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Umit Muhammet Kocyigit, Yakup Budak, Meliha Burcu Gürdere, Fatih Ertürk, Belkız Yencilek, Parham Taslimi, İlhami Gülçin & Mustafa Ceylan

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Synthesis of chalcone-imide derivatives and investigation of their anticancer and antimicrobial activities, carbonic anhydrase and acetylcholinesterase enzymes inhibition profiles

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ABSTRACT

The new 1-(4-(3-(aryl)acryloyl)phenyl)-1H-pyrrole-2,5-diones (**5a–g**) were prepared from 4'-aminchalcones (**3a–g**) and screened for biological activities. All compounds (**3a–g** and **5a–g**), except **3d** and **3e** displayed good cytotoxic activities with IC_{50} values in the range of 7.06–67.46 μM. IC_{50} value of 5-fluorouracil (5-FU) was 90.36 μM. Moreover, most of compounds **5a–g** showed high antibacterial activity with 8–20 mm of inhibition zone (19–25 mm of Sulbactam-Cefoperazone (SCF)). In addition, they showed good inhibitory action against acetylcholinesterase (AChE), and human carbonic anhydrase I, and II (hCA I and hCA II) isoforms. Also, these compounds demonstrated effective inhibition profiles with Ki values of 426.47–699.58 nM against hCA I, 214.92–532.21 nM against hCA II, and 70.470–229.42 nM against AChE. On the other hand, acetazolamide, clinically used drug, showed a Ki value of 977.77 ± 227.4 nM against CA I, and 904.47 ± 106.3 nM against CA II, respectively. Also, tacrine inhibited AChE showed a Ki value of 446.56 ± 58.33 nM.

Introduction

Today, cancer is one of the major health problems in the world. One of the ways for treatments of cancer is chemotherapy (Gallorini et al. 2012). Chemotherapy causes intense side effects, due to its cytotoxic effect on normal cells (Joseph et al. 2013). For this reason, it is important that anticancer drugs showed antiproliferative and cytotoxic activity in tumour cells without affecting normal tissues. Although numerous cytotoxic agents have been developed, there is a need to develop more potent and safer chemotherapeutic agents (Joseph et al. 2013). Previously, many compounds containing maleimide unit have been synthesised, for example, Jha et al. have prepared (2007) the maleimide based chalcone derivatives and reported their cytostatic activities against human Molt 4/C8 and CEM T-lymphocytes and murine L1210 cell lines. In addition, Patel and Dholakiya (2011, 2012) and Patel et al. (2012) have synthesised the maleimide and dibromomaleimide based chalcones and reported their antimicrobial activities.

The interconversion of carbon dioxide (CO_2) and carbonic acid (H_2CO_3) is automatically balanced to impound the parity between soluble H_2CO_3, CO_2, and bicarbonate (HCO_3^-) (Scozzafava et al. 2015a, Taslimi et al. 2017, Topal et al. 2017). The last ion (HCO_3^-) is physiologically the most significant

form, being both a buffer and a substrate for multiple carboxylation enzymes, which involved in biosynthetic pathways, such as amino acid and fatty acids biosynthesis and nucleotide synthesis (Oktay et al. 2017, Polat Köse and Gulcin 2017). Actually, the carbonic anhydrases (CAs, E.C.4.2.1.1) are enzymes that catalyse the quick interconversion of water and CO_2 to HCO_3^- and protons (H^+) (Scozzafava et al. 2015b; Ozmen Ozgun et al. 2016).
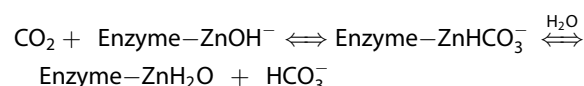
$$CO_2 + H_2O \overset{CA}{\Longleftrightarrow} H_2CO_3 \Longleftrightarrow HCO_3^- + H^+$$
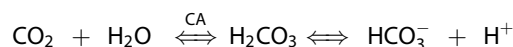
The first step of the hydration includes the offensive of a nucleophilic hydroxide (–OH) coordinated to the Zn^{2+} ion, outstanding to the conversion of CO_2 into HCO_3^- (Akıncıoğlu et al. 2015, Meleddu et al. 2015, Akocak et al. 2017).

$$CO_2 + Enzyme-ZnOH^- \Longleftrightarrow Enzyme-ZnHCO_3^- \overset{H_2O}{\Longleftrightarrow}$$
$$Enzyme-ZnH_2O + HCO_3^-$$

