Journal of Enzyme Inhibition and Medicinal Chemistry

ISSN: 1475-6366 (Print) 1475-6374 (Online) Journal homepage: http://www.tandfonline.com/loi/ienz20

Synthesis and bioactivity studies on new 4-(3-(4-Substitutedphenyl)-3a,4-dihydro-3H-indeno[1,2-c]pyrazol-2-yl) benzenesulfonamides

Halise Inci Gul, Mehtap Tugrak, Hiroshi Sakagami, Parham Taslimi, Ilhami Gulcin & Claudiu T. Supuran

To cite this article: Halise Inci Gul, Mehtap Tugrak, Hiroshi Sakagami, Parham Taslimi, Ilhami Gulcin & Claudiu T. Supuran (2016) Synthesis and bioactivity studies on new 4-(3-(4-Substitutedphenyl)-3a,4-dihydro-3H-indeno[1,2-c]pyrazol-2-yl) benzenesulfonamides, Journal of Enzyme Inhibition and Medicinal Chemistry, 31:6, 1619-1624, DOI: 10.3109/14756366.2016.1160077

To link to this article: http://dx.doi.org/10.3109/14756366.2016.1160077

Journal of Enzyme Inhibition and Medicinal Chemistry

www.tandfonline.com/ienz
ISSN: 1475-6366 (print), 1475-6374 (electronic)

J Enzyme Inhib Med Chem, 2016; 31(6): 1619–1624
 DOI: 10.3109/14756366.2016.1160077

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Synthesis and bioactivity studies on new 4-(3-(4-Substitutedphenyl)-3a,4-dihydro-3*H*-indeno[1,2-c]pyrazol-2-yl) benzenesulfonamides

Halise Inci Gul[1], Mehtap Tugrak[1], Hiroshi Sakagami[2], Parham Taslimi[3], Ilhami Gulcin[3,4], and Claudiu T. Supuran[5]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ataturk University, Erzurum, Turkey, [2]Division of Pharmacology, Meikai University School of Dentistry, Sakado, Saitama, Japan, [3]Ataturk University, Faculty of Science, Department of Chemistry, Erzurum, Turkey, [4]College of Science, Department of Zoology, King Saud University, Riyadh, Saudi Arabia, and [5]Neurofarba Department and Laboratorio di Chimica Bioinorganica, Università degli Studi di Firenze, Sesto Fiorentino, Italy

Abstract

A series of new 4-(3-(4-substitutedphenyl)-3a,4-dihydro-3*H*-indeno[1,2-c]pyrazol-2-yl) benzenesulfonamides (**7**–**12**) was synthesized starting from 2-(4-substitutedbenzylidene)-2,3-dihydro-1*H*-inden-1-one (**1**–**6**) and 4-hydrazinobenzenesulfonamide. The substituted benzaldehydes from which the key intermediate was prepared by introducing 2- or 4-substituents such as fluorine, hydroxy, methoxy, or the 3,4,5-trimethoxy moieties. The compounds were tested for their cytotoxicity, tumor-specificity and potential as carbonic anhydrase (CA, EC 4.2.1.1) inhibitors. The 3,4,5-trimethoxy and the 4-hydroxy derivatives showed interesting cytotoxic activities, which may be crucial for further anti-tumor activity studies, whereas some of these sulfonamides strongly inhibited both human (h) cytosolic isoforms hCA I and II.

Keywords

Benzenesulfonamide, carbonic anhydrase/enzyme inhibition, cytotoxicity, indane, pyrazole, tumor selectivity

History

Received 8 February 2016
Revised 25 February 2016
Accepted 26 February 2016
Published online 23 March 2016

Introduction

Cancer is the second cause of death all over the world. Although radiation and surgery are used for the treatment of cancer, chemotherapy is the most widely used therapeutic approach for it. Available anticancer drugs in markets have several problems such as side effects, toxicity, cross resistance, and low selectivity[1].

The sulfonamides are an important class of drugs known with antibacterial, anti-carbonic anhydrase, diuretic, anti-diabetic or hypoglycemic, and antithyroid activities[2–5]. A large number of sulfonamide derivatives have recently been reported to show remarkable antitumor activity both *in vivo* and/or *in vitro*. Some of these sulfonamide derivatives are currently being evaluated in clinical trial leading to consider them as novel alternative anti-cancer drugs, devoid of the side effects of presently available pharmacological agents[5]. Recently, new pyrazolines bearing benzene sulfonamides were synthesized and their anticancer activities were investigated[6]. In this study it was observed promising anti-proliferative activities with GI_{50} values less than 2 μM particularly against MOLT-4 (1.94), 5R (1.28) in leukemia cancers, EKVX (1.88) in non-small cell lung cancer, COLO 205 (1.69) in colon cancer for the compound 2f (4–(3-(3-chloro-6-hydroxy-2,4-dimethylphenyl)-5–(4-(dimethylamino)phenyl-4,5-dihydro-1*H*-pyrazol-1-yl) benzenesulfonamide). In the another literature[7], it was reported that substituted pyrazoline compound (4–(5-(2,5-dimethylphenyl)-3-(trifluoromethyl)-4, 5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide) and (1–(4-aminosulfonylphenyl)-3-trifluoromethyl-5-[3,5-di-(tri-fluoromethyl)-phenyl]-4,5-dihydro-pyrazole) showed improved antitumoral activity in the treatment of cancer, especially for colon and/or prostate cancer, although these compounds do not inhibit cyclooxygenase-1 and/or cyclooxygenase-2.

Indane or indanone-bearing compounds had been reported to show their several bioactivities including cytotoxic/anticancer activities[8–15], inhibition of β-amyloid plaques, which were stimulated by acetylcholinesterase[16], and effects on mitochondrial respiration by inhibition of reactive oxygen species[17].

Chalcones are widely used precursor molecules for the preparation of pyrazoles and pyrazolines. Chalcones and their derivatives have several bioactivities such as cytotoxic/anticancer activities[18–24], topoisomerase I inhibitory[25], carbonic anhydrase I and II inhibitory[15,26] activities.

