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New halogenated chalcones with cytotoxic and carbonic anhydrase inhibitory properties: 6-(3-Halogenated phenyl-2-propen-1-oyl)-2(3*H*)-benzoxazolones

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Abstract

In this study, novel halogenated chalcones, 6-(3-halogenated phenyl-2-propen-1-one)-2(3*H*)-benzoxazolones (**2a**–**n**), were synthesized for the first time (except **2a**), and their chemical structures were characterized by ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, and high-resolution mass spectrometry spectra. Cytotoxic activities and carbonic anhydrase (CA) inhibitory effects of the compounds were studied to identify new possible drug candidate molecules. Cytotoxicity results pointed out that compound **2m**, 6-[3-(3-bromophenyl)-2-propenoyl]-2(3*H*)-benzoxazolone, had the highest cytotoxicity (CC_{50}) and potency selectivity expression (PSE) values. Thus, compound **2m** can be considered as a lead compound of the series in terms of cytotoxicity. When the CA inhibition results of the compounds were evaluated, it was found that the K_i values of the compounds ranged from 30.5 ± 11.3 to 65.5 ± 25.6 μM toward hCA I, and they ranged from 7.3 ± 1.8 to 58.8 ± 12.3 μM toward hCA II. However, the K_i values of the reference drug, acetazolamide (AZA), were 30.2 ± 7.8 and 4.4 ± 0.6 μM toward hCA I and hCA II, respectively. According to the results obtained, compounds **2a**–**n** had lower K_i values than AZA, whereas compounds **2a**, **2b**, **2e**–**g**, **2l**, and **2n** had similar K_i values, compared with AZA. So, the compounds **2a**, **2b**, **2e**–**g**, **2l**, and **2n** can be considered as lead molecules of this series for further considerations.

KEYWORDS

benzoxazolone, carbonic anhydrase, cytotoxicity, halogenated chalcone

1 | INTRODUCTION

According to the World Health Organization report, it is predicted that 16.5 million people will die due to cancer by 2040.[1] Thus, studies on the treatment of cancer continue intensively worldwide. Although costly research and new treatment options are increasing in this area, the treatment rates of cancer patients can only be achieved by 20–25%.[2] Anticancer drugs in clinics have low selectivity, drug resistance problem, and side effects such as vomiting, hair loss, and pain.[3] Thus, the researchers are focusing on the development of new anticancer drug candidates that have high selectivity and low toxicity.

Carbonic anhydrases (CAs) are metalloenzymes acting as catalysts for the interconversion between carbon dioxide and bicarbonate. CAs play several important roles in many physiological and physiopathological functions in all organisms.[4] Until now, eight genetically different CA families (α-, β-, γ-, δ-, ζ-, η-, θ-, and the recently reported ι-CAs) have been identified.[4–6] In mammals, 16 different α-CA isoforms were isolated with their different catalytic activities. Of them, some are cytosolic (CA I, CA II, CA III, CA VII, and CA XIII), some are membrane-bound isoforms (CA IV, CA IX, CA XII, CA XIV, and CA XV), CA V is mitochondrial, and CA VI is secreted in saliva and milk.[7] Three of these isoforms are known as CA-related proteins (CARPs); they are noncatalytic (CARP VIII, CARP IX, and

CARP XI) and do not have zinc in the active site of the enzyme.[4,6,7] These isozymes are widely distributed in different organs and tissues, and they have an important role in many physiological processes like pH and CO_2 homeostasis, biosynthetic reactions, calcification, and electrolyte secretion.[8–10] Thus, inhibition or activation of these isozymes is a potential target for treatment and/or diagnosis of many diseases.[7] As a result, CA inhibitors/activators have many clinical uses as antiglaucoma drugs (hCA I, II, IV, XII), diuretics (hCA II, IV, XII, XIV), antiepileptic drugs (hCA II, IV, XIV, XIV), and antiobesity drugs.[11–15]

The α,β-unsaturated ketones have alkylation ability, especially toward thiols.[16,17] They have no reactivity or far less reactivities for amino and hydroxyl groups of available nucleic acids,[16,18,19] whereas classical alkylating agents alkylate hydroxyl and amino groups of nucleic acids, and lead to mutagenicity and carcinogenicity.[20] The level of glutathione (a thiol compound) increases before the cell division.[21] This can be an advantage for α,β-unsaturated ketone function bearing compounds such as chalcones, as they are thiol-selective alkylators to perform selective toxicity against tumor tissues.[22–24]

Chalcones are major components of natural products, such as fruits, vegetables, tea, and so forth.[25,26] Chemically, chalcones are open-chain flavonoids with two aromatic rings that are linked by a three-carbon α,β-unsaturated carbonyl system.[27] The α,β-unsaturated carbonyl system is responsible for the biological activities of the chalcones.[26,28] Various chalcone derivatives have been reported with many biological activities such as anti-inflammatory,[29,30] antimicrobial,[31] antifungal,[32] antioxidant,[33] antimalarial,[34] antileishmanial,[35,36] anticancer,[37–39] cytotoxic,[40–42] CA inhibiting,[43–45] and anti-invasive[46] activities.

