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FULL PAPER

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Synthesis of new 7-amino-3,4-dihydroquinolin-2(1*H*)-one-peptide derivatives and their carbonic anhydrase enzyme inhibition, antioxidant, and cytotoxic activities

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Abstract

Six new monopeptides, seven new dipeptides, and two deprotected monopeptide dihydroquinolinone conjugates were prepared by the benzothiazole-mediated method and their structures were confirmed by nuclear magnetic resonance, mass, infrared spectroscopy, and elemental analysis methods. The human carbonic anhydrase (hCA) I and hCA II enzyme inhibition activities of the compounds were determined using the stopped-flow instrument. The synthesized peptide–dihydroquinolinone conjugates **2**, **3**, **6**, **10**, **13**, and **15** showed inhibition against the hCA II enzyme in the range of 15.7–65.7 μM. However, none of the compounds showed inhibition of hCA I at a concentration of 100 μM. The antioxidant activities of the compounds were also examined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method at concentrations of 12.5–125 μg/ml, but when compared with the standard antioxidant compounds α-tocopherol and butylated hydroxyanisole (BHA), weak antioxidant activities were detected. The cytotoxic effects of four compounds against the A549 and BEAS-2B cell lines were also investigated. Among the compounds studied, compound **7** was found to be most effective, with the IC_{50} values on the A549 cells for 48 and 72 h being 26.87 and 9.979 μg/ml, respectively, and the IC_{50} values on the BEAS-2B cells being >100 μg/ml. None of the tested compounds showed antimicrobial activity in the concentration range (800–1.56 μg/ml) studied.

KEYWORDS

antioxidant, carbonic anhydrase, cytotoxicity, peptide, peptide–dihydroquinolin-2-one conjugates, quinolones

1 | INTRODUCTION

The first drug containing quinoline structure and possibly derived from natural sources, quinine was isolated from cinchona tree bark in 1820.[1] The effect of chloroquine, a quinoline derivative, against the COVID-19 virus has recently led to an increase in interest in the synthesis of quinoline derivatives. Possible impacts of quinoline derivatives such as chloroquine and hydroxychloroquine on the COVID-19 virus are reported to act by affecting the viral receptor S protein to which the virus binds.[2] Quinoline derivatives have a wide variety of biological activities such as antimalarial,[3–5] antifungal,[6–9] antitumor,[9–13] antitubercular,[13,14] HIV replication inhibitor,[15] antibacterial,[16,17] antiparasitic,[18] antiviral,[19] and carbonic anhydrase enzyme inhibition[20–22] properties according to the substituents they contain. For example, the antibiotic properties of quinolines were first determined in the 1960s. Although they were initially active in a narrow spectrum, they were transformed into broad-spectrum antibiotic-effective compounds through structural modifications.[23] Heterocyclic compounds are generally compounds with various physiological properties and are often found in the structure of many natural compounds and drugs. For a long time, we have been working on the synthesis and investigation of the biological properties of compounds containing benzimidazole,[24,25] benzothiazole,[26] furan,[27] coumarin,[28] quinolone,[28] quinine,[29] morpholine,[30] piperidine,[30] pyridine,[31] and isatine.[32] Carbonic anhydrase is an enzyme found in red blood cells, gastric mucosa, pancreatic cells, and renal tubules that catalyzes the interconversion of carbon dioxide (CO_2) and carbonic acid (H_2CO_3). Carbonic anhydrase plays an important role in respiration by affecting the transport of CO_2 in the blood.[33] Therefore, the synthesis of compounds that inhibit the carbonic anhydrase enzyme is an important research topic. The zinc ion, which is in the active center of carbonic anhydrase enzymes, plays a significant role in the catalytic reaction. Therefore, the preparation of new compounds with properties that can bind with the zinc ion in the active center and that has excellent inhibition-effectiveness is an extremely important research area. The existence of a possible relationship between the carbonic anhydrase enzyme and cancer has added extra importance to these studies. Related to the most commonly used artificial antioxidant substances BHT (2,6-di-tert-butyl-4-methylphenol), BHA (2-tert-butyl-4-methoxyphenol) and propyl gallate (propyl 3,4,5-trihydroxybenzoate) studies have increased the suspicions of carcinogens about these compounds. The emergence of new and biocompatible antioxidant substances has led to an increase in the search. Within the framework of these searches, it was aimed to investigate their antioxidant properties by preparing compounds containing a series of new peptide–dihydroquinolinone structures, inspired by peptides, which are natural antioxidants.[34–36] In this study, we aimed to prepare mono- and dipeptide derivatives containing dihydroquinolin-2-one and to investigate their antioxidant capacities, antimicrobial properties, and inhibition properties against human carbonic anhydrase I (hCA I) and II (hCA II) enzymes. The cytotoxic activities of some mono- and dipeptide–dihydroquinolin-2-one conjugates were also tested.

