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Microwave-assisted synthesis and bioevaluation of new sulfonamides

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ABSTRACT

In this study, 4-[5-(4-hydroxyphenyl)-3-aryl-4,5-dihydro-1*H*-pyrazol-1-yl]benzenesulfonamide derivatives (**8-14**) were synthesized for the first time by microwave irradiation and their chemical structures were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS. Cytotoxic activities and inhibitory effects on carbonic anhydrase I and II isoenzymes of the compounds were investigated. The compounds **9** (PSE = 4.2), **12** (PSE = 4.1) and **13** (PSE = 3.9) with the highest potency selectivity expression (PSE) values in cytotoxicity experiments and the compounds **13** (K_i = 3.73 ± 0.91 nM toward hCA I) and **14** (K_i = 3.85 ± 0.57 nM toward hCA II) with the lowest *Ki* values in CA inhibition studies can be considered as leader compounds for further studies.

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Carbonic anhydrase; cytotoxicity; microwave; pyrazoline; sulfonamide

Introduction

Cancer is a disease characterized by uncontrolled cell division, metastasis and known as a second cause of death in the world after cardiovascular diseases. It is estimated that number of people die from cancer will be 22 million people in the year of 2030 according to WHO's report[1]. Although several chemotherapeutics are available in the market, there is no drug free from the side effects or having the superior selectivity to the cancer cells comparing to normal ones in clinics[2–4].

The compounds having the pyrazole or pyrazoline core and/or sulfonamide moiety in its chemical structure have been reported with several bioactivities such as anticancer, antiinflammatory, monoamine oxidase inhibitory, antifungal, cyclooxygenase-2 inhibitory, carbonic anhydrase inhibitory and analgesic activities[5–15]. Our research group also reported encouraging cytotoxic activities of several pyrazoline–benzenesulfonamide bearing compounds recently[16–18].

The carbonic anhydrases (CAs, EC 4.2.1.1) are superfamily of metalloenzymes present in Archaea, prokaryotes and eukaryotes, and in all life kingdoms. These enzymes differ in their localization, catalytic activity and susceptibility to different classes of inhibitors. Up to now, six genetically distinct CA families are known, α-, β-, ɤ-, δ-, ζ-, and η-CAs. The mammalian enzymes belonging to α -CA family consist of 16 active members[4,19–21]. Some of them are cytosolic as CA I-III, CA VII and CA XIII, others are membrane bound as CA IV, CA IX, CA XII and CA XIV, two are mitochondrial such as CA VA/VB, and one is secreted in saliva as CA VI[4,19–21]. CA has a crucial role in the cell physiology and in the pathology of several diseases. Inhibition of hCA II isoenzyme is important at decreasing the ocular pressure in glaucoma while inhibition of hCA IX and XII isoenzymes are important target for stopping the development of cancer[4,19–21]. CA inhibitors (CAIs) have generally sulfonamide moiety in its chemical structure, however, some phenolic compounds and coumarin derivatives were also reported with inhibitory profiles on CAs in the literatures[22–28].

In the present study, it was aimed to synthesize the compounds having the chemical structure of 4-[5–(4-hydroxyphenyl)-3-aryl-4,5-dihydro-1*H*-pyrazol-1-yl]benzenesulfonamide since the chemical structure designed include pyrazoline, sulfonamide and phenolic pharmacophores in a single molecule to investigate their cytotoxic activities and inhibition profiles of the compounds on hCA I and II isoenzymes.

Experimental

Materials and methods

Reactions were carried out in a CEM Discover Microwave Synthesis System, 908010 (Matthews, NC). Chemical structures of the compounds were determined by ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectroscopies using a Varian Mercury Plus spectrometer (Varian inc., Palo Alto, CA). Chemical shifts (δ) were reported in ppm and coupling constants (*J*) were expressed in hertz (Hz). HRMS-ESI Mass spectra were recorded on HPLC-TOF Waters Micromass LCT Premier XE (Waters Corporation, Milford, MA. Melting points were determined using an Electrothermal 9100/IA9100 instrument (Bibby Scientific Limited, UK) and are uncorrected. The reactions were monitored using silicagel HF254–366 TLC (thin-layer chromatography) plates (E. Merck, Germany).