The drugs and drug candidate compounds developed by medicinal chemistry studies include a significant number of halogenated structures.[47,48] Some halogen-substituted chalcones were reported with strong cytotoxic activities[28,49–51] in a limited number of studies.

The benzoxazolone heterocycle is described as a "privileged scaffold" in the literature due to its chemical reactivity, electronic charge distribution, and wide range of bioactivities.[52] 2(3*H*)-Benzoxazolone and its derivatives exhibit many activities such as myorelaxant,[53,54] analgesic,[55,56] antiviral,[57] anticancer,[58,59] and anti-inflammatory[60] activities. In a very limited number of studies, some chalcones bearing 2(3*H*)-benzoxazolone with different substituents on the phenyl ring were reported with their strong cytotoxic activities.[3,58,61,62] Both the selective and strong anticancer activities of these compounds pointed out the importance of this chemical moiety in designing new chalcone compounds for possible drug candidate molecules. Thus, we chose the chalcone structure bearing the 2(3*H*)-benzoxazolone heterocycle as the main chemical skeleton for further investigation.

In this study, we aimed to synthesize new chalcones, 6-(3-halogenated phenyl-2-propen-1-one)-2(3*H*)-benzoxazolones (**2a**–**n**), which have an α,β-unsaturated carbonyl system and halogen moiety, and to investigate their cytotoxicities (toward both tumor cell lines and nontumor cells) and inhibition profiles toward hCA I and II. We hope to find out new possible drug candidate molecule(s) that may stimulate further studies of the researchers working on these fields.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

Compounds **2a**–**n**, 6-(3-halogenated phenyl-2-propen-1-one)-2(3*H*)-benzoxazolones, were synthesized successfully for the first time (except compound **2a**) according to Scheme 1.

As shown in Scheme 1, 2(3*H*)-benzoxazolone was acylated by Friedel–Crafts reaction. A direct acylation of the 2(3*H*)-benzoxazolone ring is regioselective and always leads to a 6-acetyl derivative[62] in good yield and purity. Chalcones **2a**, **2b**, **2d**–**g**, and **2j**–**l** were synthesized by classical Claisen–Schmidt condensation reaction, whereas chalcones **2c**, **2h**, **2m**, and **2n** were synthesized by a microwave irradiation method. Claisen–Schmidt condensation reaction occurred between suitable aldehyde and 6-acetyl-2(3*H*)-benzoxazolone.

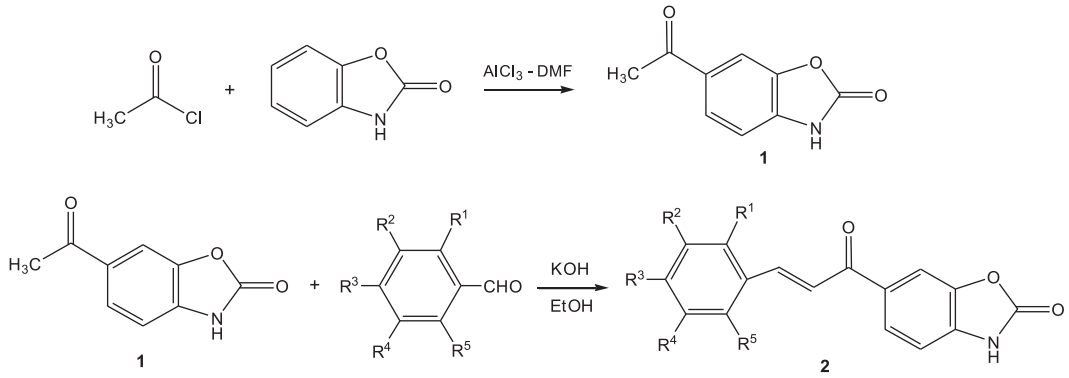
According to ^{1}H nuclear magnetic resonance (NMR) spectra of compounds **2a**–**n**, all synthesized compounds (except **2l**) were geometrically pure and had *E* configuration (coupling constant J = 15.4–16.1 Hz for vinyl protons, observed at 7.67–8.07 ppm). The aromatic ring protons and olefinic protons appeared at 7.0–8.0 ppm, as expected. ^{13}C NMR spectra of the compounds showed that carbons of carbonyl groups of the compounds appeared at about 187 ppm, as expected. High-resolution mass spectrometry (HRMS) results also confirmed the chemical structures of the compounds with high purity. Spectral data are presented in detail below.