Pyrazolines are prominent nitrogen bearing five membered heterocylic compounds with antimicrobial[27], anti-inflammatory[28], antihipertansive[29] activities. Medicinally important pyrazolines are 1,3,5-trisubstituted derivatives and their antiinflammatory[30,31], dual antimicrobial and antiinflammatory[27], analgesic and antimicrobial[32], and selective COX-2 inhibitory (i.e. Celecoxib)[33] activities were reported.

The carbonic anhydrases (CAs) are the metalloenzymes containing zinc ions (Zn^{2+}), which classically participate in the maintenance of pH homeostasis. CAs catalyze the reversible hydration of carbon dioxide (CO_2) in two-step reaction to yield bicarbonate (HCO_3^-) ion and proton (H^+)[34]. The inter-conversion

Address for correspondence: Halise Inci Gul, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ataturk University, 25240, Erzurum, Turkey. Tel: +90 442 231 5219. Fax: +90 442 231 5201. E-mail: incigul1967@yahoo.com

of these chemical species is shown in following equation, which however is too slow to meet the physiological needs of most biochemical processes[35].

$$CO_2 + H_2O \overset{CA}{\Leftrightarrow} H_2CO_3 \Leftrightarrow HCO_3^- + H^+$$

CAs have six genetically and distinct enzyme families: the α-, β-, γ-, ε-, ζ- and η-CA. Mammals, including humans, generally contain α-CAs, the most popular CA family. Until now, sixteen different α-CA isoenzymes have been identified in various tissues and organs with different expression levels, kinetic and molecular properties and oligomeric rearrangements[34]. According to the known cellular localization, some of them are cytosolic (CA I, CA II, CA III, CA VII and CA XIII), other CA isoenzymes are membrane bound (CA IV, CA IX, CA XII and CA XIV), two of CAs are mitochondrial (CA VA and CA VB) and one of CAs is salivary (CA VI)[36]. CA XV is not synthesized in humans and other primates and is abundantly found in rodents and other vertebrates as an isoform. Three acatalytic forms are also reported and named as CA related proteins (CARP), CARP VIII, X and XI, which are found in the cytosol[37].

The two important CA isozymes (CA I and CA II) are present at higher concentrations in the cytosol in erythrocytes. hCA I, and II have various medical applications and shows optimal activity at physiological pH and temperatures. Carbonic anhydrase inhibitors (CAIs) have many clinical usages of major diseases such as diuretics, antiglaucoma, gastroduodenal ulcers, anti-obesity drugs, acid-base disequilibria, and antiepileptic. CAIs are useful for the treatment of some neurological disorders such as idiopathic intracranial hypertension[38,39]. The inhibition and activation mechanisms of CAs are well-understood processes at the molecular level. Usually most classes of CAIs bind to the metal center thus causing disruption of the CO_2 hydration reaction[3]. The classical CAIs are the primary sulfonamides, RSO_2NH_2, which are in clinical use for more than seventy years as diuretics and systemically acting anti-glaucoma drugs[34].

The aim of this study was to design and synthesize new compounds including pyrazoline, sulfonamide, and indane pharmacophores all together to investigate their cytotoxicities, potential carbonic anhydrase inhibition properties to find out a leader compound/s for further studies.

Materials and methods

Melting points were determined using an Electrothermal 9100 (IA9100, Bibby Scientific Limited, Stone, UK) instrument and are uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained using a Varian Mercury Plus spectrometer (Varian Inc., Palo Alto, CA). Chemical shifts (δ) are reported in ppm. Mass spectra were undertaken on an HPLC-TOF Waters Micromass LCT Premier XE (Waters Corporation, Milford, MA) mass spectrometer using an electrospray ion source (ESI). All reactions were carried out in CEM Discover microwave synthesis systems (CEM, Matthews, NC).

General procedure for the synthesis of 2–(4-substituted-benzylidene)-2,3-dihydro-1*H*-inden-1-one (1–6)

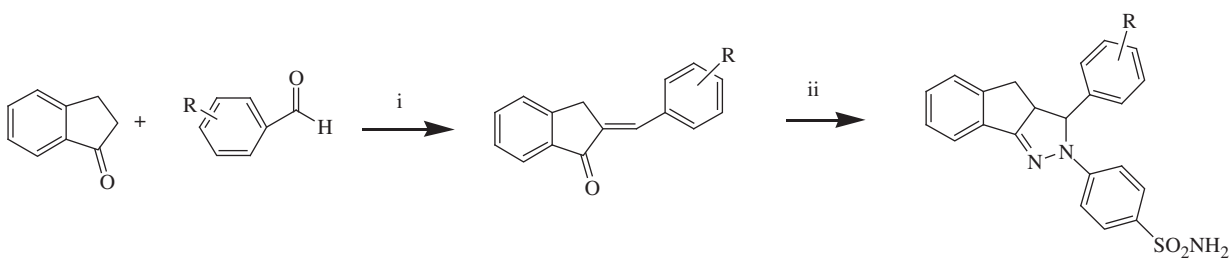
Aqueous solution of sodium hydroxide (10% w/v, 10 mL) was added into the ethanol (6 mL) solution of 1-indanone (20 mmol) and suitable substitute benzaldehyde (20 mmol) (Scheme 1). The mixture was stirred overnight at room temperature and then it was poured on ice-water (100 mL) in the beaker. The mixture was neutralized with hydrochloric acid (10% w/v, 10 mL). The colored precipitate formed was filtered and crystallized from water-ethanol for the compounds (**1**–**6**)[13–15,17,40,41]. Chemical structure of the compounds **1**–**6** were confirmed by ^{1}H NMR, ^{13}C NMR, HRMS and the literature reported melting points of the compounds. Data are not presented here.

