Synthesis, cytotoxicities, and carbonic anhydrase inhibition potential of 6-(3-aryl-2-propenoyl)-2(3H)-benzoxazolones

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ABSTRACT

In this study, new chalcone compounds having the chemical structure of 6-(3-aryl-2-propenoyl)-2(3H)-benzoxazolones (1–8) were synthesised and were characterised by 1H-NMR, 13C-NMR, and HRMS spectra. Cytotoxic and carbonic anhydrase (CA) inhibitory effects of the compounds were investigated. Cytotoxicity results pointed out that compound 4, 6-[3-(4-trifluoromethylphenyl)-2-propenoyl]-3H-benzoxazol-2-one, showed the highest cytotoxicity (CC50) and potency-selectivity expression (PSE) value, and thus can be considered as a lead compound of this study. According to the CA inhibitory results, IC50 values of the compounds 1–8 towards hCA I were in the range of 29.74–69.57 μM, while they were in the range of 18.14–48.46 μM towards hCA II isoenzyme. Kᵢ values of the compounds 1–8 towards hCA I were in the range of 28.37 ± 6.63–70.58 ± 6.67 μM towards hCA I isoenzyme and they were in the range of 10.85 ± 2.14–37.96 ± 2.36 μM towards hCA II isoenzyme.

Introduction

Cancer is the second cause of death in the world after the cardiovascular system diseases. According to The World Health Organisation (WHO) report, 13.1 million people will die because of cancer by 2030. Anticancer drugs in clinics have several adverse effects such as nausea, vomiting, hair loss, and pain, in addition to low selectivity and drug resistance problems. Their associated limitations and adverse effects are still prompting the researchers to develop more potent, selective, and safer anticancer drug candidates. Chemotherapeutic drugs commonly used for cancer treatment in clinics are alkylation anticancer agents. These compounds interact with amino and hydroxyl groups which are available nucleic acids and proteins and lead to unwanted side effects in other tissues except neoplasms.

α,β-unsaturated ketones are bioactive moieties having alkylation ability. They have an affinity for thiols while they are either inert or far less reactive towards amino and hydroxyl groups which are available nucleic acids. It was reported that before the cell division, level of glutathione, which is a thiol compound, increases. That is why it can be supposed that compounds which are thiol selective alkylators may perform selective toxicity against tumour tissues and these types of compounds may have advantages over available anticancer drugs in the market.

Chalcones are 1,3-diylyl-2-propan-1-ones, which consist of two aromatic rings connected by a three-carbons chain including α,β-unsaturated carbonyl system. Depending on substitution on the aryl ring, chalcones have a wide range of biological activities such as antiinflammatory, antimicrobial, antioxidant, cytotoxic/anticancer, chemopreventive, topoisomerase inhibiting, carbonic anhydrase (CA) inhibiting, and acetylcholine esterase inhibiting activities.

Benzoxazolones are considered as “privileged scaffolds” in the design of pharmaceutical probes. Benzoxazolones have high flexibility for chemical modifications allowing changes to the characteristics of side-chains on a rigid platform. As a result, benzoxazolones exhibit various biological activities such as anti-HIV, anticancer, analgesic, antiinflammatory, antimicrobial, and antioxidant activities. The functionalisation of the nitrogen atom at the third position of the benzoxazolone moiety is of interest since the electronic characteristics of this atom can be decisive for the biological activity.

Some chalcones bearing 2(3H)-benzoxazolone were reported with strong cytotoxic activity. However, there is a very limited number of studies about them. One of them is Ivanova and co-workers’ study. They reported antileukemic effects of benzoxazolone derived chalcones on BV-173 cell-line by inducing apoptotic cell death. The fact that these compounds showed selective and strong anticancer activity pointed out the importance of benzoxazolone bearing chalcone molecules in designing new anticancer candidate molecules.