2.2 | Biology

2.2.1 | Cytotoxic/anticancer activity

The cytotoxicities of 13 synthesized compounds have been investigated in vitro against oral squamous cancer cell line (HSC-2) and human normal oral cells (human gingival fibroblast [HGF] and human periodontal ligament fibroblast [HPLF]) as reported.[41,63] Doxorubicin (DXR) and 5-fluorouracil (5-FU), which are clinically in use for the treatment of several cancers, were used as reference drugs. Cytotoxicity results of compounds **2a**–**n** are presented in Table 1. All synthesized compounds, **2a**–**n**, showed a higher cytotoxicity than 5-FU, whereas they showed lower cytotoxicity than DXR. According to Table 1, cytotoxicities of the compounds were in the range 6.6–25.5 μM toward HSC-2 cell line. Synthesized compounds were 1.5–5.7 times more cytotoxic than 5-FU. When the cytotoxicity results of the compounds were considered, compound **2m**, 6-[3-bromophenyl-2-propenoyl]-2(*3H*)-benzoxazolone, was found to be the most potent cytotoxic molecule of the series toward HSC-2 cell line according to CC_{50} and potency selectivity expression (PSE) values.

The first point to be considered for the compounds is whether they are tumor cytotoxins. Therefore, the compounds were also evaluated against HGF and HPLF nonmalignant cells, as normal cells surround the tumor cells in an organism. Therefore, tumor selectivity (TS) values were calculated by dividing the average CC_{50} value toward normal cells by the average CC_{50} value toward cancer cell



2a: $R^1 = R^2 = R^3 = R^4 = R^5 = H$
2b: $R^1 = R^3 = Cl$; $R^2 = R^4 = R^5 = H$
2c: $R^2 = R^3 = Cl$; $R^1 = R^4 = R^5 = H$
2d: $R^1 = R^5 = Cl$; $R^2 = R^3 = R^4 = H$
2e: $R^1 = F$; $R^2 = R^3 = R^4 = R^5 = H$
2f: $R^2 = F$; $R^1 = R^3 = R^4 = R^5 = H$
2g: $R^1 = R^3 = F$; $R^2 = R^4 = R^5 = H$
2h: $R^1 = R^4 = F$; $R^2 = R^3 = R^5 = H$
2j: $R^1 = R^5 = F$; $R^2 = R^3 = R^4 = H$
2k: $R^1 = R^2 = F$; $R^3 = R^4 = R^5 = H$
2l: $R^1 = Br$; $R^2 = R^3 = R^4 = R^5 = H$
2m: $R^2 = Br$; $R^1 = R^3 = R^4 = R^5 = H$
2n: $R^3 = Br$; $R^1 = R^2 = R^4 = R^5 = H$

SCHEME 1 Synthesis of compounds **1** and **2a–n**. DMF, dimethylformamide

lines as reported[3,28,40,44,51,64,65] (Table 1). According to results, the TS values of all compounds were >1, which implies that they were all tumor-selective compounds. It can be seen from Table 1 that bromine substituted two compounds at third and fourth positions on the phenyl ring (**2m** and **2n**), respectively. These compounds had a similar TS value to DXR or a higher TS value than DXR (Table 1), whereas they had lower TS values than 5-FU. According to these results, compounds **2m** and **2n** that have TS values over 10 and

TABLE 1 Cytotoxic activities of compounds **2a–n** toward human OSCC cell lines and human oral normal cells

	CC_{50} (μM)								
	OSCC		Human normal oral cells						
	HSC-2	*SD*	HGF	*SD*	HPLF	*SD*	Mean	TS	PSE
Compounds	(*A*)						(*B*)	(*B*/*A*)	(*B*/*A*^{2}) × 100
2a	22.1	1.2	71.0	9.5	83.3	14.0	77.2	3.5	16
2b	8.9	2.0	54.7	23.3	63.3	21.5	59.0	6.6	74
2c	12.5	1.2	68.3	38.0	75.0	3.0	71.7	5.7	46
2d	8.9	1.0	71.0	9.5	75.3	3.5	73.2	8.3	93
2e	18.9	1.0	70.3	0.6	74.0	4.4	72.2	3.8	20
2f	13.3	6.0	76.7	12.9	94.7	27.2	85.7	6.4	48
2g	8.5	0.9	75.0	15.6	92.3	5.5	83.7	9.8	115
2h	25.5	4.5	43.0	26.9	70.3	26.0	56.7	2.2	9
2j	9.5	1.3	62.3	5.0	85.0	15.0	73.7	7.8	82
2k	7.5	0.5	42.3	16.2	73.0	32.8	57.7	7.7	103
2l	13.3	2.6	59.0	5.6	86.0	35.5	72.5	5.4	41
2m	6.6	0.8	52.0	19.1	87.3	19.7	69.7	10.5	158
2n	14.7	4.5	351.3	84.3	40.0	0.0	195.7	13.3	90
5-FU[a]	37.7	0.0	>1,000	0.0	>1,000	0.0	>1,000	>28.3	80.7
DXR[a]	0.5	0.1	0.7	0.0	>10	0.0	>5.3	>10.4	>2,030

Abbreviations: 5-FU, 5-fluorouracil; DXR, doxorubicin; HGF, human gingival fibroblast; HPLF, human periodontal ligament fibroblast; OSCC, oral squamous cell carcinoma; PSE, potency selectivity expression; *SD*, standard deviation; TS, tumor selectivity.
[a]Used as reference drugs.

similar TS values to DXR can be considered as leads of the series in terms of cytotoxicities.