General procedure for the synthesis of pyrazoline derivatives (7–12)

A solution of 2–(4-substituted benzylidene)-2,3-dihydro-1*H*-inden-1-one (**1**–**6**, 1.00 mmol) and 4-hydrazinobenzensulfonamide hydrochloride (1.10 mmol) in ethanol (50 mL) was heated in (100 °C, 200 Watt, 3–7 barr) for 10–120 min [20 min, 3 barr (**7**), 60 min, 7 barr (**8, 11**); 30 min, 7 barr (**9**); 10 min, 7 barr (**10**); 120 min, 3 barr (**12**)]. The reactions were monitored by TLC. When the reaction was stopped, the volume of the reaction mixture was concentrated to the half and the precipitate formed was filtered, washed with cold ethanol, and the compounds were purified by crystallization from ethanol to obtain **7**–**12**. Chemical structures of the compounds **7**–**12** were confirmed by ^{1}H NMR, ^{13}C NMR, and HRMS.

4–(3-Phenyl)-3a,4-dihydro-3*H*-indeno[1,2-c]pyrazol-2-yl) benzenesulfonamide (7)

M.p. 243–246 °C. Yield: 8.4% ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.76 (d, 1H, Ar-H, $J = 8.4$ Hz), 7.67 (d, 2H, Ar-H, $J = 9.1$ Hz), 7.34–7.19 (m, 6H, Ar-H), 7.03 (bs, 4H, Ar-H), 5.59 (d, 1H, C_3-H, $J = 10.9$ Hz), 4.28–4.21 (m, 1H, C_{3a}-H), 2.91 (dd, 1H, C_4-H_a, $J = 15.9$, 8.7 Hz), 2.17 (dd, 1H, C_4-H_b, $J = 15.9$, 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 163.9, 151.7, 148.1, 134.3, 131.0, 130.7, 129.8, 128.9, 128.4, 128.3, 127.9, 127.3, 126.6, 122.9, 112.5, 67.5, 55.2, 29.9; Mass spectrum: 390.12 (M^++1); HRMS (ESI-MS) Calc.: 390.1276 for $C_{22}H_{20}N_3O_2S$ $[M+H]^+$, found: 390.1281.



1-6 **7-12**

Scheme 1. 4–(3-(4-substitutedphenyl)-3a,4-dihydro-3*H*-indeno[1,2-c]pyrazol-2-yl) benzenesulfonamide 7–12. (i) aq. NaOH 10%, EtOH, r.t, 12 h; (ii) 4-hydrazinobenzensulfonamide hydrochloride, EtOH, 100 °C, 200 Watt, 3-7 barr, 10'-120'. R: H (**1**, **7**), 4-OCH_3 (**2**, **8**), 2-OCH_3 (**3**, **9**), 3,4,5-$(OCH_3)_3$ (**4**, **10**), 4-F (**5**, **11**), 4-OH (**6**, **12**).

4–(3-(4-Methoxyphenyl)-3a,4-dihydro-3*H*-indeno[1,2-c] pyrazol-2-yl)benzenesulfonamide (8)

M.p. 172–176 °C. Yield: 19.3% ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 7.73–7.71 (m, 1H, Ar-H), 7.64–7.62 (m, 2H, Ar-H), 7.35–7.30 (m, 2H, Ar-H), 7.26–7.24 (m, 1H, Ar-H), 7.06 (d, 2H, Ar-H, $J = 8.4$ Hz), 6.96 (bs, 2H, Ar-H), 6.77 (d, 2H, Ar-H, $J = 7.7$ Hz), 5.74 (d, 1H, C_3-H, $J = 10.9$ Hz), 4.29–4.23 (m, 1H, C_{3a}-H), 3.69 (s, 3H, OCH_3), 2.94 (dd, 1H, C_4-H_a, $J = 15.9$, 8.9 Hz), 2.14 (dd, 1H, C4-H_b, $J = 15.9$, 7.4 Hz); ^{13}C NMR (100 MHz, CD_3OD, ppm) δ 164.3, 159.6, 152.0, 148.1, 131.6, 131.1, 130.5, 128.5, 127.6, 127.4, 126.5, 126.3, 122.1, 114.1, 112.3, 67.3, 54.9, 54.4, 29.3; Mass spectrum: 420.13 (M^++1); HRMS (ESI-MS) Calc.: 420.1382 for $C_{23}H_{22}N_3O_3S$ $[M + H]^+$, found: 420.1399.

4–(3-(2-Methoxyphenyl)-3a,4-dihydro-3*H*-indeno[1,2-c] pyrazol-2-yl)benzenesulfonamide (9)

M.p. 245–247 °C. Yield: 3.6% ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 7.67–7.65 (m, 1H, Ar-H), 7.56 (d, 1H, Ar-H, $J = 9.1$ Hz), 7.34–7.28 (m, 2H, Ar-H), 7.21–7.17 (m, 1H, Ar-H), 7.07 (d, 1H, Ar-H, $J = 8.1$ Hz), 7.01 (s, 2H, Ar-H), 6.87 (bs, 2H, Ar-H), 6.67 (t, 1H, Ar-H, $J = 7.5$ Hz), 6.44 (d, 1H, Ar-H, $J = 7.7$ Hz), 5.95 (d, 1H, C_3-H, $J = 10.9$ Hz), 4.32–4.30 (m, 1H, C_{3a}-H), 3.89 (s, 3H, OCH_3), 2.99 (dd, 1H, C_4-H_a, $J = 16.2$, 8.4 Hz), 1.93 (dd, 1H, C_4-H_b, $J = 16.2$, 7.5 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ 164.8, 157.9, 152.1, 147.3, 132.9, 131.3, 131.0, 129.8, 128.4, 128.0, 127.3, 122.7, 121.4, 121.2, 112.2, 111.8, 62.4, 56.3, 54.2, 29.9; Mass spectrum: 420.13 (M^++1); HRMS (ESI-MS) Calc.: 420.1382 for $C_{23}H_{22}N_3O_3S$ $[M + H]^+$, found: 420.1373.