In the latter step, a proton is transferred from the Zn-bound water molecule to an acceptor (Meleddu et al. 2015, Del Prete et al. 2017).

$$Enzyme-ZnH_2O \Longleftrightarrow Enzyme-ZnOH^- + BH^+$$

This simple reaction is necessary for the adjustment of the several chemical types connected with CO_2 in the human

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body and its transport among biological membranes such as the inter-, intra-, and extra-cellular spaces (Meleddu et al. 2015, Küçük and Gulcin 2016). CA metalloenzyme family is encoded from seven different independent gene families including α-, β-, γ-, δ-, ζ-, n-, and θ-CAs (Boztaş et al. 2015, Yıldırım et al. 2015, Taslimi et al. 2016). The hCAs, which belong to α-CA family, include 16 different isoforms, of which there are different cytosolic isoforms (CA I, II, III, VII, and XIII), two are mitochondrial (CA VA and VB) isoforms, five are membrane-bound isoforms (CA IV, IX, XII, XIV, and XV), and one is secreted into saliva (CA VI) (Scozzafava et al. 2015b; Gocer et al. 2017). Three acatalytic forms, namely CARP VIII, X, and XI are the only known CA-related proteins (CARP). Also they are the just CAs available in mammals, demonstrating different subcellular and kinetic features, tissue repartition, and sensibility to inhibitors (Güney et al. 2014, Topal and Gülçin 2014, Göcer et al. 2016).

Acetylcholine (ACh) is a physiological neural system neurotransmitter, and it has been recognised as an important element in the homeostatic control of the essential immune response (Akıncioglu et al. 2017, Bayrak et al. 2017). Following tissue infection or harm, ACh is released from the vagus nerve, outstanding to dose-dependent inhibition of pro-inflammatory cytokine production (Gül et al. 2017a, Işık et al. 2017). ACh hydrolysis is catalysed by a related less-particular enzyme, acetylcholinesterase (AChE, E.C.3.1.1.7) and butyrylcholinesterase (BChE, E.C.3.1.1.8), which are present in pancreas, blood serum, central nervous system, and liver (Oztaskin et al. 2015, Aksu et al. 2016). AChE is consistently associated with cholinoceptive neurons and cholinergic properties. Some of the AChE enzyme inhibitors (AChEIs) have pharmaceutical applications and are exclusively considerable for the treatment of Alzheimer's disease (AD) (Polat Köse et al. 2015).

In the present study, we aimed to synthesise a series of novel chalcone-imide derivatives and investigate their biological activities including anticancer and antimicrobial activities, carbonic anhydrase, and AChE inhibition profiles.
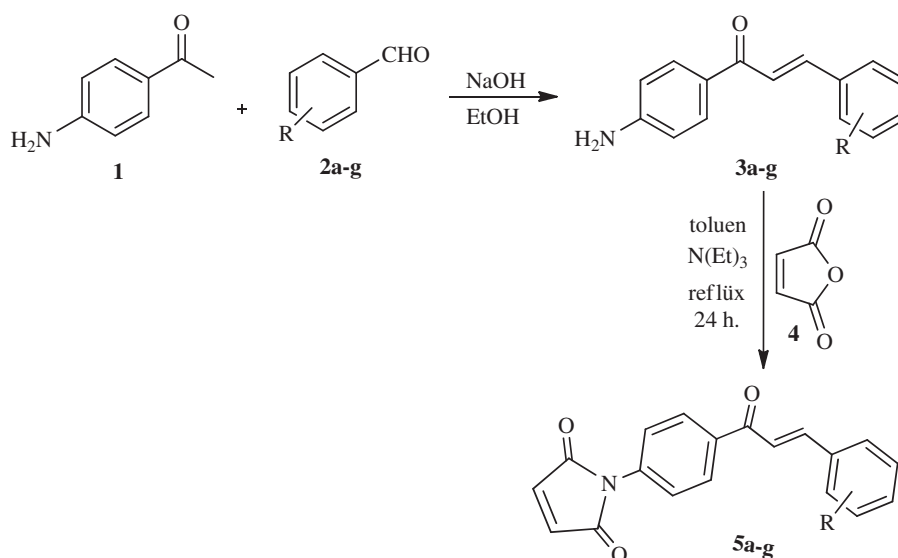
Results and discussion

Chemistry

First, 4′-aminochalcone derivatives (3a–g) were synthesised by well-known Claisen–Schmidt condensation (Gürdere et al. 2012, 2016a, 2016b). Treatment of 4′-aminoacetophenone (1) with corresponding benzaldehyde derivatives (2a–g) in basic medium (NaOH in EtOH) at room temperature for approximately 3 h gave the 4′-aminochalcone derivatives (3a–g) in good yields. The structures of chalcone derivatives (3a–g) were explained on the basis of spectral data and comparison with their authentic samples and literature data (Thirunarayanan et al. 2012, Suwito et al. 2014).