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Supplemental data for this article can be accessed here.

Synthesis of the chalcone derivatives (1–7, Scheme 1)

The compounds designed were synthesized by Claisen–Schmidt condensation under basic condition[16–18,28–31]. An aqueous solution of NaOH (10% w/v, 10 mL) was added into the mixture of an appropriate acetophenone (16 mmol) and 4-hydroxybenzaldehyde (16 mmol) in ethanol (5 mL). The reaction flask was kept at 0–5 °C. The reaction mixture was stirred at room temperature for 24–48 h. Reactions were monitored by TLC. When the reaction was stopped, the reaction content was poured into ice–water mixture (50 mL) and neutralized with aqueous HCl solution (10% w/v). The precipitated compounds were filtered, washed with water and dried. The solid compounds obtained were crystallized from ethanol–water. The purities of the compounds were checked by TLC and used for the synthesis of pyrazoline derivatives without further purification.

Synthesis of the 1,3,5-trisubstituted pyrazoline derivatives (8–14, Scheme 1)

A solution of *para*-hydrazinobenzenesulfonamide hydrochloride (2 mmol) in ethanol (20 mL) was irradiated for 5 min at 200 °C, 300W, 13 bar. A suitable chalcone derivative (2 mmol) was dissolved in ethanol (5 mL) and added into the reaction tube. The mixture was irradiated for 7 min at 200 °C, 300W, 7 bar. The progress of the reactions were monitored by TLC ($CHCl_3$:MeOH;4.8:0.2). When the reactions stopped, the content of the flask was concentrated to its half of the volume and cooled at +4 °C for 1 h. After cooling, the compounds **8**, **9**, **10** and **14** were obtained in solid form. The crude compounds of **8**, **9**, **10** and **14** were filtered, dried and crystallized from suitable solvent or solvents [ethanol (**8**, **9**), chloroform-methanol (**10**), dichloromethane-methanol (**14**)] to obtain desired pure compound. The compounds having halogen substituent such as **11**, **12** and **13** were solidified using a hexan-diisopropylether and then purified by crystallization using dichloromethane–methanol as solvent system.

4-[5-(4-Hydroxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl]benzenesulfonamide (8)

Light beige solid. M.p. 210–211 °C. Yield: 10%. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 7.78 (d, 2H, *J* = 7.0 Hz), 7.64 (d, 2H, *J* = 8.8 Hz), 7.43–7.36 (m, 3H), 7.10 (dd, 4H, *J* = 12.4, 8.8 Hz), 6.73 (d, 2H, *J* = 8.4 Hz), 5.41 (dd, 1H, *J* = 12.4, 5.7 Hz), 3.91 (dd, 1H, *J* = 17.6, 12.3 Hz), 3.15 (dd, 1H, *J* = 17.6, 5.7 Hz).^{13}C NMR (100 MHz, CD_3OD, ppm) δ 157.1, 148.7, 147.1, 143.5, 132.5, 132.3, 131.7, 127.6, 127.3, 126.9, 122.9, 115.7, 112.5, 63.4, 43.1. HRMS (ESI-MS) calculated for $C_{21}H_{20}N_3O_3S$ $[M+H]^+$ 394.1225, found: 394.1234

4-[5-(4-Hydroxyphenyl)-3-(4-methylphenyl)-4,5-dihydro-1H-pyrazol-1-yl]benzene sulfonamide (9)