Lead compounds should have both high and selective cytotoxicity for tumors. To identify the most promising compounds in terms of both high potencies and selective cytotoxicities, the PSE values have been defined and calculated, as shown in our previous studies.[3,39–41,63–65] PSE values of the compounds were in the range of 9–158 (Table 1). The PSE values of the compounds in the series were >30, except compounds **2a**, **2e**, and **2h**. Thus, these 10 compounds can be used to develop new analogs.

PSE values of the compounds pointed out that the replacement of a hydrogen by a halogen at any position of the phenyl ring increased the PSE values 1.3–9.9 times (except compound **2h**).

The other point to be considered is whether there is any relationship between cytotoxicities and structural properties of the compounds. According to the results presented in Table 1, the halogen substitution at the different position of phenyl ring was found to be a generally useful modification to increase cytotoxicity (1.2–3.3 times) and selectivity (1.1–3.8 times; except compound **2h** for both cytotoxicity and selectivity).

Compound **2m**, wherein bromine was substituted at the third position of the phenyl ring, was the most expressive compound of our series due to its high cytotoxicity and PSE value (158). CC_{50} and PSE values of compound **2m** were 3.3 and 9.9 times higher than nonsubstituted compound **1**, respectively. Although compound **2m** was 13.2 times less cytotoxic than DXR, it was 5.7 times more cytotoxic than the other reference compound, 5-FU. However, the PSE value of compound **2m** was about two times higher than 5-FU. Additionally, compounds **2g** (PSE: 115) and **2k** (PSE: 102) were other impressive compounds with PSE values above 100. As a result, compound **2m** that had the highest PSE value, 158, can be considered as a possible drug candidate of this series for further studies.

2.2.2 | CA inhibitory effects

Inhibition effects of compounds **2a**–**n** on hCA I and hCA II are presented in Table 2. Acetazolamide (AZA) was used as a reference drug. Compounds **2a**–**n** showed lower CA inhibitory effects than the reference drug, AZA. The IC_{50} value of AZA was 16.6 μM toward hCA I, whereas it was 8.4 μM toward hCA II. According to Table 2, compound **2g** with 2,4-difluoro substituents on the phenyl ring showed the best inhibitory activity (27.2 μM) toward hCA I, and compound **2d** carrying 2,6-dichloro substituents on the phenyl ring showed the best activity (29.1 μM) toward hCA II.

K_i values of the compounds (inhibitory potency) ranged from 30.5 ± 11.3 to 65.5 ± 25.6 μM toward hCA I isoenzyme, whereas they ranged from 7.23 ± 1.8 to 58.8 ± 12.3 μM toward hCA II isoenzyme. K_i values of AZA were 30.2 ± 7.8 μM and 4.4 ± 0.6 μM toward hCA I and hCA II, respectively. According to Table 2, compounds **2a**–**n** had smaller K_i values than AZA, whereas compounds **2a**, **2b**, **2e**–**g**, **2l**, and **2n** had similar K_i values to AZA. Thus, compounds **2a**, **2b**, **2e**–**g**, **2l**, and **2n** can be considered as lead molecules of this series for further considerations.

TABLE 2 Inhibitory effects of compounds **2a**–**n** on hCA I and hCA II isoenzymes

	IC_{50} (μM)				K_i (μM)	
Compounds	**hCA I**	r^2	**hCA II**	r^2	**hCA I**	**hCA II**
2a	47.5	.9406	48.1	.9854	30.5 ± 11.3	36.2 ± 10.3
2b	43.3	.9485	39.6	.9456	38.8 ± 10.5	39.0 ± 1.5
2c	47.8	.9665	44.4	.9265	46.4 ± 6.7	56.2 ± 8.7
2d	42.8	.9607	29.1	.9478	61.7 ± 3.3	34.1 ± 2.2
2e	53.7	.9559	52.9	.9436	42.8 ± 10.4	9.4 ± 2.3
2f	67.9	.9558	53.7	.9425	38.2 ± 11.4	58.8 ± 12.3
2g	27.2	.9346	72.6	.9425	38.3 ± 5.8	25.6 ± 4.7
2h	36.7	.9374	29.7	.9538	43.6 ± 2.5	14.0 ± 2.6
2j	73.7	.9597	55.4	.9384	57.2 ± 7.7	45.4 ± 8.5
2k	64.2	.9558	38.5	.9336	65.5 ± 25.6	45.3 ± 2.2
2l	48.1	.9248	39.2	.9448	35.6 ± 10.6	55.0 ± 9.2
2m	48.5	.9663	31.4	.9374	51.5 ± 16.5	48.1 ± 2.5
2n	28.8	.9364	29.5	.9326	33.5 ± 4.5	7.3 ± 1.8
AZA[a]	16.6	.9887	8.4	.9825	30.2 ± 7.8	4.4 ± 0.6

Abbreviation: AZA, acetazolamide.