Then, the reaction of 4′-aminochalcone derivatives (3a–g) with maleic anhydride (4) in the presence of a few drops of NEt$_3$ in toluene at reflux temperature for 24 h gave the target compounds 1-(4-(3-(aryl)acryloyl)phenyl)-1H-pyrrole-2,5-dione derivatives (5a–g). The crude solid product was purified by crystallisation with ethanol/n-hexane (7/3). The structures of 5a–g were explained on the basis of spectral data (IR and NMR) and elemental analysis. All spectral data are in good agreement with proposed structures (Scheme 1 and Table 1). The NMR spectrum of compounds 5a–g were given in the Supplementary materials.

Anticancer studies

All synthesised compounds, amine-chalcone (3a–g) and chalcone-imide (5a–g), were tested for their potential growth inhibitory activity against C6 (glio carcinoma cell in rats) using proliferation BrdU ELISA assay (Aydın et al. 2015). The tests were performed at 10–75 μM concentrations and 5-fluorouracil (5-FU) was used as standard and the results are presented in Table 1. The growth inhibition effects of the compounds were shown to increase the activities depending on dose increasing. According to IC$_{50}$ values, compound 3e was inactive, compounds 3d and 5d showed low activity, and the others exhibited very high activity. Among the compounds 3a–g, the most

Scheme 1. Synthesis of chalcone-imide derivatives (5a–g).

active compound was **3f**, which contains methoxy group, with IC_{50} value of 7.06 μM, followed by **3g** having furan ring (9.04 μM), **3b** containing bromine atom (18.82 μM), **3a** having methyl group (33.60 μM), and **3c** containing chlorine atom (67.46 μM) compared to standard 5-FU (IC_{50}: 90.36 μM).

In addition, in the series of **5a–g**, compound **5g** having furan ring showed the highest activity with IC_{50} value of 12.08 μM, followed by **5b** containing bromine atom (IC_{50}: 18.66 μM), **5a** having methyl atom (33.24 μM), **5f** containing methoxy group (34.20 μM), **5e** having bromine atom (37.94 μM), **5c** containing chlorine atom (41.00 μM), and **5d** having methyl group (97.82 μM) compared to standard 5-FU (IC_{50}: 90.36 μM). Furthermore, when the compounds **5a–g** compared with compounds **3a–g**, it was observed that the maleimide ring increased the activity of compounds **5c–e** while decreased the activity of the other compounds **5a,b** and **5f,g**. These results indicate that all compounds except **3e**, **3d**, and **5d** emerged as promising anticancer agents that merit further research and development for control of C6 cell lines (Table 2).

Antimicrobial studies

Compounds **5a–g** were screened for *in vitro* antimicrobial activities by disc diffusion method (Karaman *et al.* 2010, Ceylan *et al.* 2011, 2017) using Mueller-Hilton agar medium at the 50 μg/disc concentration. The test was assayed against Gram-positive and Gram-negative bacteria by agar plate disc diffusion method. In the tests, while DMSO was used as negative control, Sulbactam-Cefoperazone (SCF) was used as

Table 2. IC_{50} values of **3a–g** and **5a–g** against C6.

Compounds	C6	Compounds	C6
3a	33.60	**5a**	33.24
3b	18.82	**5b**	18.66
3c	67.46	**5c**	41.00
3d	189.74	**5d**	97.82
3e	–	**5e**	37.94
3f	7.06	**5f**	34.20
3g	9.04	**5g**	12.08
5-FU	90.36	**5-FU**	90.36

"–" not active.

Table 1. Synthesised chalcone-imide derivatives (**5a–g**).

Entry	Reagent	Products	Yield (%)	M.P. (°C)
1			76	162–165
2			88	200–203
3			83	139–141
4		5a	74	138–140
5	5b	5c	84	210–212
6	5d	5e	68	145–148
7	5f	5g	80	187–190

Table 3. Antimicrobial activity of compounds **5a–g**.