CA is a metalloenzyme that catalyses the interconversion between CO₂ and bicarbonate. CAs play an important role in many physiological and pathological processes such as biosynthetic reactions (such as gluconeogenesis, lipogenesis, and ureagenesis), respiration and transport of CO₂/bicarbonate, electrolyte...
secretion in a variety of tissues/organs, calcification, and tumorigenicity.\textsuperscript{35,36}

There are eight CA families which are genetically different such as the $\alpha$, $\beta$, $\gamma$, $\delta$, $\zeta$, $\eta$, and $\theta$, and the recently reported $\tau$-CAs.\textsuperscript{37}$\alpha$-CAs are available in human and it has 15 subtypes. Three of them (CA VIII, X, and XI) are non-catalytic as do not possess a zinc ion in their active site. The other 12 CA isozymes have differences in terms of catalytic activity, location, and cell distributions. According to the location, these are cytosolic isozymes (CA I, II, III, VII, and XIII), membrane-bounded isozymes (CA IV, IX, XII, and XIV), secreted salivary isozyme (CA VI), and mitochondrial isozyme (CA V).\textsuperscript{38–40}

Activation or inhibition of several CAs is a potential strategy for diagnosis and/or treatment of several diseases such as glaucoma, epilepsy, several neurological diseases, and obesity. Antiglaucomal agents (CA II, IV, and XII), diuretics (CA II, IV, XII, and XIV), antiepileptics (CA VII, and XIV) inhibit several isoenzymes of CA. Some chalcone compounds have been reported as attractive scaffolds for the development of new CA inhibitors.\textsuperscript{21,41}$\beta$-CA inhibitors commonly include sulfonylurea\textsuperscript{35,42} or phenol functional group. However, there are several studies reporting that chalcone compounds without these functional groups showed potent CA inhibiting effect.\textsuperscript{43–46} In addition, there was no study about CA inhibiting potency of benzoxazolone or its chalcone derivatives.

In this study, it was aimed to synthesise some chalcone compounds having the chemical structure of 6-(3-aryl-2-propenoyl)-2(3H)-benzoxazolones (1–8) in which $\alpha,\beta$-unsaturated ketone moiety is available to evaluate their cytotoxicity (towards both tumour cell lines and non-tumour cells) and inhibition profile towards CA hCAI and II with the expectation to find out a new candidate molecule/s which may direct further designs.

\section*{Experimental}

\subsection*{Chemistry}

The nuclear magnetic resonance (NMR) spectra (\textsuperscript{1}H-NMR, and \textsuperscript{13}C-NMR) were recorded on a Bruker AVANCE III 400 MHz (Bruker, Karlsruhe, Germany) spectrometer (400 MHz \textsuperscript{1}H) and 100 MHz \textsuperscript{13}C). Chemical shifts are given as $\delta$ values in ppm against tetramethylsilane as the internal standard and $J$ values were expressed in Hz. Mass spectra of the compounds were taken using a liquid chromatography ion trap-time of flight tandem mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ionisation (ESI) source, operating in both positive and negative ionisation mode. Shimadzu’s LCMS Solution software was used for data analysis. Melting points were determined using an Electrothermal 9100 instrument (IA9100, Bibby Scientific Limited, Staffordshire, UK) and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) using silica gel 60 HF254 (Merck KGaA, Darmstadt, Germany).

\subsection*{Synthesis of 6-acetyl-2(3H)-benzoxazolone}

Dimethylformamide (13 ml, 172 mmol) was slowly added on aluminium chloride (80 g, 600 mmol). The mixture was heated at 45 °C for 5 min. 2(3H)-benzoxazolone (8.1 g, 60 mmol) and acetyl chloride (6.4 ml, 90 mmol) were added into the solution of aluminium chloride (Scheme 1). Then the reaction mixture was heated at 80 °C for 3 h and poured on ice water (200 ml) with HCl (30 ml, 37%). The precipitated crude product was collected by filtration and it was air-dried and crystallised from ethanol. (Yield: 77%, m.p: 231–234 °C, brown crystals).\textsuperscript{27}