[a]AZA was used as a standard inhibitor for both hCA I and II isoenzymes. r^2 is a statistical measure of how close the data are to the fitted regression line; it is also known as the coefficient of determination or the coefficient of multiple determinations for multiple regressions.[67]

3 | CONCLUSIONS

Novel compounds, 6-(3-halogenated phenyl-2-propen-1-one)-2(3*H*)-benzoxazolones (**2a**–**n**), were reported in this study for the first time (except compound **2a**) with their cytotoxicities and inhibitory effects on hCA I and II isoenzymes. According to the cytotoxicity results, compound **2m**, 6-[3-(3-bromophenyl)-2-propenoyl]-2(3*H*)-benzoxazolone, was the most expressive compound of the study with a remarkable PSE value (158) for further studies. However, according to the K_i values of compounds **2a**–**n**, obtained by CA inhibition studies, compounds **2a**, **2b**, **2e**–**g**, **2l**, and **2n** can be considered as leading compounds of the series to develop new hCA I inhibitors due to similar K_i values to AZA, whereas the compounds were not found effective to develop new hCA II inhibitors with their current structure according to their K_i values compared with AZA.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

The NMR spectra (^{1}H NMR and ^{13}C NMR) were recorded on a Bruker AVANCE III 400 MHz (Bruker, Karlsruhe, Germany) spectrometer [400 MHz (^{1}H) and 100 MHz (^{13}C)]. Chemical shifts were given as δ values in parts per million. The internal standard was tetramethylsilane and *J* values were expressed in hertz. Mass spectra of the compounds were taken using a liquid chromatography ion trap–time-of-flight tandem mass spectrometer (Shimadzu, Kyoto, Japan), equipped with an electrospray ionization (ESI) source, operating in both positive and negative ionization mode. Shimadzu's LCMS Solution software was used for data analysis. Melting points were determined using an Electrothermal 9100 instrument (IA9100; Bibby Scientific Limited, Staffordshire, UK), and were uncorrected. Reactions were monitored by thin-layer chromatography (TLC) using silica gel 60 HF254 (Merck KGaA).

The InChI keys of the investigated compounds, together with some biological activity data, are provided as Supporting Information.

4.1.2 | Synthesis of 6-acetyl-2(3*H*)-benzoxazolone (1) (Scheme 1)

Dimethylformamide (13 ml, 172 mmol) was slowly added into aluminum chloride (80 g, 600 mmol), and the mixture was heated at 45°C for 5 min. 2(3*H*)-Benzoxazolone (8.1 g, 60 mmol) and acetyl chloride (6.4 ml, 90 mmol) were added into this solution (Scheme 1). Then the reaction mixture was heated at 80°C for 3 hr and poured on ice water (200 ml) with HCl (30 ml, 37%). The precipitated crude product was filtered and air-dried, and crystallized from ethanol.[3] (Yield: 77%, m.p: 231–234°C, brown crystals.)[3,58]

4.1.3 | Synthesis of chalcone compounds 2a, 2b, 2d–g, and 2j–l (Scheme 1)

An aqueous solution of KOH (10%, 5 ml) was added to the mixture of 6-acetyl-2(3*H*)-benzoxazolone (ketone, 5.6 mmol) and a suitable aldehyde [benzaldehyde (**2a**), 2,4-dichlorobenzaldehyde (**2b**), 2,6-dichlorobenzaldehyde (**2d**), 2-fluorobenzaldehyde (**2e**), 3-fluorobenzaldehyde (**2f**), 2,4-difluorobenzaldehyde (**2g**), 2,6-difluorobenzaldehyde (**2j**), 2,3-difluorobenzaldehyde (**2k**), 2-bromobenzaldehyde (**2l**)] in ethanol (5 ml) in a 1:1 mol ratio (Scheme 1). The mixture was stirred at room temperature for 24 hr. Reactions were followed by TLC. After the reaction finished, the content of the reaction flask was poured on ice water (100 ml) and neutralized by HCl (37%). The precipitated solid product was collected by filtration and washed with cold water.[3,58] The crude compounds were purified by crystallization from a suitable solvent (acetonitrile/ethanol for compounds **2a**, **2b**, **2e**, **2g**, **2j**, **2l**, acetonitrile/methanol for compounds **2d** and **2f**, and ethyl acetate/methanol for compound **2k**).

*6-(3-Phenyl-2-propenoyl)-2(3*H*)-benzoxazolone (**2a**)*

Yield 77%. Mp: 230–232°C. ^{1}H NMR (dimethyl sulfoxide [DMSO]-d_6) δ (ppm) 12.03 (bs, 1H, NH), 8.11 (d, 1H, arom. H, J = 1.2 Hz), 8.07 (dd, 1H, arom. H, J_1 = 8.2 Hz, J_2 = 1.6 Hz), 7.99 (d, 1H, Ar–CH=, J = 15.5 Hz), 7.89–7.91 (m, 2H, arom. H), 7.74 (d, 1H, =CHCO, J = 15.5 Hz), 7.47 (d, 1H, arom. H, J = 1.2 Hz), 7.45 (d, 2H, arom. H, J = 2.5 Hz), 7.24 (d, 1H, arom. H, J = 8.2 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm) 187.8, 154.9, 144.2, 143.9, 135.5, 135.2, 132.3, 131.0, 129.38, 129.37, 129.36, 126.2, 122.3, 110.0. HRMS (ESI–MS) *m/z* calculated $[M+H]^+$ 266.0812; measured $[M+H]^+$ 266.0803.