Entry	Product	*S. aureus* ATCC 29213	*P. vulgaris* KUEN 1329	*B. subtilis* ATCC 6633	*P. aeruginosa* ATCC 9027	*C. albicans* ATCC 1213	*E. coli* AÜ tıp
1	**5a**	18	12	15	14	18	14
2	**5b**	18	16	16	14	15	14
3	**5c**	16	13	15	20	15	10
4	**5d**	18	16	14	18	17	18
5	**5e**	–	–	–	–	–	8
6	**5f**	10	8	–	–	–	10
7	**5g**	20	12	10	15	19	8
	SCF	25	20	21	20	19	26

Table 4. The enzyme inhibition values of chalcone-imide derivatives (**5a**, **5c–g**) against human carbonic anhydrase isoenzymes I and II (hCA I and II) and acetyl-choline esterase (AChE) enzyme.

Compounds	IC$_{50}$ (nM)						Ki (nM)		
	hCA I	r^2	hCA II	r^2	AChE	r^2	hCA I	hCA II	AChE
5a	710.04	0.9550	455.92	0.9807	236.43	0.9822	581.15 ± 219.1	345.72 ± 96.66	115.28 ± 5.612
5c	724.13	0.9719	466.98	0.9850	244.70	0.9748	549.82 ± 86.01	532.21 ± 81.52	229.42 ± 43.17
5d	697.88	0.9797	412.25	0.9909	139.43	0.9739	426.47 ± 72.10	276.40 ± 29.40	70.801 ± 9.652
5e	680.74	0.9723	342.89	0.9900	150.32	0.9954	580.02 ± 90.67	219.87 ± 20.27	93.683 ± 18.50
5f	755.72	0.9745	418.73	0.9865	181.51	0.9810	563.14 ± 100.1	214.92 ± 2.172	70.470 ± 10.77
5g	760.70	0.9643	523.81	0.9792	225.58	0.9966	699.58 ± 115.8	364.23 ± 46.93	169.28 ± 38.64
AZA[a]	912.28	0.9678	838.44	0.9892	–	–	977.77 ± 227.4	904.47 ± 106.3	–
TAC[b]	–	–	–	–	546.57	0.9788	–	–	446.56 ± 58.33

[a]AZA was used as standard inhibitor for human carbonic anhydrase I and II isoenzymes (hCA I and II).
[b]TAC was used as standard inhibitor for acetylcholine esterase (AChE) enzyme.

positive controls (Table 3). All compounds except **5e** and **5f** displayed very high activity (with 16–20 mm inhibition zone) (SCF: 25 mm), the others compounds displayed low activity against *S. aureus* ATCC 29213. Compounds **5b** (having bromine atom) and **5d** (having methyl group) demonstrated good activity (with 16 mm of inhibition zone), **5e** is inactive, and the others showed low activity (with 8–12 mm inhibition zone) against *P. vulgaris* KUEN 1329 (SCF: 20 mm). Compounds **5a–d** exhibited moderate activity (with 14–16 mm of inhibition zone) against *B. subtilis* ATCC 6633, while the others are inactive (SCF: 21 mm). Compound **5c** (containing chlorine atom) showed the same activity with standard with 20 mm of inhibition zone, compound **5d** displayed very good activity with 18 mm of inhibition zone, compounds **5a, b** and **5g** demonstrated moderate activity 14–15 mm of inhibition zone and the others are inactive against *P. aeruginosa* ATCC 9027 (SCF: 20 mm). Compounds **5e** and **5f** are inactive, compounds **5a** and **5g** (having methoxy group) exhibited the same activity with Standard (18 and 19 mm of inhibition zone, respectively, (SCF: 19 mm)), compounds **5b–d** showed good activity with 15–17 mm of inhibition zone against *C. albicans* ATCC 1213. The most active compound is **5d** with 18 mm of inhibition zone, and the others demonstrated low to moderate activity with 8–14 mm of inhibition zone against *E. coli* AÜ tıp (SCF: 26 mm). Summary, from these results, further research could be performed with compound **5g** for *S. aureus,* compounds **5c** and **5d** for *P. aeruginosa* and compounds **5a, 5d**, and **5g** for *C. albicans*.