4–(3-(3,4,5-Trimethoxyphenyl)-3a,4-dihydro-3*H*-indeno[1,2-c]pyrazol-2-yl)benzenesulfonamide (10)

M.p. 266–269 °C. Yield: 41.5% ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 7.71–7.69 (m, 1H, Ar-H), 7.58 (d, 2H, Ar-H, $J = 9.1$ Hz), 7.36–7.32 (m, 3H, Ar-H), 7.05–7.01 (m, 4H, Ar-H), 5.80 (d, 1H, C_3-H, $J = 10.6$ Hz), 4.30–4.27 (m, 1H, C_{3a}-H), 2.97 (dd, 1H, C_4-H_a, $J = 16.1$, 8.7 Hz) 2.02 (dd, 1H, C_4-H_b, $J = 16.1$, 7.7 Hz); 9 hydrogen peaks of three methoxy groups were under the peak of solvents DMSO-d_6. ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ 164.6, 153.7, 152.3, 147.8, 137.3, 133.2, 131.2, 131.1 131.0, 128.5, 127.9, 127.4, 122.9, 112.6, 67.4, 60.6, 56.4, 56.5, 54.9; Mass spectrum: 480.15(M^++1); HRMS (ESI-MS) Calc.: 480.1593 for $C_{25}H_{26}N_3O_5S$ $[M + H]^+$, found: 480.1599.

4–(3-(4-Florophenyl)-3a,4-dihydro-3*H*-indeno[1,2-c] pyrazol-2-yl)benzenesulfonamide (11)

M.p. 162–165 °C. Yield: 16.5% ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 7.73 (d, 1H, Ar-H, $J = 4.2$ Hz), 7.65 (d, 2H, Ar-H, $J = 9.1$ Hz), 7.34–7.32 (m, 2H, Ar-H), 7.26 (d, 1H, Ar-H, $J = 4.3$ Hz), 7.07–6.97 (m, 6H, Ar-H), 5.81 (d, 1H, C_3-H, $J = 10.6$ Hz), 4.32–4.25 (m, 1H, C_{3a}-H), 2.97 (dd, 1H, C_4-H_a, $J = 15.8$, 8.9 Hz), 2.09 (dd, 1H, C_4-H_b, $J = 15.8$, 7.6 Hz); ^{13}C NMR (100 MHz, CD_3OD, ppm) δ 164.2, 151.9, 147.9, 131.9, 130.9, 130.8, 130.6, 129.3, 127.7, 127.5, 126.4, 122.2, 115.6, 115.4, 112.3, 66.9, 54.8, 29.4; Mass spectrum: 408.11(M^++1); HRMS (ESI-MS) Calc.: 408.1182 for $C_{22}H_{19}N_3O_2SF$ $[M + H]^+$, found: 408.1174.

4–(3-(4-Hydroxyphenyl)-3a,4-dihydro-3*H*-indeno[1,2-c] pyrazol-2-yl)benzenesulfonamide (12)

M.p. 267–271 °C. Yield: 8.8% ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 7.73–7.71 (m, 1H, Ar-H), 7.64 (d, 2H, Ar-H, $J = 9.1$ Hz), 7.34–7.25 (m, 3H, Ar-H), 7.06 (d, 2H, Ar-H, $J = 8.0$ Hz), 6.87 (bs, 2H, Ar-H), 6.64 (d, 2H, Ar-H, $J = 7.3$ Hz), 5.70 (d, 1H, C_3-H, $J = 10.6$ Hz), 4.28–4.21 (m, 1H, C_{3a}-H), 2.94 (dd, 1H, C_4-H_a, $J = 16.0$, 8.9 Hz), 2.21–2.13 (m, 1H, C_4-H_b); ^{13}C NMR (100 MHz, CD_3OD, ppm) δ 164.4, 157.1, 152.0, 148.1, 131.5, 131.1, 130.4, 128.5, 127.5, 127.3, 126.4, 125.3, 122.1, 115.4, 112.3, 67.5, 54.9, 29.3; Mass spectrum: 406.12 (M^++1); HRMS (ESI-MS) Calc.: 406.1225 for $C_{22}H_{20}N_3O_3S$ $[M + H]^+$, found: 406.1214.

Assay for cytotoxicity

The compounds were assayed towards human oral squamous cell carcinoma cell lines (Ca9-22, HSC-2, HSC-3, HSC-4), and human oral normal mesenchymal cells [gingival fibroblast (HGF), pulp cell (HPC) and periodontal ligament fibroblast (HPLF)] based on a literature procedure with some minor modifications[42,43]. In brief, cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS). Varying concentrations of the compound in dimethylsulfoxide were added to the medium and incubated at 37 °C for 48 h. The viable cell numbers were determined by the MTT method except for HL-60 cells, the viable cell number of which was counted with a hemocytometer after staining with 0.15% trypan blue. The 50% cytotoxic concentration (CC_{50}) value was determined from the growth curves plotted at different concentrations of each compounds in triplicate wells.

Carbonic anhydrase enzyme assay

The Carbonic Anhydrase (CA) I, and II isoenzymes were purified from fresh human blood erythrocytes using by Sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatography[44,45]. This method contains the purification of CA isoenzymes *via* a single step described previously[46].

CA isoenzyme activity was determined spectrophotometricaly at 348 nm as described by Verpoorte et al.[47]. According to this method the absorbance changes were measured during the time of 3 min at 25 °C as *p*-nitrophenylacetate (PNA) converted to 4-nitrophenylate ion. These type of spectrophotometric determinations are described in detail in our previous studies[48].

Bradford method was used to quantify the amount of protein during the purification steps. This spectrophotometric assay has been explained previously[49]. Bovine serum albumin was used as standard protein[50].

After the purification process of the CA isoenzymes, SDS-polyacrylamide gel electrophoresis (SDS–PAGE) has been carried out[51]. Stacking and resolving gel containing 3% and 10% acrylamide, and 0.1% SDS was used for running the process using a Minigel system (Mini-PROTEAN® system Casting stand, Catalog 1658050, Bio-Rad Laboratories, Inc., China). The method used for visualization of protein has been explained in detail in our previous studies[52]. According to this method, the gel was fixed then stained with Coomassie Brilliant Blues R-250 later on the gel stained by using standard methods for detecting protein bands that are belong to purified CA isoenzymes[53].