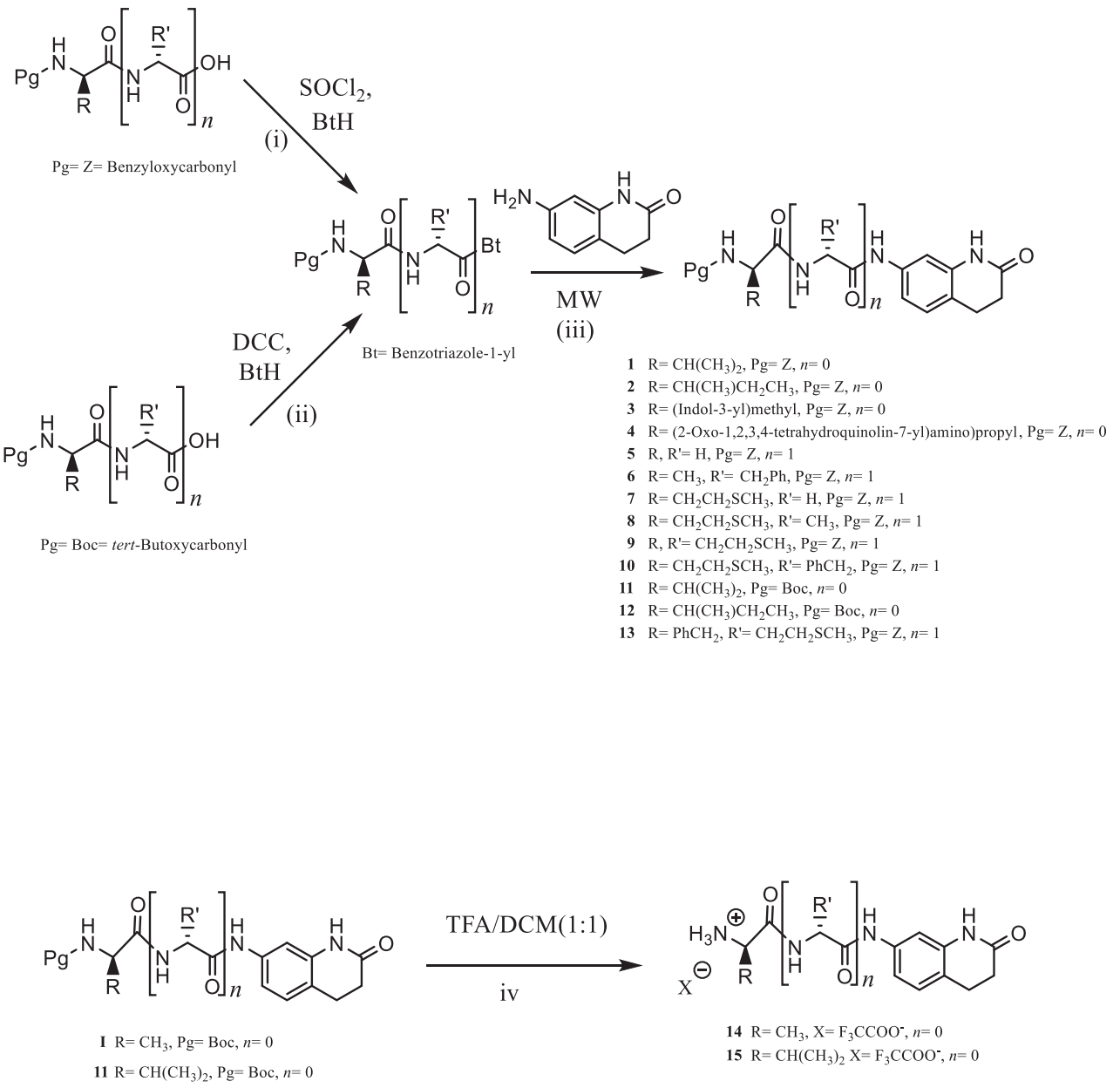
2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The mono- and dipeptide–quinolone derivatives reported in this study were prepared in good yields from the reaction of 7-amino-3,4-dihydroquinolin-2(1*H*)-one with benzotriazole-activated N-protected peptide under microwave heating conditions. Removal of the Boc protecting group for compounds **I**,[21] **II**,[21] and **11** was done in a mixture of trifluoroacetic acid (TFA) and dichloromethane (1:1) with stirring at room temperature for 2 h according to the literature method.[37]