Biochemical studies

The inhibition profiles of both isoenzymes (hCA I and II) and AChE have been taken under biochemical investigation. This study clearly indicates that chalcone and imide-chalcone derivatives (**5a, 5c–g**) show good cytotoxic properties, antibacterial activities and hCA I, hCA II, and AChE inhibitory effects. Both physiologically relevant hCA I, and II isoforms and AChE were studied in the enzyme inhibition part of this work. CA I, due to its diffuse repartition in the blood and gastrointestinal tract is one of the important off-targets for such pharmacologic agents, however, CA II isoform was selected because of its antiglaucoma drug targets (Kocyigit et al. 2017). Also, AChE as primary cholinesterase in the body was defined for its considerable applications in development and drug discovery for treatment of AD (Garibov et al. 2016).

Cytosolic hCA I isoenzyme was potently inhibited by chalcone-imide derivatives (**5a, 5c–g**). Ki values were found in range between 426.47 ± 72.10 and 699.58 ± 115.8 nM (Table 4). The best inhibition for this isoform was determined by novel (E)-1-(4-(3-(mtolyl)acryloyl)phenyl)-1H-pyrrole-2,5-dione (**5d**), with Ki value of 426.47 ± 72.10 nM. On the other hand, acetazolamide (AZA) was defined for broad-specificity CA inhibitor owing to its common inhibition of CAs, which showed Ki value of 977.77 ± 227.4 nM against hCA I. The CA I is associated with retinal oedema and cerebral, and the inhibition of CA I may be a precious factor for fighting these situations (Talaz et al. 2009, Gocer et al. 2017, Gül et al. 2017b).

The accessible and physiologically predominant cytosolic isoform hCA II is affiliated with multiple diseases. For hCA II, chalcone-imide derivatives (**5a, 5c–g**) had Ki values from 214.92 ± 2.172 to 532.21 ± 81.52 nM. Additionally, AZA (5-aceta-mido-1,3,4-thiadiazole-2-sulfonamide), which is used for the treatment of altitude sickness, cystinuria, idiopathic intracranial hypertension, glaucoma, epileptic seizure, dural estasia, and central sleep apnoea, had a medium potency CA II inhibition for this isoform, with a Ki value of

904.47 ± 106.3 nM. In this study, the most inhibition effect against CA II was observed by (E)-1-(4-(3-(3-methoxyphenyl)acryloyl)phenyl)-1H-pyrrole-2,5-dione (**5f**) with Ki values of 214.92 ± 2.172 nM.

Most of the prevalent available drugs on the market such as rivastigmine, tacrine, galantamine, and donepezil are defined to treat AD, which are AChE inhibitors. All the obtained compounds have determined AChE inhibiting activity with IC_{50} values in the range of 139.43–244.70 nM. Enhanced selectivity for AChE enzyme results in the functional improvement in symptomatic therapy of muscle weakness. Chalcone-imide derivatives (**5a**, **5c–g**), effectively inhibited AChE enzyme with Ki values in the range of 70.470 ± 10.77–229.42 ± 43.17 nM (Table 4). In this work, we found that tacrine compound, which are clinical factors as AChE inhibitors showed Ki values of 446.56 ± 58.33 nM. Acetylthiocholine iodide (AChI) was used as substrate of this study. All chalcone-imide derivatives (**5a**, **5c–g**) show similar inhibition profile against AChE. (E)-1-(4-(3-(2-chlorophenyl)acryloyl)phenyl)-1H-pyrrole-2,5-dione (**5c**), which shows the weakest AChE inhibition, had two times AChE inhibition effects than that of Tacrine. The best inhibition for AChE enzyme was determined by (E)-1-(4-(3-(3-methoxyphenyl)acryloyl)phenyl)-1H-pyrrole-2,5-dione (**5f**), with Ki value of 70.470 ± 10.77 nM.

Conclusion

In the present study, a series of the chalcone-imide derivatives **5a–g** were synthesised, characterised and their anticancer activity against C6 rat gliocarcinoma, antimicrobial activity (against some human pathogen microorganism) were investigated. All compounds except **3d** and **5d** showed very high anticancer activity with IC_{50} values in the range of 7.06–67.46 μM compared to 5-FU (IC_{50}: 90.36 μM). In addition, most of the compounds exhibited moderate to high antimicrobial activity against microorganism compared to standard (SCF). The most active compounds were **5g** for *S. aureus,* compounds **5c** and **5d** for *P. aeruginosa* and compounds **5a**, **5d**, and **5g** for *C. albicans.* Also, the chalcone-imide derivatives (**5a**, **5c–g**) used in the present study described effective inhibition profiles against both hCA isoenzymes and AChE enzyme. In this study, nanomolar level of Ki values was recorded for each chalcone-imide derivative (**5a**, **5c–g**), and these compounds can be a selective inhibitor of AChE enzyme and both cytosolic CA isoenzymes.