The effects of novel benzenesulfonamides (**7**–**12**) derivatives were examined using the hydratase activity and recorded in triplicate analysis at each concentration used[54]. For this purpose, different concentrations of novel benzenesulfonamides (**7**–**12**) derivatives were determined in preliminary assays. CA isoenzyme activities were measured in the presence of different quantity of them. The control sample activity in the absence of a novel benzenesulfonamides (**7**–**12**) derivatives were taken as 100%[55]. For each novel benzenesulfonamides (**7**–**12**), an activity (%)-[Benzenesulfonamides] was drawn using Excel program. IC_{50} of each novel benzenesulfonamides (**7**–**12**) derivatives was calculated from graphs. IC_{50} value is a measure of the effectiveness of benzenesulfonamides (**7**–**12**) derivatives in inhibiting both

CA isoenzymes[56]. For determination of Ki values, three different benzenesulfonamides (**7**–**12**) concentrations were used. Ki values reflect the binding affinity of benzenesulfonamides (**7**–**12**) to both CA isoenzymes. In this way, Value is converted to an absolute inhibition constant Ki value. In this experiment, PNA was used as substrate at five different concentrations. Then, Lineweaver–Burk curves were drawn[57].

Result and discussion

Condensation between 1-indanone and the appropriate benzaldehyde afforded the compounds **1**–**6**. These compounds reacted with 4-hydrazinobenzensulfonamide hydrochloride to produce pyrazoline derivatives, the compounds **7**–**12**. ^{1}H NMR, ^{13}C NMR, and HRMS spectroscopies confirmed the chemical structures.

The cytotoxicity data (Table 1), hCA I and II inhibition percentages data (Table 2) of the compounds were presented in Tables 1 and 2, respectively.

When the cytotoxicity data of the compounds were considered, the first question to be addressed is whether the compounds **7**–**12** have anti-neoplastic properties. The results portrayed in Table 1 reveal that in general the CC_{50} of **7**–**12** are in the range of 4.6–58.0 μM towards Ca9–22, HSC-2, HSC-3, and HSC-4 cells. The potency of the compounds **7**–**12** towards tumor cell lines was compared with a reference compound 5-Fluorouracil (5-FU). Compounds **7** was more potent than 5-FU towards HSC-4 cells.

The second aspect of these compounds to be considered is whether they are tumor-specific cytotoxins since tumors are surrounded by different types of normal cells. Selectivity index (SI) figures were generated which are quotients of average CC_{50} values of normal cells and CC_{50} figure of a compound towards a specific cell line. The results in Table 1 reveal that SI values of greater than 1 were obtained in general. Exceptions were **12** towards HSC-2, HSC-3, and HSC-4 cell lines and **7** towards HSC-2 cells.

When the most-selective compounds (SI) toward tumorous cells were considered, the following sets of combination were found to be the best: **12** (SI: 6.9) towards Ca9–22, **10** (SI: 1.9) towards HSC-2, **7** (SI: 3.8) towards HSC-3, **7** (SI: 7.2) towards HSC-4 cell lines.

Tumor-specificity (TS) value reflects the selectivity of the compounds against cancer tissues rather than normal ones. In this study, two types TS values were calculated. First, TS was also calculated by dividing the mean CC_{50} value of each compound against three human oral normal cells (Column D) to mean CC_{50} value against four human OSCC cell lines (Column B) (Table 1). Second, TS was calculated by dividing the CC_{50} value of each compound against HGF cells (Column C) to the CC_{50} value against Ca9–22 cell line (Column A), both cells being originated from the same tissue (gingiva) (Table 1). All compounds showed lower TS values than reference drug 5-FU by these two types of criteria for TS. According to TS values obtained by first calculation method, the order of potency of TS values of the compounds was as follows: The compound number (TS value): **10** (2.3) > **7** (1.9) > **9** (1.7) > **11** (1.6) > **8** (1.5) > **12** (1.3). When the second calculation was considered, the order of potency of TS values of the compounds was as follows: **12** (6.9) > **10** (3.9) > **8** and **9** (2.6) > **11** (2.1) > **7** (1.4).

When the esterase assay with 4-nitrophenyl acetate as substrate were applied to the compounds **7**–**12**, all benzenesulfonamide compounds **7**–**12** behaved as powerful inhibitors against slow cytosolic isoenzyme hCA I with Ki values in ranging of 324.61 ± 47.16 – 550.21 ± 103.2 nM. Compound **10** (Ki: 324.61 ± 47.16 nM), which is 3,4,5-trimethoxy derivative, and compound **11** (Ki: 328.92 ± 31.02), which is 4-fluoro

Table 1. Cytotoxicity, tumor-specificity and Log *P* values of new 4–(3-(4-substitutedphenyl)-3a,4-dihydro-3*H*-indeno[1,2-c]pyrazol-2-yl) benzenesulfonamides (**7**–**12**).