\subsection*{Synthesis of chalcone compounds 1, 2, 3, 4, and 6}

To the mixture of 6-acetyl-2(3H)-benzoxazolone (ketone, 5.6 mmol) and a suitable aldehyde [benzaldehyde (1), 4-methylbenzaldehyde (2), 4-methoxybenzaldehyde (3), 4-trifluorobenzaldehyde (4), 4-isopropylbenzaldehyde (6)] in ethanol (5 ml) in 1:1 mol ratios, aqueous solution of KOH (10%, 5 ml) was added (Scheme 1). Reaction content was stirred at room temperature for 24 h. Reactions were followed by TLC. When the reaction finished, the content of the reaction flask was poured on ice water (100 ml) and neutralised by HCl (37%). The precipitated solid product was filtered and washed with cold water. The crude compounds were purified by crystallisation from a suitable solvent (Acetonitrile: ethanol for compounds 1–4; acetonitrile: methanol for compound 6)\textsuperscript{27}.

\begin{flushleft}
\textbf{Scheme 1.} Synthesis of the compounds 1–8. Ar: Phenyl (1); 4-methylphenyl (2); 4-Methoxyphenyl (3); 4-trifluoromethylphenyl (4); 3-hydroxyphenyl (5); 4-isopropylphenyl (6); 4-dimethylaminophenyl (7); 4-benzoxoxygenphenyl (8).
\end{flushleft}
144.2, 143.9, 135.5, 135.2, 132.3, 131.0, 129.38, 129.37, 129.36, 126.2, 122.3, 110.0. HRMS (ESI-MS) m/z calculated [M + H]+ 266.0812; measured 266.0803.

6-[3-(4-Methylphenyl)-2-propenoyl]-3H-benzoxazol-2-one (2)
Yield 83%. Mp: 258–260°C. 1H-NMR (DMSO-d6) δ (ppm) 8.08 (d, 1H, arom. H, J = 1.5 Hz), 8.05 (dd, 1H, arom. H, J1 = 8.1 Hz, J2 = 1.5 Hz), 7.93 (d, 1H, Ar-CH=, J = 15.5 Hz), 7.78 (d, 2H, arom. H, J = 8.0 Hz), 7.70 (d, 1H, =CHCO, J = 15.5 Hz), 7.27 (d, 2H, arom. H, J = 8.0 Hz), 7.22 (d, 1H, arom. H, J = 8.1 Hz), 2.35 (s, 3H, CH3). 13C-NMR (DMSO-d6) δ (ppm) 187.7, 155.2, 144.2, 144.0, 141.1, 135.9, 132.5, 132.3, 129.98, 129.97, 129.42, 129.41, 126.1, 121.2, 110.0, 109.8, 21.6. HRMS (ESI-MS) m/z calculated [M + H]+ 280.0968; measured 280.0967.

6-[3-(4-Methoxyphenyl)-2-propenoyl]-3H-benzoxazol-2-one (3)
Yield 58%. Mp: 210–213°C. 1H-NMR (DMSO-d6) δ (ppm) 8.08 (d, 1H, arom. H, J = 1.4 Hz), 8.04 (dd, 1H, arom. H, J1 = 8.2 Hz, J2 = 1.4 Hz), 7.86 (d, 2H, arom. H, J = 8.8 Hz), 7.84 (d, 1H, Ar-CH=, J = 15.5 Hz), 7.71 (d, 1H, =CHCO, J = 15.5 Hz), 7.22 (d, 1H, arom. H, J = 8.2 Hz), 7.01 (d, 2H, arom. H, J = 8.8 Hz), 3.82 (s, 3H, OCH3). 13C-NMR (DMSO-d6) δ (ppm) 187.6, 161.8, 155.0, 144.2, 143.9, 135.3, 132.6, 131.3, 127.9, 126.0, 119.7, 114.8, 110.0, 109.9, 55.9. HRMS (ESI-MS) m/z calculated [M + H]+ 296.0917; measured 296.0918.