The synthesis scheme of the N-protected mono- and dipeptide–quinolin-2-one derivatives is summarized in Scheme 1.

The structures of the newly synthesized compounds were elucidated using spectroscopic methods and elemental analysis measurements. In the nuclear magnetic resonance (NMR) spectra of the compounds, the carbamate NH peak in the dihydroquinolinone ring was generally shifted to the downfield in the range of 0.24–0.34 ppm compared with the carbamate NH peak in the starting compound (9.84 ppm). In compound **3** containing the amino acid L-tryptophan, the NH resonance at position 1 of the indole was observed at 10.58 ppm lower than the carbamate NH peaks. The observation of carbamate NH resonances at 10.20 and 10.18 ppm and amide NH resonances at 10.15 and 9.94 ppm in compound **4** containing L-glutamic acid with two dihydroquinolinone rings confirms the structure. The amino resonance observed at 4.97 ppm in the starting compound 7-amino-3,4-dihydroquinolin-2(1*H*)-one was observed in peptide–dihydroquinoline conjugates at around 10 ppm slightly lower than in carbamate NH resonances. In peptide–dihydroquinoline conjugates (**1**–**13**), the shift of this peak to the downfield by about 5 ppm confirms that the amino group is acylated. While the aliphatic methylene peaks in the quinoline ring of the dihydroquinolinone derivatives resonated in the range of 2.82–2.85 ppm adjacent to the carbonyl group, the other methylene resonated in the range of 2.44–2.47 ppm. The aliphatic methylene carbons in the quinoline ring of the dihydroquinolinone derivatives resonated between 31.1–34.1 ppm and 24.1–23.1 ppm, respectively. The singlet peaks of the *tert*-butoxy group of around 1.3 ppm disappeared in compounds **14** and **15** in which the Boc protecting group was removed. Similarly, the quaternary carbon peak at around 78 ppm and the carbamate carbonyl peak at around 156 ppm seen in the ^{13}C NMR spectra of compounds **I** and **11** disappeared in compounds **14** and **15**. After removing the Boc protecting group, the free amino group formed was converted into trifluoroacetate salt by taking a proton from TFA. This was confirmed by a broad singlet peak corresponding to three protons at 8.3 ppm in the proton NMR spectra of compounds **14** and **15** and their element analysis results.



SCHEME 1 Synthesis pathways of the new dihydroquinolinone–peptide conjugates. Conditions and reagents: (i) room temperature (rt), 2 h in tetrahydrofuran (THF); (ii) rt, 12 h in H_2CCl_2; (iii) 70°C, 1 h in THF; and (iv) rt, 3 h

In compounds **14** and **15**, carbon peaks belonging to the trifluoroacetate group were seen as quartet in the carbon-13 spectra at around 160 and 116 ppm. All other protons and carbon peaks were present in the spectra, confirming the proposed structures.

2.2 | Biology

2.2.1 | Carbonic anhydrase inhibition

Among the biological activities, human carbonic anhydrase (hCA; EC 4.2.1.1) inhibition has been the subject of several investigations since the discovery of the biological importance of this enzyme in several living organisms.[38] As many heterocyclic compounds exhibit CA inhibitor properties,[39,40] we synthesized novel type mono- and dipeptide–dihydroquinolinone conjugates to explore their possible carbonic anhydrase enzyme inhibition capacities against human carbonic anhydrase hCA I, II, IX, and XII. To investigate the inhibitory capacity of all novel mono- and dipeptide–dihydroquinolin-2-one conjugates (**1**–**15**) prepared, two human CA isoforms (hCA I and hCA) were evaluated using a stopped-flow CO_2 hydrase assay. The inhibition results of the compounds are presented in Table 1 together with the result of acetazolamide (AAZ) used as the standard inhibitor. When the results in Table 1 are analyzed, the following structure–activity relationships can be obtained. While none of the newly synthesized peptide–dihydroquinolinone conjugates showed