*6-[3-(2,4-Dichlorophenyl)-2-propenoyl]-2(3*H*)-benzoxazolone (**2b**)*

Yield 86%. Mp: 248–250°C. ^{1}H NMR (DMSO-d_6) δ (ppm) 8.27 (d, 1H, arom. H, J = 8.5 Hz), 8.11 (d, 1H, arom. H, J = 1.4 Hz), 8.05 (d, 1H, Ar–CH=, J = 15.5 Hz), 8.04 (dd, 1H, arom. H, J_1 = 8.2 Hz, J_2 = 1.4 Hz), 7.94 (d, 1H, =CHCO, J = 15.5 Hz), 7.73 (d, 1H, arom. H, J = 2.0 Hz), 7.54 (dd, 1H, J_1 = 8.5 Hz, J_2 = 2.0 Hz), 7.23 (d, 1H, J = 8.2 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm) 187.4, 154.9, 143.9, 137.4, 136.0, 135.8, 135.6, 131.9, 131.8, 130.3, 129.9, 128.4, 126.4, 125.4, 110.1, 110.0. HRMS (ESI–MS) *m/z* calculated $[M+H]^+$ 334.0032; measured $[M+H]^+$ 334.0032.

*6-[3-(2,6-Dichlorophenyl)-2-propenoyl]-2(3*H*)-benzoxazolone (**2d**)*

Yield 90%. Mp: 235–236°C. ^{1}H NMR (DMSO-d_6) δ (ppm) 7.87 (dd, 1H, arom. H, J_1 = 8.2 Hz, J_2 = 1.7 Hz), 7.82 (d, 1H, Ar–CH=, J = 16.0 Hz), 7.78 (d, 1H, arom. H, J = 1.7 Hz), 7.65 (d, 1H, =CHCO, J = 16.0 Hz), 7.59 (d, 2H, arom. H, J = 7.6 Hz), 7.42 (t, 1H, arom. H, J = 7.6 Hz), 7.09 (d, 1H, arom. H, J = 8.2 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm) 187.3, 158.9, 145.4, 142.9, 136.2, 134.5, 132.9, 131.3, 131.2, 129.53, 129.53, 126.1, 110.3, 108.1. HRMS (ESI–MS) *m/z* calculated $[M+H]^+$ 334.0032; measured $[M+H]^+$ 334.0030.

*6-[3-(2-Fluorophenyl)-2-propenoyl]-2(3H)-benzoxazolone (**2e**)*

Yield 47%. Mp: 218–219°C. ^{1}H NMR (DMSO-d_6) δ (ppm) 12.11 (bs, 1H, NH), 8.18–8.10 (m, 2H, arom. H), 8.06 (d, 1H, Ar–CH═, J = 15.6 Hz), 8.05 (d, 1H, arom. H, J = 8.1 Hz), 7.83 (d, 1H, ═CHCO, J = 15.6 Hz), 7.55–7.48 (m, 1H, arom. H), 7.34 (d, 1H, arom. H, J = 1.4 Hz), 7.29 (d, 1H, arom. H, J = 8.2 Hz), 7.24 (d, 1H, arom. H, J = 8.1 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm) 187.6, 163.0, 159.7, 154.9, 143.9, 135.6, 135.2, 133.1, 132.0, 129.5, 126.3, 125.4, 124.3, 122.8, 116.5, 110.0. HRMS (ESI–MS) m/z calculated $[M+H]^+$ 284.0717; measured $[M+H]^+$ 284.0714.

*6-[3-(3-Fluorophenyl)-2-propenoyl]-2(3H)-benzoxazolone (**2f**)*

Yield 47%. Mp: 229–230°C. ^{1}H NMR (DMSO-d_6) δ (ppm) 8.13 (d, 1H, arom. H, J = 1.5 Hz), 8.08 (dd, 1H, arom. H, J_1 = 8.5 Hz, J_2 = 1.5 Hz), 8.05 (d, 1H, Ar–CH═, J = 15.5 Hz), 7.88 (d, 1H, arom. H, J = 8.5 Hz), 7.73 (d, 1H, ═CHCO, J = 15.5 Hz), 7.70 (d, 1H, arom. H, J = 7.7 Hz), 7.53–7.46 (m, 1H, arom. H), 7.30 (dd, 1H, arom. H, J_1 = 8.3 Hz, J_2 = 2.3 Hz), 7.24 (d, 1H, arom. H, J = 8.3 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm) 187.7, 164.6, 161.4, 154.9, 143.9, 142.7, 137.8, 135.6, 132.1, 131.3, 126.2, 123.7, 117.7, 115.2, 114.9, 110.0. HRMS (ESI–MS) m/z calculated $[M+H]^+$ 284.0717; measured $[M+H]^+$ 284.0716.