6-[3-(4-Trifluoromethylphenyl)-2-propenoyl]-3H-benzoxazol-2-one (4)
Yield 80%. Mp: 257–259°C. 1H-NMR (DMSO-d6) δ (ppm) 7.24 (d, 1H, arom. H, J = 8.2 Hz), 7.78 (d, 1H, =CHCO, J = 15.7 Hz), 7.80 (d, 2H, arom. H, J = 8.2 Hz), 8.06 (d, 1H, arom. H, J = 1.6 Hz), 8.09 (d, 1H, Ar-CH=, J = 15.7 Hz), 8.12–8.14 (m, 3H, arom. H). 13C-NMR (DMSO-d6) δ (ppm) 187.7, 154.9, 143.9, 142.2, 139.2, 135.7, 131.9, 130.5, 129.9, 126.2, 124.9, 122.7, 119.1, 110.1. HRMS (ESI-MS) m/z calculated [M + H]+ 334.0686; measured 334.0687.

6-[3-(4-Isopropylphenyl)-2-propenoyl]-3H-benzoxazol-2-one (6)
Yield 33%. Mp: 220–222°C. 1H-NMR (DMSO-d6) δ (ppm) 12.08 (bs, 1H, NH), 8.10 (d, 1H, arom. H, J = 1.4 Hz), 8.06 (dd, 1H, arom. H, J1 = 8.2 Hz, J2 = 1.4 Hz), 7.93 (d, 1H, Ar-CH=, J = 15.5 Hz), 7.82 (d, 2H, arom. H, J = 8.2 Hz), 7.72 (1H, =CHCO, J = 15.5 Hz), 7.34 (d, 2H, arom. H, J = 8.2 Hz), 7.24 (d, 1H, arom. H, J = 8.2 Hz), 2.98–2.89 (m, 1H, CH2), 1.22 (d, 6H, CH3, J = 6.9 Hz). 13C-NMR (DMSO-d6) δ (ppm) 187.8, 154.9, 151.9, 144.3, 143.9, 135.3, 132.9, 132.4, 129.55, 129.54, 127.35, 126.12, 121.3, 110.0, 33.9, 24.1. HRMS (ESI-MS) m/z calculated [M + H]+ 308.1281; measured 308.1286.

Synthesis of the compounds 5, 7, and 8
To the mixture of 6-acetyl-2(3H)-benzoxazolone (ketone, 5.6 mmol) and a suitable aldehyde [3-hydroxybenzaldehyde (3), 4-dimethylaminobenzaldehyde (4), 4-benzoyl oxybenzaldehyde (8)] in 1:1 molar ratios in ethanol (2 ml), aqueous solution of KOH (10%, 2 ml) was added. Then the mixture was irradiated by microwave (50–80°C, 25–60W) for 15 min (compound 7), 20 min (compound 8) and 30 min (compound 5). Reactions were followed by TLC. When the reaction finished, the content of the reaction flask was poured on ice-water (100 ml) and neutralised by HCl (37%). The solid precipitated was filtered and washed with cold water. The crude compounds were purified by crystallisation from acetonitrile.

Biological activity
Cytotoxicity test
Materials
The following chemicals and reagents were obtained from the indicated companies: Dulbecco’s modified Eagle’s medium (DMEM) from GibCO BRL (Grand Island, NY); foetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), doxorubicin (DXR), and dimethyl sulphoxide (DMSO) from Wako Pure Chem. Ind. (Osaka, Japan); and culture plastic dishes and plates (96-well) were purchased from Becton Dickinson (Franklin Lakes, NJ).

Cell culture
Human normal oral mesenchymal cells, gingival fibroblast (HGF), and periodontal ligament fibroblast (HPLF) established from the first premolar tooth extracted from the lower jaw of a 12-year-old girl and human OSCC cell line HSC-2 (derived from tongue), purchased from Riken Cell Bank (Tsukuba, Japan), were cultured at 37°C in DMEM supplemented with 10% heat-inactivated FBS, 100 units/ml penicillin G, and 100 µg/ml streptomycin sulphate under
a humidified 5% CO₂ atmosphere. HGF and HPLF cells at 10–18 population doubling levels were used in this study.