White solid. M.p. 250–251 °C. Yield: 16%. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 7.64 (*t*, 4H, *J* = 8.8 Hz), 7.21 (d, 2H, *J* = 8.2 Hz), 7.08 (*t*, 4H, *J* = 8.2 Hz), 6.72 (d, 2H, *J* = 8.4 Hz), 5.36 (dd, 1H, *J* = 12.0, 5.8 Hz), 3.87 (dd, 1H, *J* = 17.4, 12.0 Hz), 3.11 (dd, 1H, *J* = 17.4, 5.8 Hz), 2.36 (s, 3H, CH_3). ^{13}C NMR (100 MHz, CD_3OD, ppm) δ 156.9, 150.0, 147.4, 139.5, 132.8, 129.7, 129.2, 127.5, 127.2, 126.9, 125.9, 115.7, 112.3, 63.1, 43.4, 20.2. HRMS (ESI-MS) Calculated for $C_{22}H_{22}N_3O_3S$ $[M+H]^+$ 408.1382, found: 408.1370

4-[5-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl] benzenesulfonamide (10)

White solid. M.p. 236–238 °C. Yield: 11%. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 7.70 (d, 2H, *J* = 9.1 Hz), 7.62 (d, 2H, *J* = 8.8 Hz), 7.07 (d, 2H, *J* = 8.8 Hz), 7.06 (d, 2H, *J* = 8.4 Hz), 6.95 (d, 2H, *J* = 9.1 Hz), 6.72 (d, 2H, *J* = 8.4 Hz), 5.32 (dd, 1H, *J* = 12.0, 5.8 Hz), 3.84 (dd, 1H, *J* = 17.4, 12.0 Hz), 3.82 (s, 3H, OCH_3), 3.09 (dd, 1H, *J* = 17.4, 5.8 Hz). ^{13}C NMR (100 MHz, CD_3OD, ppm) δ 161.0, 156.9, 149.9, 147.5, 132.9, 131.5, 127.5, 127.2, 126.9, 125.1, 115.7, 113.9, 112.2, 63.0, 54.6, 43.5. HRMS (ESI-MS) Calculated for $C_{22}H_{22}N_3O_4S$ $[M+H]^+$ 424.1331, found: 424.1342

4-[5-(4-Hydroxyphenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl] benzenesulfonamide (11)

Light beige solid. M.p. 138–140 °C. Yield: 12%. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 7.79 (dd, 2H, *J* = 9.0, 5.3 Hz), 7.64 (d, 2H, *J* = 9.0 Hz), 7.16–7.05 (m, 6H), 6.72 (d, 2H, *J* = 8.4 Hz), 5.38 (dd, 1H, *J* = 12.1, 5.9 Hz), 3.88 (dd, 1H, *J* = 17.6, 12.1 Hz), 3.12 (dd, 1H, *J* = 17.6, 5.9 Hz). ^{13}C NMR (100 MHz, CD_3OD, ppm) δ 162.3, 157.0, 148.9, 132.7, 132.0, 128.1, 127.9, 127.2, 126.9, 115.7, 115.5, 115.3, 112.4, 63.3, 43.4. HRMS (ESI-MS) Calculated for $C_{21}H_{19}N_3O_3SF$ $[M+H]^+$ 412.1131, found: 412.1140

4-[5-(4-Hydroxyphenyl)-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl] benzenesulfonamide (12)

Light beige solid. M.p. 153–155 °C. Yield: 6%. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 7.75 (d, 2H, *J* = 8.4 Hz), 7.64 (d, 2H, *J* = 9.1 Hz), 7.41 (d, 2H, *J* = 8.4 Hz), 7.11 (d, 2H, *J* = 9.1 Hz), 7.07 (d, 2H, *J* = 8.8 Hz), 6.73 (d, 2H, *J* = 8.4 Hz), 5.40 (dd, 1H, *J* = 12.1, 5.9 Hz), 3.88 (dd, 1H, *J* = 17.6, 12.1 Hz), 3.12 (dd, 1H, *J* = 17.6, 5.9 Hz). ^{13}C NMR (100 MHz, CD_3OD, ppm) δ 157.0, 149.9, 147.3, 132.7, 132.5, 132.0, 129.1, 128.5, 127.2, 126.9, 125.9, 115.7, 112.4, 63.2, 43.3. HRMS (ESI-MS) Calculated for $C_{21}H_{19}N_3O_3SCl$ $[M+H]^+$ 428.0836, found: 428.0848