	CC_{50} (μM)														
	Human oral squamous cell carcinoma cell lines								Human normal oral cells						
	Ca9-22 (**A**)	SI	HSC-2	SI	HSC-3	SI	HSC-4	SI	mean (**B**)	mean SI	HGF (**C**)	HPLF	HPC	mean (**D**)	TS(D/B)	TS(C/A)	*Log P
7	19.3 ± 4.7	1.7	37.1 ± 1.3	0.9	8.6 ± 5.2	3.8	4.6 ± 0.6	7.2	17.4	3.4	27.4 ± 24.7	8.1 ± 2.3	63.3 ± 19.8	33	1.9	1.4	3.68
8	18.7 ± 4.7	2.5	41.2 ± 1.8	1.1	32.8 ± 1.6	1.4	34.8 ± 7.8	1.3	31.9	1.5	48.7 ± 15.3	38.0 ± 2.0	52.0 ± 10.3	46,2	1.5	2.6	3.55
9	14.0 ± 2.0	2.4	25.6 ± 1.1	1.3	23.0 ± 0.7	1.5	17.1 ± 2.2	2.0	19.9	1.8	36.0 ± 1.6	36.0 ± 7.5	30.3 ± 6.0	34,1	1.7	2.6	3.55
10	24.7 ± 4.7	2.8	35.2 ± 1.9	1.9	29.4 ± 3.8	2.3	27.8 ± 2.9	2.4	29.3	2.3	97.3 ± 25.1	41.7 ± 3.2	65.3 ± 18.5	68,1	2.3	3.9	3.3
11	21.3 ± 0.6	1.9	35.2 ± 2.0	1.2	26.1 ± 7.8	1.6	19.2 ± 3.3	2.2	25.5	1.7	45.0 ± 2.6	38.3 ± 2.3	41.3 ± 4.6	41,6	1.6	2.1	3.84
12	58.0 ± 20.8	6.9	>400	<1	>400	<1	>400	<1	314.5	<2.5	>400	>400	>400	400	1.3	>6.9	3.29
Average	26	3	>95,7	>1.2	>86,6	>1.9	>83,9	>2.7	73.1	2.2	>109,1	>93,7	>108,7	103,8			
5-FU	12.1 ± 0.7	72.3	15.2 ± 2.7	57.6	7.2 ± 1.0	121.6	7.3 ± 0.17	119.9	10.5	92.8	>1000	>1000	626.3 ± 87.9	875.4	83.8	83.3	

The CC_{50} values refer to the concentrations of the compounds in micromoles, which kill 50% of the cells. The letters SI indicate the selectivity index, i.e. the quotient of the average CC_{50} figures towards HGF, HPC and HPLF nonmalignant cells divided by the CC_{50} figure of the compound towards a specific tumor cell line.
*Log *P* values were calculated using ChemDraw Ultra (Version 12.0, Cambridge Soft Corporation, Cambridge, MA).

Table 2. Inhibition of human carbonic anhydrase isoenzymes (hCA I and II) by new 4–(3-(4-substitutedphenyl)-3a,4-dihydro-3*H*-indeno[1,2-c]pyrazol-2-yl) benzenesulfonamides (**7–12**).

	IC_{50} (nM)				K_I (nM)	
Compounds	hCA I	r^2	hCA II	r^2	hCA I	hCA II
7	644.65	0.9846	514.47	0.9725	547.35 ± 154.2	500.87 ± 122.5
8	502.17	0.9717	391.08	0.9543	456.93 ± 117.3	400.58 ± 172.4
9	510.31	0.9755	463.54	0.9801	458.30 ± 69.20	470.71 ± 136.1
10	413.97	0.9693	330.78	0.9726	324.61 ± 47.16	262.92 ± 72.05
11	535.13	0.9814	418.73	0.9624	328.92 ± 31.02	318.06 ± 120.1
12	498.92	0.9783	410.78	0.9610	550.21 ± 103.2	487.73 ± 214.1
AZA*	656.87	0.9908	610.57	0.9958	460.27 ± 192.8	455.28 ± 146.0

*Acetazolamide (AZA) was used as a standard inhibitor for all hCA.

derivative, inhibited hCA I activity more potently than reference drug AZA (Ki: 460.27 ± 192.8), which is used for the treatment of idiopathic intracranial hypertension, cystinuria, glaucoma, altitude sickness, epileptic seizure, periodic paralysis, central sleep apnea and dural estasia. Since hCA I isoenzyme is found in many tissues and involved in retinal and cerebral edema, its inhibition by the compounds **7–12** may be a valuable tool for fighting against these symptoms. On the other hand, the compounds **7–12** demonstrated Ki values ranging between 262.92 ± 72.05 and 500.87 ± 122.5 nM towards hCA II. The compounds **10** (Ki: 262.92 ± 72.05 nM) and **11** (Ki: 318.06 ± 120.1 nM) inhibited hCA II activity more potently than reference compound AZA (Ki: 455.28 ± 146.0 nM), like in the case of hCA I experiment. Since CAII isoenzyme involved in several diseases, such as glaucoma, edema, epilepsy, and altitude sickness, its inhibitory property of **7–12** may be applicable for fighting these diseases.

As a result, the compounds **10**, which is 3,4,5-trimethoxy derivative, and **12**, which is 4-hydroxy derivative, seem candidate cytotoxic compounds for further studies in terms of tumor-specificity according to two types of TS calculations while the compounds **10** and **11**, which is 4-fluoro derivative, seem candidate compounds as both hCA I and II inhibitors for further studies.

Declaration of interest

The authors report no conflict of interest and are responsible for the contents and writing of the paper. This research work was supported by Ataturk University Research Found, Turkey (Project No BAP: 2012/74.