Experimental

General methods

All the reagents and solvents for synthesis were purchased from Sigma-Aldrich (St. Louis, MO) and Fluka. Melting points were determined on Electrothermal 9100 apparatus. IR spectrums (KBr disc) were recorded on a Jasco FT/IR-430 spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance DPX-400 instrument (Billerica, MA). As internal standards served TMS (δ 0.00) for ^{1}H NMR and $CDCl_3$ (δ 77.0) for ^{13}C NMR spectroscopy, J values are given in Hz.

Elemental analyses were obtained from a LECO CHNS 932 Elemental analyser (St Joseph, MI) (Supplementary material).

General procedure for the synthesis of 1-(4-(3-(aryl)acryloyl)phenyl)-1H-pyrrole-2,5-dione derivatives (5a–g)

To the solution of maleic anhydride (0.19 g, 2 mmol) and toluene (3 ml) was added chalcone derivatives (0.5 g, 2 mmol) and 15 drops of N(Et)$_3$. The reaction mixture was heated at reflux temperature for 24 h. Toluene was evaporated, and poured into ice/water and stand up for 3 h. The solid was filtered and crystallised with ethanol/n-hexane (7/3).

(E)-1-(4-(3-(o-tolyl)acryloyl)phenyl)-1H-pyrrole-2,5-dione (5a)

Yellowish solid, Yield: 76%. M.P. 0.162–165 °C. IR (KBr, cm^{-1}): 3091, 2981, 1718, 1658, 1606, 1590, 1513, 1396, 1378, 1336, 1324, 1220, 1180, 1145, 1033, 1016. ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.15–8.11 (m, 3H), 7.70 (d, J = 7.2 Hz, 1H), 7.56 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 15.6 Hz, 1H), 7.33–7.22 (m, 3H), 6.89 (s, 2H), 2.47 (s, 3H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 189.2, 169.0 (2C), 142.8, 138.4, 136.9, 135.2, 134.4 (2C), 133.7, 130.9, 130.4, 129.3 (2C), 126.4, 125.4 (2C), 122.6, 19.9. Anal. calc. for C$_{20}$H$_{15}$NO$_3$: C, 75.70; H, 4.76; N, 4.41. Found: C, 75.65; H, 4.72; N, 4.36.

(E)-1-(4-(3-(2-bromophenyl)acryloyl)phenyl)-1H-pyrrole-2,5-dione (5b)

Colourless solid, Yield: 88%. M.P. 200–203 °C. IR (KBr, cm^{-1}): 1740, 1643, 1611, 1586, 1527, 1394, 1386, 1246, 1224, 1160, 1148. ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.15 (d, J = 8.4 Hz, 2H), 7.76 (d, J = 7.6 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.61 (d, J = 8.4 Hz, 1H), 7.45 (d, J = 15.6 Hz, 2H), 7.38–7.31 (m, 4H), 6.93 (s, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 189.2 (2C), 168.9, 143.6, 136.7, 135.3, 134.9, 134.4 (2C), 133.6, 131.4, 129.5 (2C), 127.9, 127.7, 125.9, 125.4 (2C), 124.6. Anal. calc. for C$_{19}$H$_{12}$BrNO$_3$: C, 59.71; H, 3.16; N, 3.66. Found: C, 59.65; H, 3.02; N, 3.58.

(E)-1-(4-(3-(2-chlorophenyl)acryloyl)phenyl)-1H-pyrrole-2,5-dione (5c)

Brown crystals, Yield: 83%. M.P. 183–185 °C. IR (KBr, cm^{-1}): 3095, 1720, 1660, 1606, 1592, 1513, 1394, 1376, 1317, 1272, 1220, 1180, 1143, 1033, 1016. ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.242 (d, J = 16 Hz, 1H), 8.13 (d, J = 8.8 Hz, 2H), 7.78–7.76 (m, 1H), 7.59 (d, J = 8.4 Hz, 2H), 7.50 (d, J = 15.6, 1H), 7.46–7.44 (m, 1H), 7.37–7.31 (m, 2H), 6.91 (s, 2H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 189.3, 168.9 (2C), 141.1, 136.6, 135.6, 135.4, 134.4 (2C), 133.0, 131.4, 130.3, 129.5 (2C), 127.8, 127.1, 125.4 (2C), 124.3. Anal. calc. for C$_{19}$H$_{12}$ClNO$_3$: C, 67.56; H, 3.58; N, 4.15. Found: C, 67.49; H, 3.42; N, 4.06.