*6-[3-(2,4-Difluorophenyl)-2-propenoyl]-2(3H)-benzoxazolone (**2g**)*

Yield 89%. Mp: 250–251°C. ^{1}H NMR (DMSO-d_6) δ (ppm) 8.28–8.20 (m, 1H, arom. H), 8.06 (d, 1H, arom. H, J = 4.2 Hz), 8.04 (d, 1H arom. H, J = 7.0 Hz), 8.00 (d, 1H, Ar–CH═, J = 15.8 Hz), 7.70 (d, 1H, ═CHCO, J = 15.8 Hz), 7.42–7.34 (m, 1H, arom. H), 7.27 (d, 1H, arom. H, J = 7.0 Hz), 7.23 (d, 1H, arom. H, J = 8.2 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm) 187.5, 155.1, 144.0, 136.0, 134.2, 131.9, 131.1, 131.0, 126.3, 124.0, 119.8, 119.6, 113.1, 112.8, 110.1, 109.9. HRMS (ESI–MS) m/z calculated $[M+H]^+$ 302.0623; measured $[M+H]^+$ 302.0612.

*6-[3-(2,6-Difluorophenyl)-2-propenoyl]-2(3H)-benzoxazolone (**2j**)*

Yield 53%. Mp: 252–254°C. ^{1}H NMR (DMSO-d_6) δ (ppm) 12.13 (bs, 1H, NH), 7.94 (d, 2H, arom. H, J = 9.8 Hz), 7.86 (d, 1H, Ar–CH═, J = 15.9 Hz), 7.67 (d, 1H, ═CHCO, J = 15.9 Hz), 7.60–7.50 (m, 1H, arom. H), 7.27 (d, 1H, arom. H, J = 8.3 Hz), 7.25 (s, 1H, arom. H), 7.24 (d, 1H, arom. H, J = 8.3 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm) 187.8, 163.2, 159.8, 154.8, 144.0, 135.8, 133.0, 131.8, 129.4, 127.7, 126.3, 113.0, 110.1, 109.7. HRMS (ESI–MS) m/z calculated $[M+H]^+$ 302.0623; measured $[M+H]^+$ 302.0613.

*6-[3-(2,3-Difluorophenyl)-2-propenoyl]-2(3H)-benzoxazolone (**2k**)*

Yield 39%. Mp: 245–247°C. ^{1}H NMR (DMSO-d_6) δ (ppm) 8.08 (s, 1H, arom. H), 8.05 (d, 1H, Ar–CH═, J = 15.4 Hz), 8.04 (d, 1H, arom. H, J = 8.4 Hz), 7.98–7.94 (m, 1H, arom. H), 7.78 (d, 1H, ═CHCO, J = 15.4 Hz), 7.58–7.48 (m, 1H, arom. H), 7.35–7.28 (m, 1H, arom. H), 7.23 (d, 1H, arom. H, J = 8.4 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm) 187.4, 154.9, 151.9, 148.9, 147.5, 143.9, 135.8, 133.9, 131.8, 126.4, 125.6, 125.2, 124.6, 119.7, 119.4, 110.1. HRMS (ESI–MS) m/z calculated $[M+H]^+$ 302.0623; measured $[M+H]^+$ 302.0616.

*6-[3-(2-Bromophenyl)-2-propenoyl]-2(3H)-benzoxazolone (**2l**)*

Yield 55%. Mp: 239–240°C. ^{1}H NMR (DMSO-d_6) δ (ppm) 12.12 (bs, 1H, NH), 8.23 (dd, 1H, arom. H, J_1 = 8.0 Hz, J_2 = 1.4 Hz), 8.12 (d, 1H, arom. H, J = 1.4 Hz), 8.08 (d, 1H, arom. H, J = 8.2 Hz), 8.00 (s, 2H, α,β-protons),[66] 7.74 (d, 1H, arom. H, J = 8.0 Hz), 7.52–7.47 (m, 1H), 7.42–7.36 (m, 1H), 7.25 (d, 1H, arom. H, J = 8.2 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm) 187.6, 154.9, 143.9, 141.5, 135.7, 135.4, 133.8, 132.6, 131.9, 129.3, 128.7, 126.4, 125.9, 125.1, 110.1, 110.08. HRMS (ESI–MS) m/z calculated $[M+H]^+$ 343.9917; measured $[M+H]^+$ 343.9922.

4.1.4 | Synthesis of compounds 2c, 2h, 2m, and 2n (Scheme 1)

An aqueous solution of KOH (10%, 2 ml) was added to the mixture of 6-acetyl-2(3*H*)-benzoxazolone (ketone, 5.6 mmol) and a suitable benzaldehyde [3,4-dichlorobenzaldehyde (**2c**), 2,5-difluorobenzaldehyde (**2h**), 3-bromobenzaldehyde (**2m**), and 4-bromobenzaldehyde (**2n**)] in a 1:1 mol ratio in ethanol (2 ml). Then the reaction mixture was irradiated by microwave [(50–80°C, 25–60 W) for 25 min (compounds **2c**, **2h**, and **2n**) and for 30 min (compound **2 m**)]. Reactions were followed by TLC. After the reaction finished, the content of the reaction flask was poured on ice water (100 ml) and neutralized by HCl (37%). The solid precipitated was filtered and washed with water.[3] The crude compounds were purified by crystallization from a suitable solvent [ethyl acetate/methanol (**2c**), acetonitrile/ethanol (**2h**), chloroform/methanol (**2m**), and methanol/dimethylformamide (**2n**)].