**Assay for cytotoxic activity**

Cells were inoculated at 2.5 × 10³ cells/0.1 ml in a 96-microwell plate (Becton Dickinson Labware, Franklin Lakes, NJ). After 48 h, the medium was replaced with 0.1 ml of fresh medium containing different concentrations of single test compounds. Cells were incubated further for 48 h and the relative viable cell number was then determined by the MTT method. All benzoxazolone derivatives were dissolved with DMSO at the concentration of 40 mM and stored until use. Control cells were treated with the same amounts of DMSO (0.00156, 0.03125, 0.0625, 0.125, 0.25, 0.5, and 1.0%) and the cell damage induced by DMSO was subtracted from that induced by test agents. In brief, cells were stained with MTT reagent, dissolved with DMSO, and the absorbance of the MTT-stained cell lysate was measured at 560 nm, using a microplate reader (Infinite F 50R, TECAN, Kawasaki, Japan). Control cells were treated with the same amounts of DMSO and the cell damage induced by DMSO was subtracted from that induced by test agents. The concentration of compound that reduced the viable cell number by 50% (CC₅₀) was determined from the dose-response curve and the mean value of CC₅₀ for each cell type was calculated from triplicate assays.

**Calculation of tumour specificity**

Tumour specificity (TS) was calculated using the following equation: TS = Mean CC₅₀ against three normal oral cell types (HGF, HPLF)/Mean CC₅₀ against four OSCC cell lines (HSC-2). Since HGF cells were derived from gingival tissue, the relative sensitivity of these cells was also compared (as mean CC₅₀ against HGF/mean CC₅₀ against HSC-2).

**Calculation of potency-selectivity expression**

Potency-selectivity expression (PSE) was calculated by the following equation: PSE = Mean CC₅₀ against two normal oral cell types/ (CC₅₀ against four OSCC cell lines)² × 100 (HGF, HPLF, HSC-2) and as mean CC₅₀ against HGF/CC₅₀ against HSC-2)² × 100 using the pair of cell types from the same tissue (gingiva).

**Carbonic anhydrase inhibition**

The purification of cytosolic CA isoenzymes (CA I and CA II) was previously described with a simple one-step method by a Sepharose-4B-L tyrosine-sulphanilamide affinity chromatography. The protein quantity in the column effluents was determined spectrophotometrically at 280 nm. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was applied with a Bio-Rad Mini Gel system Mini-PROTEIN system (Bio-Rad Laboratories, Inc., Shanghai, China) after purification of both CA isoenzymes. Briefly, it was performed in acrylamide for the running (10%) and the stacking gel (3%) contained SDS (0.1%), respectively. The increase in absorbance of the reaction medium was spectrophotometrically recorded at 348 nm. Also, the quantity of protein was determined at 595 nm according to the method as described previously. Bovine serum albumin was used as a standard protein. The IC₅₀ values were obtained from activity (%) versus compounds plots. For the calculation of Kᵣ values, three different concentrations were used. The Lineweaver–Burk curves were drawn and calculations were realised as before.

**Results and discussion**

**Chemistry**

The compounds 1–8, 6-(3-aryl-2-propenoyl)-2(3H)-benzoxazolones, were synthesised successfully according to Scheme 1. Aryl part was changed as phenyl (1), 4-methylphenyl (2), 4-methoxyphenyl (3), 4-trifluoromethylphenyl (4), 3-hydroxyphenyl (5), 4-isopropylphenyl (6), 4-dimethylaminophenyl (7), and 4-benzoxoxyphenyl (8). The compounds except 1, 3, 5 were reported for the first time in this study.

As indicated in Scheme 1, 6-acetyl-2(3H)-benzoxazolone was synthesised by Friedel–Crafts acylation first. The product was obtained in good yield and purity. Since direct acylation of 2(3H)-benzoxazolone is regioselective and always leads to a 6-acyl derivative.

The chalcones 1–4, and 6 were synthesised by the conventional synthesis method using Claisen–Schmidt condensation reaction between 6-acetyl-2(3H)-benzoxazolone and suitable aldehyde as shown in Scheme 1. On the other hand, chalcones 5, 6, 7, and 8 were synthesised by microwave irradiation method.