(E)-1-(4-(3-(m-tolyl)acryloyl)phenyl)-1H-pyrrole-2,5-dione (5d)

Colourless solid, Yield: 74%. M.P. 138–140 °C. IR (KBr, cm^{-1}): 3097, 2915, 1716, 1702, 1660, 1606, 1583, 1407, 1324, 1241, 1216, 1155, 1027. ^{1}H NMR (400 MHz, CDCl$_3$, ppm): δ 8.12–8.09 (dt, $J = 2.4$ Hz, 2 Hz, 1.6 Hz, 2H), 7.80 (d, $J = 16.0$ Hz, 1H), 7.57–7.56 (dt, $J = 2$ Hz, 2 Hz, 2 Hz, 2H), 7.49 (d, $J = 15.6$ Hz, 1H), 7.44 (d, $J = 7.6$ Hz, 2H), 7.31 (t, $J = 8.4$ Hz, 8 Hz, 7.6 Hz, 1H), 7.23 (d, $J = 7.6$ Hz, 1H), 6.90 (s, 2H), 2.39 (s, 3H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 189.4, 168.9, 145.4, 138.6, 136.9, 135.1, 134.6, 134.4 (2C), 131.5, 129.3 (2C), 129.0, 128.8, 125.7, 125.4 (2C), 121.4, 21.3. Anal. calc. for C$_{20}$H$_{15}$NO$_3$: C, 75.70; H, 4.76; N, 4.41. Found: C, 75.63; H, 4.68; N, 4.39.

(E)-1-(4-(3-(3-bromophenyl)acryloyl)phenyl)-1H-pyrrole-2,5-dione (5e)

Yellowish crystals, Yield: 84%. M.P. 210–212 °C. IR (KBr, cm^{-1}): 3056, 1714, 1658, 1598, 1556, 1415, 1313, 1220, 1178, 1031. ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.10–8.08 (dd, $J = 6.8$ Hz, 1.6 Hz, 2H), 7.77 (t, $J = 1.6$ Hz, 1H), 7.70 (d, $J = 15.6$ Hz, 2H), 7.57–7.55 (dd, $J = 6.8$ Hz, 1.6 Hz, 2H), 7.53–7.47 (m, 3H), 6.89 (s, 2H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 188.9, 168.9 (2C), 143.3, 136.8, 136.6, 134.4 (2C), 133.3, 130.8, 130.4, 129.3 (2C), 127.2, 125.4 (2C), 123.0, 122.9, 30.8. Anal. calc. for C$_{19}$H$_{12}$BrNO$_3$: C, 59.71; H, 3.16; N, 3.66. Found: C, 59.65; H, 3.02; N, 3.56.

(E)-1-(4-(3-(3-methoxyphenyl)acryloyl)phenyl)-1H-pyrrole-2,5-dione (5f)

Colourless solid, Yield: 68%. M.P. 145–148 °C. IR (KBr, cm^{-1}): 3091, 3023, 2933, 1712, 1658, 1602, 1577, 1369, 1263, 1220, 1137, 1049. ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.13 (d, $J = 8.4$ Hz, 2H), 7.80 (d, $J = 15.6$ Hz, 1H), 7.59 (d, $J = 8.4$ Hz, 2H), 7.51 (d, $J = 15.6$ Hz, 1H), 7.36 (t, $J = 8$ Hz, 7.8 Hz, 7.6 Hz, 1H), 7.27 (d, $J = 7.2$ Hz, 1H), 7.18 (bs, 1H), 7.01–6.98 (dd, $J = 8.2$ Hz, 2.8 Hz, 2.4 Hz, 1H), 6.92 (s, 2H), 3.88 (s, 3H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 189.5, 168.9 (2C), 159.9, 145.2, 137.0, 136.1, 135.2, 134.4 (2C), 130.0, 129.4 (2C), 125.4 (2C), 122.1, 121.1, 116.5, 113.4, 55.3. Anal. calc. for C$_{20}$H$_{15}$NO$_4$: C, 72.06; H, 4.54; N, 4.20. Found: C, 72.01; H, 4.42; N, 4.09.