4-[5-(4-Hydroxyphenyl)-3-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl] benzenesulfonamide (13)

Light beige solid. M.p. 194–196 °C. Yield: 4%. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 7.68 (d, 2H, *J* = 8.8 Hz), 7.64 (d, 2H, *J* = 8.8 Hz), 7.55 (d, 2H, *J* = 8.4 Hz), 7.11 (d, 2H, *J* = 9.1 Hz), 7.06 (d, 2H, *J* = 8.4 Hz), 6.72 (d, 2H, *J* = 8.8 Hz), 5.40 (dd, 1H, *J* = 12.2, 5.7 Hz), 3.87 (dd, 1H, *J* = 17.5, 12.2 Hz), 3.11 (dd, 1H, *J* = 17.5, 5.7 Hz). ^{13}C NMR (100 MHz, CD_3OD, ppm) δ 157.1, 148.7, 147.1, 132.5, 132.3, 131.7, 127.6, 127.3, 126.9, 122.9, 121.1, 115.7, 112.5, 63.4, 43.1. HRMS (ESI-MS) Calculated for $C_{21}H_{19}N_3O_3SBr$ $[M+H]^+$ 472.0330, found: 472.0337

4-[5–(4-Hydroxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl] benzenesulfonamide, 14

Light yellow color solid. M.p. 152–154 °C. Yield: 8%. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 7.63 (d, 2H, *J* = 8.8 Hz), 7.45 (d, 1H, *J* = 5.1 Hz), 7.22 (d, 1H, *J* = 3.7 Hz), 7.09–7.05 (m, 5H), 6.73 (d, 2H, *J* = 8.4 Hz), 5.40 (dd, 1H, *J* = 12.0, 5.5 Hz), 3.90 (dd, 1H, *J* = 17.3, 12.0 Hz), 3.14 (dd, 1H, *J* = 17.3, 5.5 Hz). ^{13}C NMR (100 MHz, CD_3OD, ppm) δ 157.1, 147.1, 145.9, 135.9, 132.5, 132.1, 127.5, 127.4, 127.3, 127.2, 126.9, 115.7, 112.4, 63.3, 43.9. HRMS (ESI-MS) Calculated for $C_{19}H_{18}N_3O_3S_2$ $[M+H]^+$ 400.0790, found: 400.0799

Biological activity

Cytotoxicity assay

The cytotoxicity of the compounds were assayed toward human oral squamous cell carcinoma cell lines derived from gingiva tissue (CA9–22) and tongue (HSC-2, HSC-3, HSC-4), and human normal

oral cells (gingival fibroblasts, HGF; periodontal ligament fibroblasts, HPLF; pulp cells, HPC) with some minor modifications[16,17,26,30–33]. In brief, cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS). Cells (2.5×10^3 cells/well) were inoculated and incubated for 48 h to achieve complete adherence. Near confluent cells were incubated for a further 48 h in the fresh culture medium containing each test compound (3.12, 6.25, 12.5, 25, 50, 100, 200, 400 μM) or 5-FU (positive control) (7.8, 15.6, 31.2, 62.5, 125, 250, 500, 1000 μM). The viable cell numbers were determined by the MTT method. Cytotoxicity induced by DMSO (0.0078, 0.0156, 0.03125, 0.0625, 0.125, 0.25, 0.5 or 1%) was subtracted from each well. The CC_{50} values were determined from the dose-response curves. The tumor selectivity (TS) was calculated by the following equation: TS = mean CC_{50} against normal cells/mean CC_{50} against cancer cells [shown as (D/B) or (C/A) in Table 1]. A potency selectivity expression (PSE) was calculated by multiplying the reciprocal of average CC_{50} values toward cancer cell lines and the average SI values toward these cell lines and expressed as a percentage [$PSE = (D/B^2) \times 100$].

Carbonic anhydrase inhibition assay

The purification of cytosolic CA isoenzymes (CA I and II) were previously described with a simple one-step method by a Sepharose-4B-L tyrosine-sulfanilamide affinity chromatography[34]. The protein quantity in the column effluents was determined spectrophotometrically at 280 nm. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was applied with a Bio-Rad Mini Gel system (Mini-PROTEAN Tetra System, China) after purification of both CA isoenzymes[35]. Briefly, it was performed in acrylamide for the running (10%) and the stacking gel (3%) contained SDS (0.1%), respectively. Activities of CA I and II isoenzymes were determined according to the method of Verpoorte et al[36]. The increase in absorbance of reaction medium was spectrophotometrically recorded at 348 nm (Shimadzu, UV-VIS Spectrophotometer, UVmini-1240, Kyoto-Japan). Also, the quantity of protein was determined at 595 nm according to Bradford method[37]. Bovine serum albumin was used as standard protein. The IC_{50} values were obtained from activity (%) versus compounds plots. For calculation of K_i values, three different concentrations were used. The Lineweaver–Burk curves were drawn and calculations were realized[38].

Results and discussion

In this study, the compounds having the chemical structure of [4-[5–(4-hydroxyphenyl)-3-aryl-4,5-dihydro-1*H*-pyrazol-1-yl]benzenesulfonamide, the compounds **8–14**] were designed and successfully synthesized by microwave irradiation with some minor modifications[17,39,40] by starting from the suitable chalcones synthesized according to the literature[16–18,28–31]. Reactions were monitored by TLC. After the confirmation of the chemical structures of the chalcones **1–7** by ^{1}H NMR spectra (See Supplementary File), they were used in synthesis of target compounds **8–14**. The chemical structures of the compounds **8–14** were elucidated by ^{1}H NMR, ^{13}C NMR and HRMS spectra (See Supplementary File). The compounds **8–14** were reported for the first time by this study. The cytotoxicity activities of **8–14** and inhibitory profiles of **8–14** on hCA I and II isoenzymes were reported in Tables 1 and 2, respectively.

Human oral squamous cell carcinoma cell lines derived from gingiva tissue (CA9–22) and tongue (HSC-2, HSC-3, HSC-4), and human normal oral cells (gingival fibroblasts, HGF; periodontal

Table 1. Cytotoxicities of sulfonamides **8–14** toward human oral squamous cell carcinoma cells and human normal oral cells.

	CC_{50} (μM)																									
	Human oral squamous cell carcinoma cells												Human normal oral cells							TS					
Comp.	(A) Ca9-22	SD	SI	HSC-2	SD	SI	HSC-3	SD	SI	HSC-4	SD	SI	(B) Mean CC_{50}	SD	(C) HGF	SD	HPLF	SD	HPC	SD	(D) Mean	SD	(D/B)	(C/A)	PSE $(D/B^2) \times 100$	Log P
8	42.0	5.0	1.8	67.0	12.2	1.1	66.3	11.9	1.1	50.3	7.1	1.5	56.4	12.3	85.0	13.5	54.3	5.0	88.3	6.8	75.9	18.7	1.3	2.0	2.3	1.17
9	25.7	4.2	1.7	33.3	2.1	1.3	39.7	0.6	1.1	31.7	2.1	1.4	32.6	5.8	43.7	4.0	39.3	4.2	49.7	3.8	44.2	5.2	1.4	1.7	4.2	1.63
10	37.7	1.5	1.3	65.3	5.7	0.8	47.0	8.7	1.1	40.7	2.1	1.2	47.7	12.4	47.3	1.5	44.0	2.0	61.0	12.5	50.8	9.0	1.1	1.3	2.2	1.08
11	36.3	2.5	1.6	46.3	10.1	1.3	43.3	5.9	1.4	39.3	4.7	1.5	41.3	4.4	66.7	3.5	43.0	4.6	67.7	0.6	59.1	14.0	1.4	1.8	3.5	1.22
12	22.3	1.5	2.0	39.0	2.6	1.1	38.3	2.5	1.2	31.3	4.5	1.4	32.8	7.8	44.0	3.6	38.3	3.8	50.7	7.4	44.3	6.2	1.4	2.0	4.1	1.76
13	20.7	0.6	1.7	37.7	11.6	0.9	34.7	2.9	1.0	25.7	6.1	1.4	29.7	7.9	34.7	4.2	29.7	0.6	39.7	8.1	34.7	5.0	1.2	1.7	3.9	1.94
14	45.7	3.1	1.8	55.0	18.5	1.5	61.3	3.1	1.4	55.3	4.9	1.5	54.3	6.5	88.3	6.8	73.0	30.3	87.3	6.7	82.9	8.6	1.5	1.9	2.8	0.85
5-FU	100.0	4.4	>167.3	45.7	19.6	>16.1	27.3	10.3	>71.5	17.3	12.2	>60.3	47.6	36.9	>1000	0.0	208.3	72.2	>1000	0.0	>736	457.1	>15.5	>10.0	>32.6	

CC_{50} values refer to the concentrations of the compounds in micromoles which reduce the viable cell number by 50%. Oral squamous cell carcinoma (OSCC) cell lines used are Ca9–22 (derived from gingiva), HSC-2, HSC-3 and HSC-4 (derived from tongue). Normal oral cells used are human gingival fibroblasts (HGF), human periodontal ligament fibroblasts (HPLF) and human pulp cells (HPC). Tumor-specificity (TS) value is calculated by dividing the mean CC_{50} value of each compound against normal cells by the mean CC_{50} value against OSCC (D/B or C/A). Selectivity index (SI) figures were generated, which are quotients of the average CC_{50} values of nonmalignant cells and CC_{50} figure of a compound toward a specific cell line. A potency selectivity expression (PSE) was calculated by multiplying the reciprocal of average CC_{50} values toward cancer cell lines and the average SI values toward these cell lines and expressed as a percentage [$PSE = (D/B^2) \times 100$]. CC_{50} value was determined from the growth curves plotted at different concentrations of each compounds in triplicate wells. 5-Fluorouracil (5-FU) were used as a reference drug. Log P values were calculated using ACD/ChemSketch Version 2015 Software. μM : micromolar.

ligament fibroblasts, HPLF; pulp cells, HPC) were used to estimate the cytotoxicities of the compounds **8–14** while 5-Fluorouracil (5-FU) was used as a reference drug.

First question to be adressed is whether the compounds have cytotoxic/anticancer properties. The cytotoxicities of the compounds toward tumor cell lines had changed in the range of 22.3–67.0 μM (Table 1). This suggests that the compounds had anticancer property. The compounds having more potent cytotoxicity than reference compound 5-Fluorouracil (5-FU), which is a drug in clinical use and times of potency (in parenthesis) were as follows: All compounds toward Ca9–22 cell line **8** (2.4), **9** (3.9), **10** (2.7), **11** (2.8), **12** (4.5), **13** (4.8), **14** (2.2) and the compounds **9** (1.4), **11** (1.0), **12** (1.2), **13** (1.2) toward HSC-2 cell line.

Tumor cells in body are surrounded by normal cells. Thus, candidate compounds aimed at future clinical application should show higher cytotoxicity against tumor cells rather than normal cells. Selectivity index (SI) figures that reflect this property was thus introduced. SI values can be calculated by dividing the average CC_{50} values toward nonmalignant cells to CC_{50} figure of a compound toward a specific cell line (Table 1). SI value which is over 1 reflects the selectivity of the compound toward tumor cell rather than normal cell[16,17,26,30–32,41]. On the basis of this information, all compounds showed SI values of 1.3–2.0 toward Ca9–22 cell line; **9**, **11** and **14** showed SI values of 1.3–1.5 toward HSC-2 cell line; **11, 12** and **14** showed SI values of 1.2–1.4 toward HSC-3 cell line; and all compounds showed SI values of 1.2–1.5 toward HSC-4 cell line.

The tumor selectivity (TS) of each compound was calculated by two methods. First calculation was made by dividing the average CC_{50} value toward three normal cells to the average CC_{50} value toward a total of four cancer cell lines (TS = Column D/Column B, Table 1)[16,17,26,30,31]. First calculation pointed out that the compound **14,** which has thienyl ring showed the highest TS value (1.5). This indicated that replacement of benzene (TS = 1.3) by its bioisoster thiophene ring (TS = 1.5) increased the TS value slightly.

The second calculation considers the difference of sensitivity between the malignant (Ca9–22) and nonmalignant (HGF) cells derived from the same tissue (gingiva). TS value was determined by dividing the CC_{50} value toward HGF cells to the CC_{50} value toward Ca9–22 cells (TS = Column C/Column A, Table 1)[16,17,26,30,31]. This type of calculation pointed out that the compounds **8**, which has non-substituted phenyl ring, and **12**, which has chlorine substituent on phenyl ring, showed the highest TS value (2.0) among the compounds tested.

Lead compounds should possess both marked cytotoxic potencies and selective toxicity for tumor cells. In order to identify such molecules, a potency selectivity expression (PSE) value of test compounds was calculated by multiplying the reciprocal of the average CC_{50} value (a measure of potency) and the average SI figure (a determination of tumor selectivity) [Column D/(Column B)^2 × 100, Table 1][16,17,26,30–32]. Among seven compounds tested, substituted compounds **9** (with methyl, PSE = 4.2), **12** (with chlorine, PSE = 4.1) and **13** (with bromine, PSE = 3.9) showed relatively higher PSE values than the others, although their PSE values were much lower than 5-FU (PSE = >32.6). Other five compounds, except methoxy-substituted compound **10**, showed slightly higher PSE values than non-substituted compound **8.** When the PSE values of the compounds were considered and compared with non-substituted compound **8,** it can be noticed that introduction of a substituent, which allows hydrogen bond formation, affected the PSE value of a compound in different ways. However, replacement of benzene in **8** by its bioisoster thiophene in **14** resulted in the increases in PSE value of the compound **14**. The differences in cytotoxicities and tumor selectivities (SI, TS, PSE) of the compounds may result from the different chemical structure of the compounds, different nature of cell lines used, and different mechanism of action of the compounds tested.

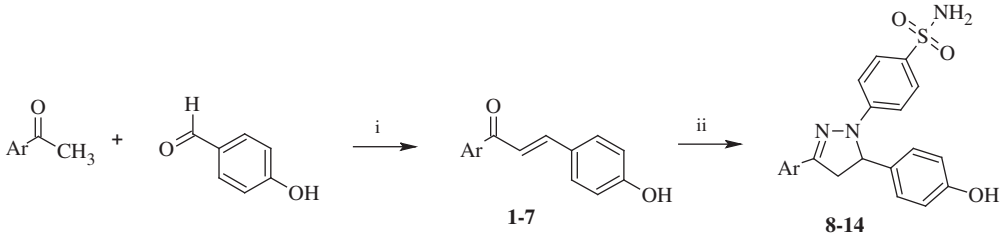
The relationship between PSE and log P values were next investigated. For correlation analysis, bivariate correlation test was applied using PASW Statistics 18 (Release 18.0.0) software. There was a positive correlation between Log *p* and PSE values. (Pearson's correlation, *R*: 0.869 (R^2: 0.755), $p = 0.011$).

When CA inhibitory profiles of the compounds were investigated, the compounds were effective at 4.22–5.68 nM toward hCA I while they were effective at 4.25–5.72 nM toward hCA II isoenzyme in terms of IC_{50} values (Table 2). Bromine-bearing compound **13** and chlorine-bearing compound **12** were the most effective inhibitors on hCA I and hCA II isoenzymes, respectively, while fluorine-bearing compound **11** was the less effective one toward both CA isoenzymes in terms of IC_{50} values. Reference compound Acetazolamid (AZA) had IC_{50} values as 190.12 nM and 199.2 nM toward hCA

Table 2. Inhibitory effects of sulfonamides **8–14** on hCA I and II isoenzymes.

	IC_{50} (nM)				*Ki* (nM)	
Compounds	hCA I	r^2	hCA II	r^2	hCA I	hCA II
8	4.81	0.9757	4.95	0.9553	4.84 ± 0.78	4.59 ± 0.83
9	5.29	0.9634	5.63	0.9570	4.59 ± 1.25	7.48 ± 2.52
10	4.84	0.9893	5.37	0.9569	3.99 ± 0.90	5.07 ± 1.69
11	5.68	0.9738	5.72	0.9855	5.10 ± 1.17	4.53 ± 0.75
12	4.91	0.9657	4.25	0.9705	4.49 ± 0.67	4.06 ± 0.60
13	4.22	0.9725	4.65	0.9834	3.73 ± 0.91	4.56 ± 1.08
14	5.13	0.9736	5.45	0.9824	4.17 ± 0.93	3.85 ± 0.57
AZA*	190.12	0.9957	199.62	0.9913	182.93 ± 1.18	194.47 ± 0.34

*Acetazolamide (AZA) was used as a standard inhibitor for both hCA I and II.



Reagents and conditions. i: NaOH (10%), C_2H_5OH, rt, 24-48h, ii: 4-Hydrazinobenzenesulfonamide hydrochloride, C_2H_5OH, 200°C, 300 Watt, 7-13 barr, 12 min. Ar: Phenyl (**1,8**), 4-methylphenyl (**2,9**), 4-methoxyphenyl (**3,10**), 4-fluorophenyl (**4,11**), 4-chlorophenyl (**5,12**), 4-bromophenyl (**6,13**), 2-thienyl (**7, 14**).

Scheme 1. Synthesis of sulfonamides **8–14** via microwave irradiation.

isoenzymes. The compounds **8** (39.5), **9** (35.9), **10** (39.3), **11** (33.5), **12** (38.7), **13** (45.1), **14** (37.1) toward hCA I and **8** (40.3), **9** (35.5), **10** (37.1), **11** (34.9), **12** (46.9), **13** (42.9), **14** (36.6) toward hCA II were more potent than AZA in terms of IC_{50} values.

When the inhibition constants (*Ki*) were considered, *Ki* values of the compounds were in the range of 3.73 ± 0.91 to 5.10 ± 1.17 nM toward hCA I and in the range of 3.85 ± 0.57 to 7.48 ± 2.52 nM toward hCA II while *Ki* values of AZA were 182.93 ± 1.18 nM (toward hCA I) and 194.47 ± 0.34 nM (towards hCA II). The compound **13** with bromine toward hCA I and the compound **14** with thiophen ring toward hCA II had the lowest *Ki* values. This suggest that the compounds **13** and **14** were the leader compounds of series for further studies in the field of CA inhibition.

Conclusions

Seven new pyrazoline-bearing sulfonamides having the chemical structure of 4-[5–(4-hydroxyphenyl)-3-aryl-4,5-dihydro-1*H*-pyrazol-1-yl]benzenesulfonamide] were synthesized and their chemical structures were confirmed by detailed spectral analyses. The compounds **8–14** were reported for the first time with their cytotoxic and CA inhibitory activities. The compounds **9** (PSE = 4.2), **12** (PSE = 4.1) and **13** (PSE = 3.9) with the highest PSE values in cytotoxicity experiments and the compound **13** (*Ki* = 3.73 ± 0.91 nM) toward hCA I and the compound **14** (*Ki* = 3.85 ± 0.57 nM) toward hCA II with the lowest *Ki* values in CA inhibition studies attract attention and they can be considered as leader compounds for further studies.

Disclosure statement

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