*6-[3-(3,4-Dichlorophenyl)-2-propenoyl]-2(3H)-benzoxazolone (**2c**)*

Yield 58%. Mp: 299–301°C. ^{1}H NMR (DMSO-d_6) δ (ppm) 8.26 (d, 1H, arom. H, J = 1.7 Hz), 8.07 (d, 1H, Ar–CH═, J = 15.4 Hz), 8.06 (s, 1H, arom. H), 8.05 (d, 1H, ═CHCO, J = 15.4 Hz), 7.84 (dd, 1H, arom. H, J_1 = 8.1 Hz, J_2 = 1.7 Hz), 7.68 (s, 1H, arom. H, J = 8.1 Hz), 7.67 (d, 1H, arom. H, J = 8.4 Hz), 7.20 (d, 1H, arom. H, J = 8.4 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm) 187.7, 155.4, 144.2, 141.5, 136.3, 133.31, 133.30, 132.5, 132.1, 130.76, 130.75, 129.9, 126.5, 124.4, 110.24, 100.23. HRMS (ESI–MS) m/z calculated $[M+H]^+$ 334.0032; measured $[M+H]^+$ 334.0025.

*6-[3-(2,5-Difluororophenyl)-2-propenoyl]-2(3H)-benzoxazolone (**2h**)*

Yield 63%. Mp: 291–293°C. ^{1}H NMR (DMSO-d_6) δ (ppm) 8.11–8.06 (m, 1H, arom. H), 8.05 (d, 1H, Ar–CH═, J = 15.5 Hz), 7.93 (dd, 1H, arom. H, J_1 = 8.2 Hz, J_2 = 1.6 Hz), 7.84 (d, 1H, arom. H, J = 1.6 Hz), 7.69 (d, 1H, ═CHCO, J = 15.5 Hz), 7.35–7.29 (m, 2H, arom. H), 7.02 (d, 1H, arom. H, J = 8.4 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm) 187.2, 160.8, 160.3, 158.9, 158.0, 156.4, 146.1, 145.6, 132.8, 129.3, 126.2, 119.3, 118.3, 115.1, 110.6, 108.0. HRMS (ESI–MS) m/z calculated $[M+H]^+$ 302.0623; measured $[M+H]^+$ 302.0610.

*6-[3-(3-Bromophenyl)-2-propenoyl]-2(3H)-benzoxazolone (**2m**)*

Yield 37%. Mp: 265–267°C. ^{1}H NMR (DMSO-d_6) δ (ppm) 12.10 (bs, 1H, NH), 8.20 (s, 1H, arom. H), 8.1 (s, 1H, arom. H), 8.08–8.07 (m, 1H,

arom. H), 8.05 (d, 1H, Ar–CH═, J = 15.6 Hz), 7.85 (d, 1H, arom. H, J = 7.7 Hz), 7.68 (d, 1H, ═CHCO, J = 15.6 Hz), 7.62 (d, 1H, arom. H, J = 8.1 Hz), 7.42–7.38 (m, 1H, arom. H), 7.22 (d, 1H, arom. H, J = 8.1 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm) 187.9, 155.2, 144.2, 142.7, 137.9, 135.9, 133.7, 132.3, 131.6, 131.4, 129.1, 126.6, 123.9, 123.1, 110.4, 110.3. HRMS (ESI–MS) m/z calculated $[M+H]^+$ 343.9917; measured $[M+H]^+$ 343.9900.

*6-[3-(4-Bromophenyl)-2-propenoyl]-2(3H)-benzoxazolone (**2n**)*

Yield 81%. Mp: 288–290°C. ^{1}H NMR (DMSO-d_6) δ (ppm) 8.07 (s, 1H, arom. H), 8.04 (d, 1H, arom. H, J = 8.2 Hz), 8.00 (d, 1H, Ar–CH═, J = 16.1 Hz), 7.85 (d, 2H, arom. H, J = 8.4 Hz), 7.68 (d, 1H, ═CHCO, J = 16.1 Hz), 7.64 (d, 2H, arom. H, J = 8.4 Hz), 7.20 (d, 1H, arom. H, J = 8.2 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm) 187.9, 155.5, 144.3, 143.0, 136.4, 134.8, 132.5, 132.2, 131.5, 126.5, 124.6, 123.3, 110.3, 110.1. HRMS (ESI–MS) m/z calculated $[M+H]^+$ 343.9917; measured $[M+H]^+$ 343.9900.

4.2 | Biological activity

4.2.1 | Cytotoxicity test

Cytotoxicity tests were realized as described in our previous studies.[3,28,39–41,51,67–69]

4.2.2 | CA inhibition

CA inhibition assays were done as described in our previous studies.[3,44,64,65,67,68,70,71]

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

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