References

1. Bange J, Zwick E, Ullrich A. Molecular targets for breast cancer therapy and prevention. Nat Med 2001;7:548–52.
2. Ghorab MM, Alsaid MS, Ceruso M, et al. Carbonic anhydrase inhibitors: Synthesis, molecular docking, cytotoxic and inhibition of the human carbonic anhydrase isoforms I, II, IX, XII with novel benzenesulfonamides incorporating pyrrole, pyrrolopyrimidine and fused pyrrolopyrimidine moieties. Bioorg Med Chem 2014;22:3684–95.
3. Carta F, Supuran CT, Scozzafava A. Sulfonamides and their isosters as carbonic anhydrase inhibitors. Future Med Chem 2014;6:1149–65.
4. Supuran CT, Scozzafava A, Inventors. Carbonic anhydrase inhibitors and their therapeutic potential. Exp Opin Ther Patents 2000;10:575–600.
5. Casini A, Scozzafava A, Mastrolorenzo A, Supuran CT. Sulfonamides and sulfonylated derivatives as anticancer agents. Curr Cancer Drug Targets 2002;2:55–75.
6. Bano S, Javed K, Ahmad S, et al. Synthesis and biological evaluation of some new 2-pyrazolines bearing benzene sulfonamide moiety as potential anti-inflammatory and anti-cancer agents. Eur J Med Chem 2011;46:5763–8.
7. Altisen RC, Constansa JF, Bafalluy RM, Rigat IC. Inventors. Substituted pyrazoline derivatives. 2005;US patent 0182119A1.
8. Bansal R, Narang G, Zimmer C, Hartmann RW. Synthesis of some imidazolyl-substituted 2-benzylidene indanone derivatives as potent aromatase inhibitors for breast cancer therapy. Med Chem Res 2011;20:661–9.
9. Saxena HO, Faridi U, Kumar JK, et al. Synthesis of chalcone derivatives on steroidal framework and their anticancer activities. Steroids 2007;72:892–900.
10. Saxena HO, Faridi U, Srivastava S, et al. Gallic acid-based indanone derivatives as anticancer agents. Bioorg Med Chem Lett 2008;18:3914–18.
11. Chanda D, Bhushan S, Guru SK, et al. Anticancer activity, toxicity and pharmacokinetic profile of an indanone derivative. Eur J Pharm Sci 2012;47:988–95.
12. Zhou DY, Ding N, Zhao SQ, et al. Synthesis and evaluation of curcumin-related compounds containing inden-2-one for their effects on human cancer cells. Biol Pharm Bull 2014;37:1977–81.
13. Tugrak M, Gul HI, Sakagami H. Synthesis and cytotoxicities of 2-[4-hydroxy-(3,5-bis-aminomethyl)-benzylidene]-indan-1-ones. Lett Drug Des Discov 2015;12:806–12.
14. Tugrak M, Yamali C, Sakagami H, Gul HI. Synthesis of mono Mannich bases of 2-(4-hydroxybenzylidene)-2,3-dihydroinden-1-one and evaluation of their cytotoxicities. J Enzyme Inhib Med Chem 2015;6:1–6.
15. Yamali C, Tugrak M, Gul HI, et al. The inhibitory effects of phenolic Mannich bases on carbonic anhydrase I and II isoenzymes. J Enzyme Inhib Med Chem 2016;10:1–4.
16. Rizzo S, Bartolini M, Ceccarini L, et al. Targeting Alzheimer's disease: novel indanone hybrids bearing a pharmacophoric fragment of AP2238. Bioorg Med Chem 2010;18:1749–60.
17. Vaskova J, Reisch R, Vasko L, et al. Effect of selected dimethylaminochalcones on some mitochondrial activities. In Vitro Cell Dev Biol Anim 2013;49:354–9.
18. Yerdelen KO, Gul HI, Sakagami H, Umemura N. Synthesis and biological evaluation of 1,5-bis(4-hydroxy-3-methoxyphenyl)penta-1,4-dien-3-one and its aminomethyl derivatives. J Enzyme Inhib Med Chem 2015;30:383–8.
19. Yerdelen KO, Gul HI, Sakagami H, et al. Synthesis and cytotoxic activities of a curcumin analogue and its bis-Mannich derivatives. Lett Drug Des Discov 2015;12:643–9.
20. Bilginer S, Gul HI, Mete E, et al. 1-(3-Aminomethyl-4-hydroxyphenyl)-3-pyridinyl-2-propen-1-ones: A novel group of tumour-selective cytotoxins. J Enzyme Inhib Med Chem 2013;28:974–80.
21. Das U, Doroudi A, Gul HI, et al. Cytotoxic 2-benzylidene-6-(nitrobenzylidene)cyclohexanones which display substantially greater toxicity for neoplasms than non-malignant cells. Bioorg Med Chem 2010;18:2219–24.
22. Gul HI, Yerdelen KO, Das U, et al. Synthesis and cytotoxicity of novel 3-aryl-1-(3′-dibenzylaminomethyl-4′-hydroxyphenyl)-propenones and related compounds. Chem Pharm Bull 2008;56:1675–81.
23. Gul HI, Yerdelen KO, Gul M, et al. Synthesis of 4′-hydroxy-3′-piperidinomethylchalcone derivatives and their cytotoxicity against PC-3 cell lines. Arch Pharm (Weinheim) 2007;340:195–201.