The structures of the compounds were confirmed by ¹H-NMR, ¹³C-NMR, and HRMS. In particular, analysis of ¹H-NMR spectra of the compounds 1–8 revealed that all compounds (except compound 7) were both geometrically pure and were configured trans, as derived from coupling constant J: 15.4–15.7 Hz for vinyl protons, observed at 7.60–8.09 ppm. The compound 7 was configured cis with coupling constant J: 10.4 Hz for vinyl protons. The aromatic ring protons were observed at the area of 7.0–8.0 ppm, as expected. The ¹³C-NMR spectra of all compounds, carbons of carbonyl groups were appeared about 187 ppm, as expected. HRMS results were also confirmed the chemical structures of the compounds.

Although all the spectral results were presented in the experimental section, the details of ¹H-NMR spectra of compound 2 are given in Figure 1 as an example.

**Cytotoxic/anticancer activity**

The cytotoxicities of the synthesised compounds, 1–8, have been investigated in vitro against oral squamous cancer cell line (HSC-2) and human normal oral cells (HGF and HPLF). The reference compounds used were DXR and 5-fluorouracil (5-FU) which are clinically in use for cancer treatment. Cytotoxicity results of compounds 1–8 were presented in Table 1. All of the compounds showed lower cytotoxicity than DXR but showed higher cytotoxicity than 5-FU. Cytotoxicities of the compounds were in the range of 4.0–30.2 μM towards HSC-2 cell line. The compounds showed 1.3–9.4 times more cytotoxic than 5-FU. When the cytotoxicities of the
The trifluoromethyl group was substituted at the 4 position of the phenyl ring. The substitution of an alkyl group at the 4 position of the phenyl ring, the increase in cytotoxicity (1.5 and 1.9 times, respectively) was lower than compounds 2 and 4 which carry 4-methyl and 4-trifluoromethyl substituents on the phenyl ring. However, when the substitution of 4 position of phenyl was dimethylamine (compound 7) cytotoxicity reduced 1.4 times comparing to the non-substituted derivative, compound 1. In addition, substituents at 4 position in compounds 3 and 8 lead to a lower increase in selectivity comparing to the compounds 2 and 4’s.

When the PSE values of the compounds were considered for the compounds having substitution at 4 position of phenyl ring, the results observed were interesting. The total electronic contributions of the substituents at 4 position of phenyl ring were as follows according to literature: $\sigma_{\text{isopropyl}} = -0.15$, $\sigma_{\text{methoxy}} = -0.17$, $\sigma_{\text{methoxy}} = -0.27$, $\sigma_{\text{dimethylamine}} = -0.83$ and $\sigma_{\text{trifluoromethyl}} = 0.54$. The compounds bearing the substituent at issue were 6, 2, 3, 7, and 4. The results presented at Table 1 show that the replacement of hydrogen by electron-withdrawing substituent at the 4 position of the phenyl ring increased the PSE values of the compounds (except for compound 2). It means as long as the Hammet value increased, the PSE value of the compound increased. There was a high positive correlation between $\sigma$ and PSE values (r: 0.7918). It also reflects that electron-withdrawing substituents at the 4 position of phenyl had a positive effect on PSE.

Table 1. Cytotoxic activities of the compounds 1–8 towards human OSCC cell lines and human oral normal cells