(E)-1-(4-(3-(furan-2-yl)acryloyl)phenyl)-1H-pyrrole-2,5-dione (5g)

Yellowish crystals, Yield: 80%. M.P. 187–190 °C. IR (KBr, cm^{-1}): 3097, 1718, 1654, 1606, 1594, 1550, 1513, 1479, 1390, 1330, 1303, 1280, 1228, 1182, 1145, 1035, 1012. ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.17–8.14 (m, 2H), 7.63 (d, $J = 15.6$ Hz, 1H), 7.60–7.56 (m, 3H), 7.46 (d, $J = 15.2$ Hz, 1H), 6.92 (s, 2H), 6.76 (d, $J = 3.6$ Hz, 1H), 6.56–6.54 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 169.0, 151.5, 145.1, 134.4 (2C), 131.1, 129.3 (2C), 125.4 (2C), 118.9, 116.6, 112.7. Anal. calc. for C$_{17}$H$_{11}$NO$_4$: C, 69.62; H, 3.78; N, 4.78. Found: C, 69.59; H, 3.74; N, 4.71.

Screening of anticancer studies

Cell culture

C6 (rat gliocarcinoma) cell line was maintained in Dulbecco's modified eagle's medium (DMEM, Sigma, St. Louis, MO), supplemented with 10% (v/v) foetal bovine serum (Sigma, Steinheim, Germany) and Pen Strep solution (Sigma, Steinheim, Germany). At confluence, cells were detached from the flasks using 4 ml of Trypsin-EDTA (Sigma, Steinheim, Germany), centrifuged and cell pellet re-suspended with 4 ml supplemented DMEM.

BrdU cell proliferation assay (BCPA)

The anticancer activity test of the synthesised compounds was performed by BCPA. 5-FU was used as standard molecule (Gürdere et al. 2016a, 2016b).

Screening of antibacterial activity

Compounds **5a–g** were screened for their *in vitro* antimicrobial activities against *Staphylococcus aureus* ATCC 29213, *Proteus vulgaris* KUEN 1329, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 1213, and *Escherichia coli* AÜ tip (Ankara University, clinical isolated) by disc diffusion method using Mueller-Hilton agar medium, according to previously described methods (Gülçin et al. 2003, 2004, 2008).

Biochemistry

In this work, both hCA I, and II isoenzymes were purified by Sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography (Genç et al. 2016). This affinity chromatography technic organises Sepharose-4B-L-tyrosine-sulfanilamide that acts as affinity matrix for selective retention of CA isoenzymes (Koçak et al. 2016). Chromatographic segregation method was used for the purification of biomolecules, such as enzymes and protein. CA activity determination was measured spectrophotometrically according to Verpoorte et al. (1967) as described previously (Sentürk et al. 2009, Ozgeris et al. 2016). *p*-Nitrophenylacetate (PNA) was consumed as substrate for both isoenzymes and enzymatically transformed to *p*-nitrophenolate ions (Turan et al. 2016). One CA activity unit is the amount of enzyme, which had absorbance change at 348 nm of PNA to 4-nitrophenylate ion over a period of 3 min at 25 °C.

Bradford technique was used for the investigation of protein amount during the purification stages (1976). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) method was used for fixation of both isoenzymes (Laemmli 1970) described in previous studies (Arabaci et al. 2014, Aktaş et al. 2017, Köksal et al. 2017). Bovine serum albumin was used as the standard protein. For determining the inhibition result of each isoenzyme, chalcone-imide derivatives (**5a**, **5c–g**) and an activity (%) [chalcone-imides] graph was drawn. To calculate Ki values, three different chalcone-imide derivative (**5a**, **5c–g**) concentrations were tested.

Also, the inhibitory effect of chalcone-imide derivatives (**5a**, **5c–g**) on AChE activity was measured according to

spectrophotometric technique of Ellman *et al.* (1961). Acetylthiocholine iodide was used as the substrate for this reaction. 5,5′-Dithio-bis(2-nitro-benzoic)acid (DTNB, D8130-1G, Sigma-Aldrich, Steinheim, Germany) was used for the measurement of the AChE activity. In addition, 10 ml of sample solution, 100 ml of Tris/HCl buffer (1.0 M, pH 8.0) dissolved in distilled water at diverse concentrations and 50 ml AChE $(5.32 \times 10^{-3}$ U) solution were mixed and incubated for 10 min at 25 °C. Then, 50 ml of DTNB (0.5 μM) was added. One AChE unit is the amount of enzyme that hydrolyses 1.0 μmol of ACh to choline and acetate per minute at pH 8.0 at 37 °C (Gülçin *et al.* 2016).

Disclosure statement

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