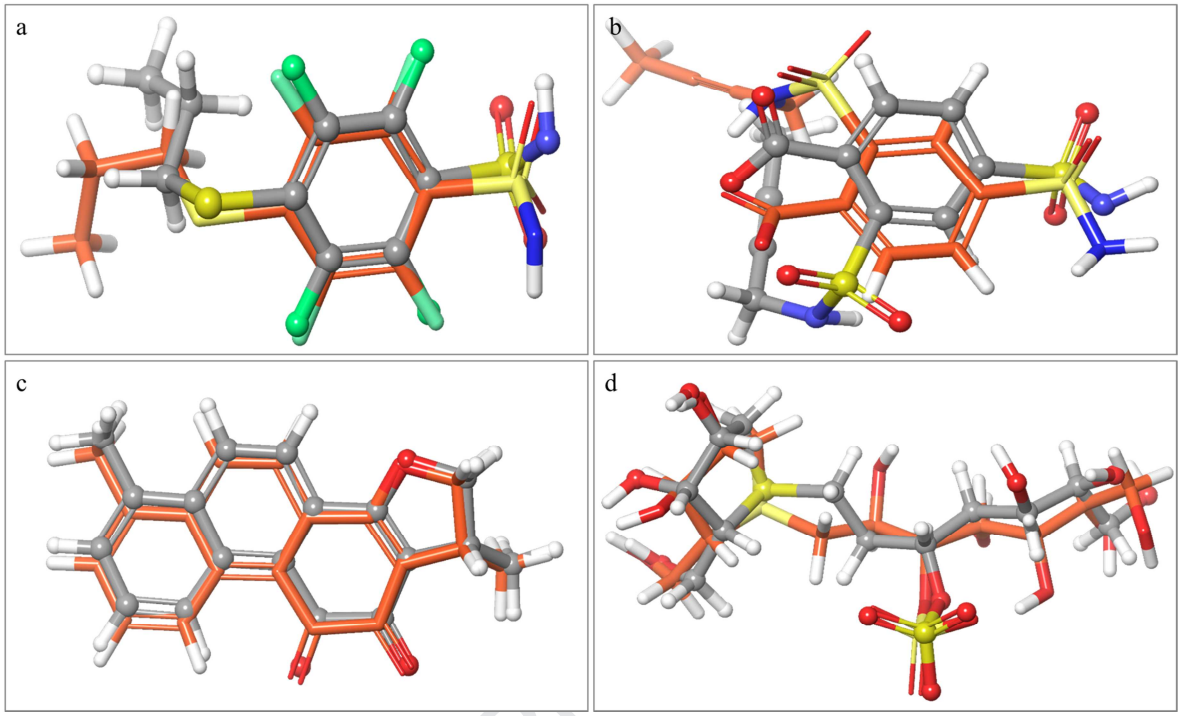

Figure 3

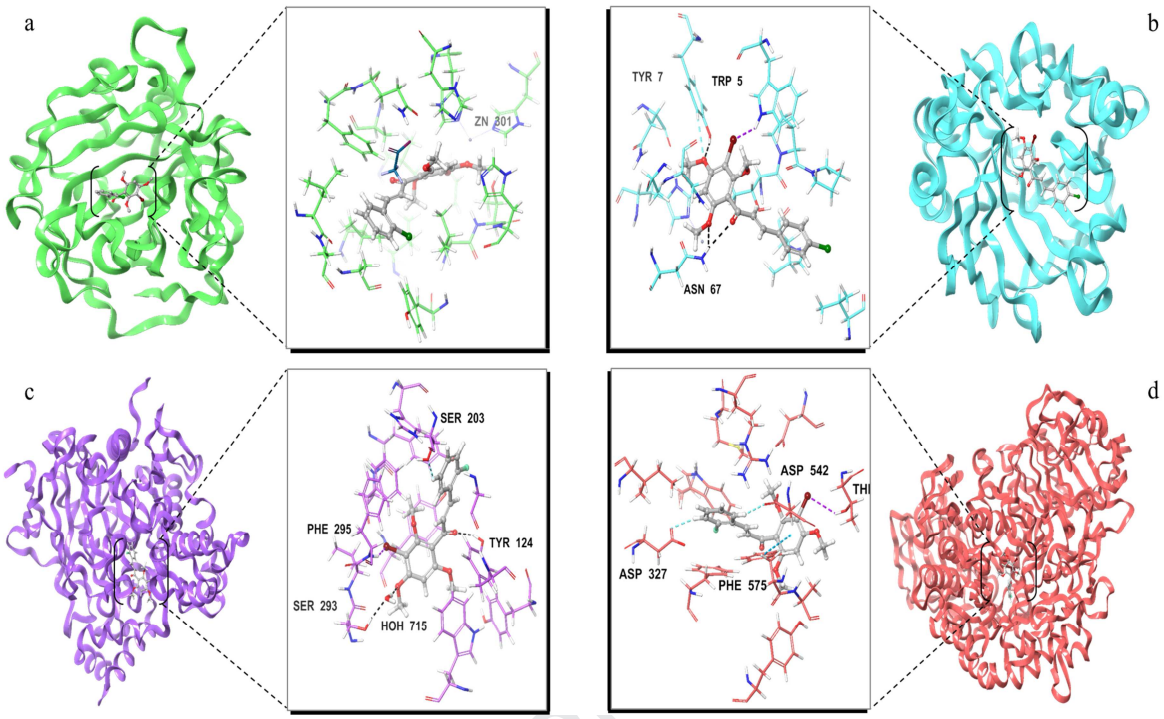

Figure 4

Highlight

- The synthesis of novel chalcone derivatives was achieved.
- They have been characterized by ^{1}H-NMR, ^{13}C-NMR, FTIR and elemental analysis.
- The binding modes of the best inhibitors were identified with molecular docking studies.
- Their inhibition effects on hCA I, hCA II, α-glycosidase and AChE were determined.

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: