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RESEARCH ARTICLE

Synthesis and bioactivity studies of 1-aryl-3-(2-hydroxyethylthio)-1-propanones

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Abstract

A series of Mannich bases having piperidine moiety were reacted with 2-mercaptoethanol, leading to 1-aryl-3-piperidine-4-yl-1-propanone hydrochlorides. The cytotoxicity and carbonic anhydrase inhibitory activities of these new compounds were evaluated. Among the compounds, only one derivative, nitro substituent bearing EU9, showed an effective cytotoxicity, although weak tumor specificity against human oral malignant versus nonmalignant cells. The compound induced apoptosis in HSC-2 oral squamous cell carcinoma cells, but not in human gingival fibroblast. Chemical modifications of this lead are thus necessary to further investigate it as a drug candidate and to obtain compounds with a better activity profile.

Keywords

Carbonic anhydrase inhibition, cytotoxicity, PARP, thiol addition

History

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Introduction

Cancer is the second cause of death after cardiovascular disorders. Although a great amount of improvements have been made in cancer chemotherapy, there is still need for new selective cytotoxic anticancer agents because of the problems available against the drugs that are on market such as gained resistance, low selectivity and stability. Mannich bases are an important group of compounds in medicinal chemistry and they may be synthesized by applying the reaction discovered by Mannich[1–5]. These compounds have a wide range of biological activities such as carbonic anhydrase (CA, EC 4.2.1.1) inhibitory[1–3], cytotoxic[2–7], anti-inflammatory[8] and anticonvulsant activities[9,10]. The reported mechanism action of the Mannich bases are based on thiol alkylation[11–14], interaction with enzymes that are important for antioxidant mechanisms[7], inhibition of mitochondrial respiration[15,16], inhibition of topoisomerase enzyme[17] as well as tubulin polimerization inhibition[18].

The CAs are that enzymes play important roles in physiological and pathological processes[19]. Sixteen CA isoforms have been identified in mammals. Many CA isoforms take part in several vital biological processes such as electrolyte secretion, acid–base balance, ion transport and lipogenesis, ureagenesis and bone resorption. Inhibitors or activators of these enzymes have medical applications such as diuretics, in the treatment of glaucoma, neurological disorders including epilepsy and antitumor drugs targeting hypoxic tumors that overexpress some CA isoforms (e.g. CA IX and XII)[19–25].

The aim of this study was to synthesize 1-aryl-3-(2-hydroxyethylthio)-1-propanones starting from mono Mannich bases (1-aryl-3-piperidine-4-yl-1-propanone hydrochlorides) and to investigate their cytotoxic and CA inhibitory activities against human (h) isoforms hCA I and II.

Materials and methods

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were taken using a Varian spectrometer (Danbury, CT). Chemical shifts (δ) were reported in ppm. Melting points were determined using an Electrothermal 9100 (IA9100, Bibby Scientific Limited, Staffordshire, UK) instrument and are uncorrected. Mass spectra was taken using a MS (ESI–MS) VG Waters Micromass ZQ (Waters Corporation, Milford, MA). All reactions were carried out in CEM Discover Microwave Synthesis Systems (CEM, Matthews, NC).

Chemistry

*Synthesis of 1-aryl-3-piperidin-4-yl-1-propanone hydrochlorides (**B1–B9**)*

A mixture of the appropriate ketone, paraformaldehyde and piperidine hydrochloride in acetic acid (10 mL) was heated in microwave oven at 70 Watt and 120 °C for 20–65 min (Scheme 1). Reactions were monitored by thin-layer chromatography (TLC) using $CHCl_3$:CH_3OH (8:2 or 9:1) solvent system. When the reaction finished, reaction solvent was removed under vacuum and the crude solid was crystallized from suitable solvent such as CH_3OH, $CHCl_3$/CH_3OH or CH_3OH/Et_2O to obtain **B1–B9**.

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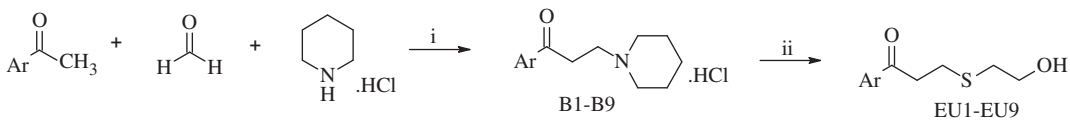

Reagents and conditions. i) Acetic acid glacial, 70W, 120°C ii) 2-Mercaptoethanol, phosphate buffer solution (pH=7.4), 37°C. Ar: C_6H_5 (EU1), 4-$CH_3C_6H_4$ (EU2), 4-$CH_3OC_6H_4$ (EU3), 4-FC_6H_4 (EU4), 4-ClC_6H_4 (EU5), 4-BrC_6H_4 (EU6), C_4H_3S(2-yl) (EU7), C_4H_3O(2-yl) (EU8), 4-$NO_2C_6H_4$ (EU9)

Scheme 1. Synthesis of the compounds **EU1–EU9**.

Table 1. Experimental data of the compounds **EU1–EU9**.

Compound Code	Aryl	**B1–B9** (mmol)	2-Mercaptoethanol (mmol)	Reaction time (minute)	Yield (%)
EU1	C_6H_5	0.39	0.39	2640	82.38
EU2	4-$CH_3C_6H_4$	0.37	0.37	5785	70.49
EU3	4-$H_3OC_6H_4$	0.35	0.35	5785	71.75
EU4	4-FC_6H_4	0.36	0.36	2750	89.39
EU5	4-ClC_6H_4	0.34	0.34	4640	81.78
EU6	4-BrC_6H_4	0.30	0.30	1325	82.39
EU7	C_4H_3S(2-yl)	0.38	0.38	2595	88.81
EU8	C_4H_3O(2-yl)	0.41	0.41	4650	67.11
EU9	4-$O_2NC_6H_4$	0.33	0.33	3150	19.16

EU1–EU9 compounds were synthesized by using **B1–B9** as starting compounds. Reactions were carried out in phosphate buffer solution (5 mL, pH = 7.4, 37 °C). Compounds were purified by column chromatography [Silicagel 60 (70–230 mesh) Ethylacetate:Hexane (8:2)].

Chemical structure of the compounds were confirmed by ^{1}H NMR and melting points reported (data were not shown since they are reported in literatures).

Synthesis of 1-aryl-3-(2-hydroxyethylthio)-1-propanones (EU1-EU9, Scheme 1)

A mixture of 2-mercaptoethanol and a suitable compound of 1-aryl-3-piperidin-4-yl-1-propanone hydrochloride **(B1–B9)** in a phosphate buffer solution (5 mL, pH = 7.4) was shaken at 37 °C. Reactions were monitored by TLC. When the reaction was stopped, reaction content was extracted with $CHCl_3$ (3×10 mL) and then with distilled water (3×10 mL). Organic phase was dried on anhydrous sodium sulfate. Solvent was removed under vacuum. Crude compounds were purified by column chromatography on silica gel 60 (70–230 mesh) using ethylacetate:hexane (8:2) solvent system as a mobile phase to obtain a suitable compound of **EU**.

During the synthesis of **EU2**, **EU3**, **EU5**, **EU8** and **EU9** Mannich bases used (**B2**, **B3**, **B5**, **B8** and **B9**) did not consumed in the reaction medium, although reactions were continued for 77–96 h. The compounds **EU2**, **EU3**, **EU5** and **EU6** were solid, while the compounds **EU1**, **EU7**, **EU8** and **EU9** were viscous liquid. **EU4** was solid at +4 °C, while it was viscous liquid at room temperature. Experimental and spectral details of **EU1–EU9** are presented in Tables 1 and 2, respectively.

Biological activity

Cytotoxicity assay

The compounds were assayed toward human oral squamous cell carcinoma cell lines (HSC-2, HSC-3, HSC-4), human promyelocytic leukemic cell line (HL-60) and human oral normal mesenchymal cells (gingival fibroblast (HGF), pulp cells (HPC) and periodontal ligament fibroblast (HPLF)) based on a literature procedure with some minor modifications[26–28]. In brief, cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) except the HL-60 cells that were cultured in RPMI 1640 medium supplemented with 10% 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. Calculation of tumor-specificity (TS) index: The TS value was calculated by dividing the mean CC_{50} value of each compound against normal cells to mean CC_{50} value against OSCC.

Immunoblot analysis

Primary antibodies against cPARP were purchased from Cell Signaling Technology (Danvers, MA), and the primary antibody against actin was purchased from Sigma-Aldrich (St. Louis, MO). The horseradish peroxidase-conjugated secondary anti-mouse immunoglobulin G (IgG) and anti-rabbit IgG antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The HSC-2 and HGF cells were cultured in six well plates for 24 h and then incubated with the compound for 24 h. The cells were scraped with a rubber policeman and collected in $10 \times$ cell lysis buffer (Cell Signaling Technology, Beverly, MA) supplemented with 1 mM phenylmethanesulfonyl fluoride plus one tablet of protease inhibitor cocktail (Complete, EDTA-free; Roche Diagnostics GmbH, Mannheim, Germany). Aliquots of the lysates (50 μg protein) were subjected to SDS-polyacrylamide gel electrophoresis, followed by immunoblotting with primary antibodies against cPARP and actin (employed as a loading control) and secondary anti-IgG antibodies, as previously described[29].

Table 2. Spectral data of the compounds **EU1–EU9**.

EU1	^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 7.87–7.85 (*m*, 2H), 7.49–7.46 (*m*, 1H), 7.38–7.34 (*m*, 2H), 3.72–3.68 (*m*, 2H), 3.50 (brs, OH, 1H), 3.21–3.16 (*m*, 2H), 2.88–2.82 (*m*, 2H), 2.70–2.66 (*m*, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 198.8, 136.6, 133.6, 128.9, 128.2, 61.2, 39.2, 35.5, 26.2. HRMS (ESI–MS): calcd. for $C_{11}H_{14}O_2S$ $[M+H]^+$ 211.0748; found 211.0793.
EU2	^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 7.79 (d, *J* = 8.4 Hz, 2H), 7.19 (d, *J* = 8.4 Hz, 2H), 3.73 (*m*, 2H), 3.20 (*t*, *J* = 6.9 Hz, 2H), 3.16 (*s*, OH, 1H), 2.88 (*t*, *J* = 6.9 Hz, 2H), 2.71 (*t*, *J* = 6.9 Hz, 2H), 2.34 (*s*, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 198.3, 144.4, 134.2, 129.6, 128.4, 61.1, 39.0, 35.6, 26.3, 21.9. HRMS (ESI–MS): calcd. for $C_{12}H_{16}O_2S$ $[M+H]^+$ 225.0905; found 225.0949.
EU3	^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ =7.77 (d, *J* = 8.8 Hz, 2H), 6.78 (d, *J* = 8.8 Hz, 2H), 3.70 (*s*, 3H), 3.65 (*t*, *J* = 6.7 Hz, 2H), 3.56 (*s*, OH, 1H), 3.08 (*t*, *J* = 6.7 Hz, 2H), 2.78 (*t*, *J* = 6.7 Hz, 2H), 2.63 (*t*, *J* = 6.7 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 197.3, 163.8, 130.5, 129.6, 114.0, 61.2, 55.6, 38.7, 35.4, 26.4. HRMS (ESI–MS): calcd. for $C_{12}H_{16}O_3S$ $[M+H]^+$ 241.0854; found 241.0898.
EU4	^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ =7.91 (dd, *J* = 8.0, 5.7 Hz, 2H), 7.06 (*t*, *J* = 8.0 Hz, 2H), 3.72 (*q*, *J* = 6.5 Hz, 2H), 3.20 (*t*, *J* = 6.5 Hz, 2H), 3.13 (b*s*, OH, 1H), 2.87 (*t*, *J* = 6.5 Hz, 2H), 2.70 (*t*, *J* = 6.5 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 197.1, 166.0 (^1J = 255 Hz), 133.1 (^4J = 3 Hz), 130.9 (^3J = 9 Hz), 116.0 (^2J = 21 Hz), 61.0, 39.0, 35.6, 26.1. HRMS (ESI–MS): calcd. for $C_{11}H_{13}FO_2S$ $[M+H]^+$ 229.0654; found 229.0699.
EU5	^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 7.83 (d, *J* = 8.8 Hz, 2H), 7.37 (d, *J* = 8.8 Hz, 2H), 3.73 (*t*, *J* = 6.5 Hz, 2H), 3.21 (*t*, *J* = 6.5 Hz, 2H), 2.98 (*s*, OH, 1H), 2.88 (*t*, *J* = 6.5 Hz, 2H), 2.72 (*t*, *J* = 6.5 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 197.4, 140.0, 135.0, 129.7, 129.2, 61.0, 39.1, 35.7, 26.1. HRMS (ESI–MS): calcd. for $C_{11}H_{13}ClO_2S$ $[M+H]^+$ 245.0325; found 245.0403.
EU6	^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 7.82 (d, *J* = 8.6 Hz, 2H), 7.62 (d, *J* = 8.6 Hz, 2H), 3.78 (*q*, *J* = 6.4 Hz, 2H), 3.26 (*t*, *J* = 6.4 Hz, 2H), 2.95 (*t*, *J* = 6.4 Hz, 2H), 2.78 (*t*, *J* = 6.4 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 197.4, 132.3, 131.7, 129.8, 126.6, 60.7, 39.1, 36.0, 25.9. HRMS (ESI-MS): calcd. for $C_{11}H_{13}BrO_2S$ $[M+H]^+$ 288.9820; found 288.9898.
EU7	^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 7.68 (dd, *J* = 3.6, 1.1 Hz, 1H), 7.60 (dd, *J* = 4.8, 1.1 Hz, 1H), 7.08 (dd, *J* = 4.8, 3.6 Hz, 1H), 3.71 (*m*, 2H), 3.18–3.15 (*m*, 2H), 2.89–2.85 (*m*, 2H), 2.72–2.68 (*m*, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 191.6, 143.9, 134.4, 132.6, 128.5, 61.1, 39.7, 35.6, 26.3. HRMS (ESI–MS): calcd. for $C_9H_{12}O_2S_2$ $[M+H]^+$ 217.0312; found 217.0356.
EU8	^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 7.57 (br s, 1H), 7.20 (d, *J* = 3.7 Hz, 1H), 6.52 (dd, *J* = 3.7, 1.8 Hz, 1H), 3.74 (*t*, *J* = 6.0 Hz, 2H), 3.13–3.08 (*m*, 2H), 2.90–2.84 (*m*, 2H), 2.74–2.70 (*m*, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 187.7, 152.6, 147.0, 117.8, 112.7, 60.9, 38.8, 35.6, 25.9. HRMS (ESI–MS): calcd. for $C_9H_{12}O_3S$ $[M+H]^+$ 201.0541; found 201.0580.
EU9	^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 8.32 (d, *J* = 9.0 Hz, 2H), 8.11 (d, *J* = 9.0 Hz, 2H), 3.79 (*t*, *J* = 6.3 Hz, 2H), 3.34 (*t*, *J* = 6.3 Hz, 2H), 2.97 (*t*, *J* = 6.3 Hz, 2H), 2.79 (*t*, *J* = 6.3 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 197.0, 141.1, 129.8, 129.3, 124.2, 60.9, 39.8, 35.9, 25.8. HRMS (ESI–MS): calcd. for $C_{11}H_{13}NO_4S$ $[M+H]^+$ 256.0599; found 256.0644.

Table 3. Cytotoxic activities of the compounds **EU1–EU9**.

	CC_{50} (μM)							
	Human OSCC cell lines				Human oral normal cells			
	HSC-2	HSC-3	HSC-4	HL-60	HGF	HPC	HPLF	TS
EU1	>400	>400	>400	>400	>400	>400	>400	><1.0
EU2	>400	>400	>400	340 ± 58	>400	>400	331 ± 32	><1.0
EU3	>400	>400	>400	338 ± 38	>400	>400	>400	><1.0
EU4	>400	>400	>400	>400	>400	>400	>400	><1.0
EU5	337 ± 4	292 ± 8	380 ± 6	167 ± 17	328 ± 5	>400	317 ± 12	>1.2
EU6	336 ± 16	302 ± 40	>400	158 ± 15	322 ± 13	>400	321 ± 21	>1.2
EU7	>400	320 ± 20	>400	>400	>400	>400	>400	><1.1
EU8	377 ± 16	363 ± 30	>400	>400	325 ± 41	>400	>400	><0.97
EU9	49 ± 6	50 ± 8	68 ± 2	15 ± 1	72 ± 2	39 ± 2.0	64 ± 16	1.3
5-FU	4.9 ± 0.91	47 ± 7.2	2.5 ± 0.25	10 ± 1.1	>100	>100	>100	>6.2
Melphalan	6.2 ± 0.32	19 ± 0.58	36 ± 1.7	1.0 ± 0.04	96 ± 6.4	83 ± 4.5	80 ± 0.58	5.5

CC_{50} values refer to the concentrations of the compounds in micromoles which reduce the viable cell number by 50%. Tumor-specific (TS) value is calculated by dividing the mean CC_{50} value of each compound against normal cells to mean CC_{50} value against OSCC. CC_{50} value was determined from the growth curves plotted at different concentrations of each compounds in triplicate wells. Human oral squamous cell carcinoma cell lines (HSC-2, HSC-3, HSC-4), human promyelocytic leukemic cell line (HL-60), human oral normal mesenchymal cells (gingival fibroblast (HGF), pulp cells (HPC), periodontal ligament fibroblast (HPLF). TS: tumour selectivity; mM: micromolar.

Carbonic anhydrase inhibition assay

An Applied Photophysics stopped-flow instrument has been used for assaying the CA-catalyzed CO_2 hydration activity by using the method of Khalifah[30]. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor, at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled and deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by nonlinear least-squares methods using PRISM (www.graphpad.com), and nonlinear least squares methods, values representing the mean of at least three different determinations, as described earlier by us[31]. All enzymes used were recombinant, produced in *E. coli* and the cell pellets were lysed and enzyme was purified through affinity chromatography using pAMBS resin as reported earlier[24,32–34].

Results and discussion

In this study, the designed compounds, 1-aryl-3-(2-hydroxyethylthio)-1-propanones, were successfully synthesized by the

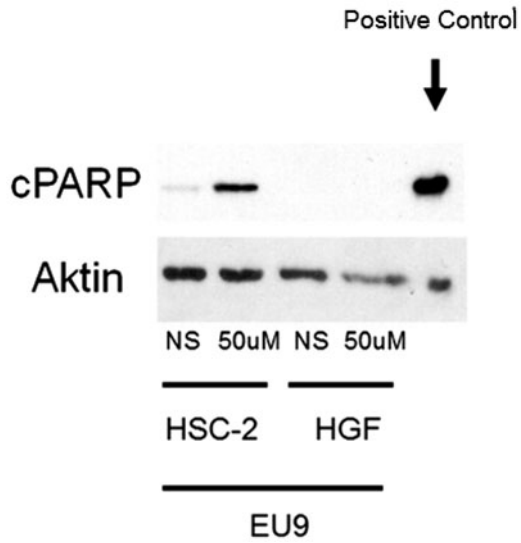

Figure 1. Effect of the compound **EU9** on HSC-2 cancer cells and HGF normal cells after. 24 h. (NS means no stimulation (control)).

Table 4. Inhibition percentages of hCA I and II by the compounds **EU1–EU9**.

	% Inhibition (10^{-5} M)	
Compound	hCA I	hCA II
EU1	27	26
EU2	26	26
EU3	25	27
EU4	24	25
EU5	24	26
EU6	23	25
EU7	26	25
EU8	27	25
EU9	30	25
Acetazolamide	87	91

reaction of a suitable Mannich base, 1-aryl-3-amino-1-propenone hydrochloride, with 2-mercaptoethanol in phosphate buffer solution (PBS) (pH = 7.4) at 37 °C. Chemical structures of the compounds were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS. **EU2**, **EU3**, **EU5**, **EU6** and **EU8** were reported for the first time, while **EU1**[35], **EU4** and **EU7** and **EU9**[36] had been reported before. The compounds were synthesized with the yield of 19–89%. **EU2**, **EU3**, **EU5** and **EU6** were solid, while **EU1**, **EU7**, **EU8** and **EU9** were viscous liquid. **EU4** was solid at +4 °C, while it was viscous liquid at room temperature.

Among the nine compounds reported here, derivatives **EU1**, **EU2**, **EU3**, **EU4**, **EU7** and **EU8** were cytotoxic above 400 μM toward both cancer and normal cells. Therefore, the calculation of tumor-specific (TS) value was practically impossible. **EU5** and **EU6** showed very weak tumor specificity (TS ≥ 1.2). **EU9** showed the highest TS value (TS ≥ 1.3), and one-order higher cytotoxicity against both cancer and normal cells as compared with the other derivatives. All compounds had lower TS values than a popular anticancer drugs 5-Fluorouracil (5-FU) and Melphalan (Table 3).

Most of the cytotoxic compounds induce apoptosis[37,38]. The breaks in single-strand DNA may be repaired by poly(ADP-ribose)polymerase (PARP1)[39]. This is the reason why PARP1 test was done by using the compound **EU9. EU9** at 50 μM concentration (CC_{50}) induced apoptosis (assessed by the cleavage of PARP1) in HSC-2 (OSCC), but not in HGF cells (normal cells) (Figure 1).

All compounds inhibited hCA I (23–30%) and hCA II (25–27%) isoenzymes with similar percentages. There was not selectivity toward any of hCA isoenzymes. Inhibitions of these izoenzymes by the compounds studied were lower than the reference compound (Table 4).

Conclusion

Several new compounds are reported here, being obtained by reaction of a suitable Mannich base, 1-aryl-3-amino-1-propenone hydrochloride, with 2-mercaptoethanol. The 1-aryl-3-(2-hydroxyethylthio)-1-propanone investigated here showed rather low tumor-specificity although they induced apoptosis, suggesting that apoptosis-inducing activity itself does not guarantee the antitumor effects. Chemical modifications of the best compound detected here, **EU9**, are thus necessary to further evaluate such derivatives for their biological activity, as cytotoxic agents or CA inhibitors[40–43].

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Declaration of interest

This study was supported by Ataturk University (BAP Project Number: 2010/166). The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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