| Compounds | HSC-2 (A) | SD | HGF (A) | SD | HPLF (A) | SD | Mean (B) | TS (B/A) | PSE (B/A2) |
|-----------|-----------|----|---------|----|----------|----|----------|-----------|------------|
| 1         | 22.1 1.2  | 71.0 9.5 | 83.3 14.0 | 77.2 3.5 | 16.0 0.5 | 7.3 0.1 | 0.27 0.2614 | 0.83 0.83 |
| 2         | 10.1 0.4  | 87.7 18.5 | 105.3 3.1 | 96.5 9.5 | 94.0 0.5 | 10.1 0.5 | 5.3 0.5 | 0.7 0.8 |
| 3         | 14.5 2.9  | 88.3 17.9 | 84.0 0.0 | 86.2 5.9 | 41.0 0.8 | 10.4 0.2 | 3.9 0.3 | 0.27 0.2614 |
| 4         | 4.0 0.6   | 24.7 0.6  | 6.3 0.5  | 25.5 6.4 | 159.0 0.0 | 159.0 0.0 | 3.5 0.5 | 0.7 0.8 |
| 5         | 6.9 0.5   | 55.4 19.7 | 50.3 3.3 | 52.9 7.7 | 111.0 0.0 | 111.0 0.0 | 7.7 0.7 | 0.83 0.83 |
| 6         | 10.1 0.5  | 53.0 5.3  | 76.0 7.0  | 64.5 6.4 | 64.5 6.4 | 64.5 6.4 | 2.9 0.3 | 0.27 0.2614 |
| 7         | 7.0 0.2   | 76.3 9.3  | 70.0 2.0  | 73.2 2.4 | 8 8 | 2.4 0.3 | 0.83 0.83 |
| 8         | 11.9 0.2  | 98.0 50.2 | 52.7 1.5 | 65.3 5.5 | 46.0 0.8 | 46.0 0.8 | 159.0 0.0 | 0.27 0.2614 |
| S-FU*     | 37.7 0.0  | >1000 1000 | 0.0 >1000 0.0 | >1000 28.3 80.7 | 28.3 80.7 | 28.3 80.7 | 5.3 0.5 | 0.7 0.8 |
| DXR*      | 0.5 0.1   | 0.7 0.0  | 0.1 >10 0.0 | >10 0.0 | >10 0.0 | >10 0.0 | >10 0.0 | >10 0.0 |

$\text{SD: standard deviation}.$

The human CA I and II inhibitory effects of the compounds 1–8 were reported for the first time in this study and the inhibition data are shown in Table 2. Acetazolamide (AZA) was used as a reference drug for both hCA I and II isoenzymes. The compounds 1–8 showed lower CA inhibitory effects than the reference drug, AZA. According to Table 2, $\text{IC}_{50}$ values of the compounds 1–8 towards hCA I were in the range of 29.74–69.57$\mu$M, while they were in the range of 18.14–48.46$\mu$M towards hCA II isoenzyme. $\text{IC}_{50}$ values of AZA were 16.58 and 8.37$\mu$M towards hCA I and hCA II, respectively. According to the $\text{IC}_{50}$ values, compound 4, wherein the trifluoromethyl group was substituted at the 4 position of the phenyl ring showed the best activity (29.74$\mu$M).
towards hCA I and compound 8 carrying 4-benzyloxy substituent on phenyl ring showed the best activity (18.14 μM) towards hCA II.

According to Table 2, Kᵢ values (inhibitory potency) of the compounds 1–8 towards hCA I were in the range of 28.37 ± 6.63 – 70.58 ± 6.67 μM towards hCA I isoenzyme and they were in the range of 10.85 ± 2.14 – 37.96 ± 2.36 μM towards hCA II isoenzyme. Kᵢ values of AZA were 30.18 ± 7.77 μM and 4.41 ± 0.55 μM towards hCA I and hCA II, respectively. So, some compounds (compounds 3, 4, 6, and 8) had similar Kᵢ values with AZA towards hCA I while all compounds had higher Kᵢ values than AZA towards hCA II which suggests that they are worse inhibitor than AZA.

Conclusions

Newly synthesized 6-(3-aryl-2-propenoyl)-2(3H)-benzoxazolones, the compounds 1–8, were reported here for the first time with their cytotoxic properties and potential inhibitory effects on hCA I and II. According to the cytotoxicity results of the compounds, compound 4 was the most impressive lead compound of the study with remarkably PSE value (159) for further testing and investigations. On the other hand, according to Kᵢ values compounds 2, 3, 4, 6, and 8 can be considered for the development of new CA I inhibitors due to similar Kᵢ values to AZA but they are not suitable derivatives for the development of new CA II inhibitors since they had higher Kᵢ values than AZA.

Disclosure statement

The authors declare no conflict of interest.

Funding

This research was supported by the Ataturk University Research Fund (Project number: 2016/118).

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