24. Das U, Gul HI, Alcorn J, et al. Cytotoxic 5-aryl-1-(4-nitrophenyl)-3-oxo-1,4-pentadienes mounted on alicyclic scaffolds. Eur J Med Chem 2006;41:577–85.
25. Gul HI, Cizmecioglu M, Zencir S, et al. Cytotoxic activity of 4′-hydroxychalcone derivatives against Jurkat cells and their effects on mammalian DNA topoisomerase I. J Enzyme Inhib Med Chem 2009;24:804–7.
26. Bilginer S, Unluer E, Gul HI, et al. Carbonic anhydrase inhibitors. Phenols incorporating 2- or 3-pyridyl-ethenylcarbonyl and tertiary amine moieties strongly inhibit *Saccharomyces cerevisiae* beta-carbonic anhydrase. J Enzyme Inhib Med Chem 2014;29:495–9.
27. Sharma PK, Kumar S, Kumar P, et al. Synthesis of 1-(4-aminosulfonylphenyl)-3,5-diarylpyrazoline derivatives as potent antiinflammatory and antimicrobial agents. Med Chem Res 2012; 21:2945–54.
28. Fioravanti R, Bolasco A, Manna F, et al. Synthesis and biological evaluation of *N*-substituted-3,5-diphenyl-2-pyrazoline derivatives as cyclooxygenase (COX-2) inhibitors. Eur J Med Chem 2010;45: 6135–8.
29. Turan-Zitouni G, Chevallet P, Kilic FS, Erol K. Synthesis of some thiazolyl-pyrazoline derivatives and preliminary investigation of their hypotensive activity. Eur J Med Chem 2000;35:635–41.
30. Reddy MV, Billa VK, Pallela VR, et al. Design, synthesis, and biological evaluation of 1-(4-sulfamylphenyl)-3-trifluoromethyl-5-indolyl pyrazolines as cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) inhibitors. Bioorg Med Chem 2008;16:3907–16.
31. Sharma PK, Kumar S, Kumar P, et al. Synthesis and biological evaluation of some pyrazolylpyrazolines as anti-inflammatory-antimicrobial agents. Eur J Med Chem 2010;45:2650–5.
32. Sahu SK, Banerjee M, Samantray A, et al. Synthesis, analgesic, anti-inflammatory and antimicrobial activities of some novel pyrazoline derivatives. Trop J Pharm Res 2008;7:961–8.
33. Penning TD, Talley JJ, Bertenshaw SR, et al. Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benze nesulfonamide (SC-58635, celecoxib). J Med Chem 1997;40:1347–65.
34. Supuran CT. Carbonic anhydrase inhibitors: an editorial. Expert Opin Ther Pat 2013;23:677–9.
35. Hisar O, Beydemir S, Gulcin I, et al. Effects of low molecular weight plasma inhibitors of rainbow trout (Oncorhynchus mykiss) on human erythrocyte carbonic anhydrase-II isozyme activity in vitro and rat erythrocytes in vivo. J Enzyme Inhib Med Chem 2005; 20:35–9.
36. Innocenti A, Gulcin I, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors. Antioxidant polyphenols effectively inhibit mammalian isoforms I-XV. Bioorg Med Chem Lett 2010;20:5050–3.
37. Boztas M, Cetinkaya Y, Topal M, et al. Synthesis and carbonic anhydrase isoenzymes I, II, IX, and XII inhibitory effects of dimethoxybromophenol derivatives incorporating cyclopropane moieties. J Med Chem 2015;58:640–50.
38. Scozzafava A, Supuran CT, Carta F. Antiobesity carbonic anhydrase inhibitors: a literature and patent review. Expert Opin Ther Pat 2013; 23:725–35.
39. Carta F, Supuran CT. Diuretics with carbonic anhydrase inhibitory action: a patent and literature review. Expert Opin Ther Pat 2013;23: 681–91.
40. Sabitha G, Reddy GSKK, Reddy KB, Yadav JS. Iodotrimethylsilane-mediated cross-aldol condensation: a facile synthesis of alpha, alpha ’-bis(substituted benzylidene)cycloalkanones. Synthesis 2004; 2:263–6..
41. Dimmock JR, Kandepu NM, Hetherington M, et al. Cytotoxic activities of Mannich bases of chalcones and related compounds. J Med Chem 1998;41:1014–26.
42. Tamura N, Hirano K, Kishino K, et al. Analysis of type of cell death induced by topoisomerase inhibitor SN-38 in human oral squamous cell carcinoma cell lines. Anticancer Res 2012;32: 4823–32.
43. Sakagami H, Shimada C, Kanda Y, et al. Effects of 3-styrylchromones on metabolic profiles and cell death in oral squamous cell carcinoma cells. Toxocol Rep 2015;2:1281–90.
44. Topal M, Gulcin I. Rosmarinic acid: a potent carbonic anhydrase isoenzymes inhibitor. Turk J Chem 2014;38:894–902.
45. Cetinkaya Y, Gocer H, Gulcin I, Menzek A. Synthesis and carbonic anhydrase isoenzymes inhibitory effects of brominated diphenylmethanone and its derivatives. Arch Pharm (Weinheim) 2014;347: 354–9.
46. Akincioglu A, Topal M, Gulcin I, Goksu S. Novel sulphamides and sulphonamides incorporating the tetralin scaffold as carbonic anhydrase and acetylcholine esterase inhibitors. Arch Pharm (Weinheim) 2014;347:68–76.
47. Verpoorte JA, Mehta S, Edsall JT. Esterase activities of human carbonic anhydrases B and C. J Biol Chem 1967;242: 4221–9.
48. Cetinkaya Y, Gocer H, Goksu S, Gulcin I. Synthesis and carbonic anhydrase isoenzymes I and II inhibitory effects of novel benzylamine derivatives. J Enzyme Inhib Med Chem 2014;29: 168–74.
49. Akbaba Y, Akincioglu A, Gocer H, et al. Carbonic anhydrase inhibitory properties of novel sulfonamide derivatives of aminoindanes and aminotetralins. J Enzyme Inhib Med Chem 2014;29: 35–42.
50. Gulcin I, Kufrevioglu OI, Oktay M. Purification and characterization of polyphenol oxidase from nettle (*Urtica dioica* L.) and inhibitory effects of some chemicals on enzyme activity. J Enzyme Inhib Med Chem 2005;20:297–302.
51. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–5.
52. Aksu K, Nar M, Tanc M, et al. Synthesis and carbonic anhydrase inhibitory properties of sulfamides structurally related to dopamine. Bioorg Med Chem 2013;21:2925–31.
53. Akincioglu A, Akbaba Y, Gocer H, et al. Novel sulfamides as potential carbonic anhydrase isoenzymes inhibitors. Bioorg Med Chem 2013;21:1379–85.
54. Gulcin I, Beydemir S. Phenolic compounds as antioxidants: carbonic anhydrase isoenzymes inhibitors. Mini Rev Med Chem 2013;13: 408–30.
55. Atasever A, Ozdemir H, Gulcin I, Irfan Kufrevioglu O. One-step purification of lactoperoxidase from bovine milk by affinity chromatography. Food Chem 2013;136:864–70.
56. Nar M, Cetinkaya Y, Gulcin I, Menzek A. (3,4-Dihydroxyphenyl)(2,3,4-trihydroxyphenyl)methanone and its derivatives as carbonic anhydrase isoenzymes inhibitors. J Enzyme Inhib Med Chem 2013;28:402–6.
57. Ozturk Sarikaya SB, Topal F, Senturk M, et al. In vitro inhibition of α-carbonic anhydrase isozymes by some phenolic compounds. Bioorg Med Chem Lett 2011;21:4259–62.