TABLE 1 Inhibition data of hCA I and hCA II, with compounds **1–15** and the standard sulfonamide inhibitor acetazolamide (AAZ) by a stopped-flow CO_2 hydrase assay

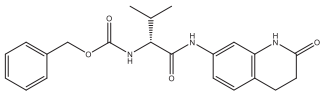
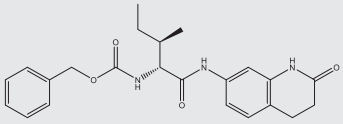
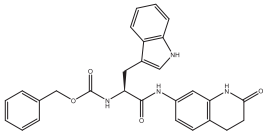
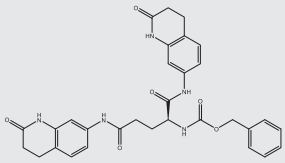
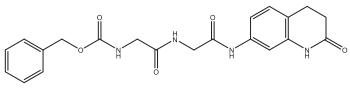
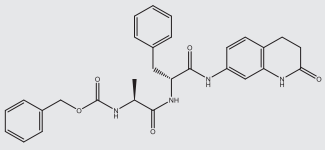
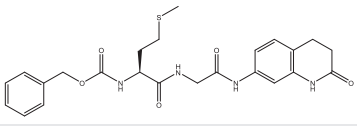
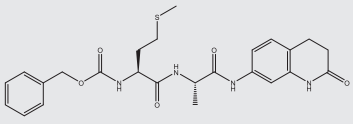
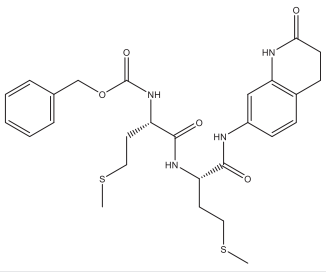
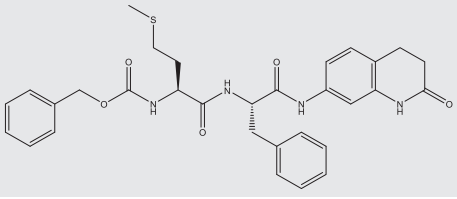
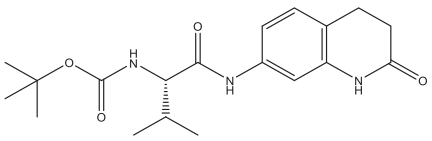
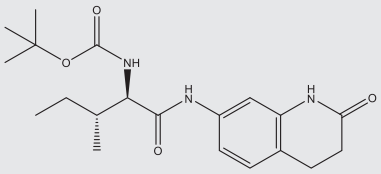
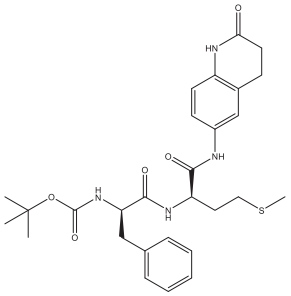
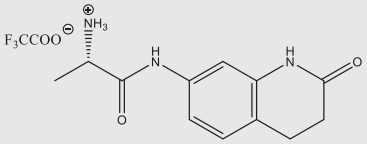
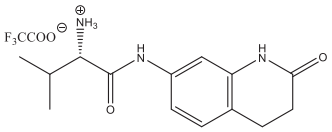
Compound no.		K_i (μM)[a] hCA I	hCA II
1		>100	>100
2		>100	60.8
3		>100	53.9
4		>100	>100
5		>100	>100
6		>100	32.5
7		>100	>100
8		>100	>100
9		>100	24.4
10		>100	15.7

TABLE 1 (Continued)

Compound no.		K_i (μM)[a] hCA I	hCA II
11		>100	>100
12		>100	>100
13		>100	23.8
14		>100	>100
15		>100	65.7
AAZ		0.25	0.012

[a]Mean from three different assays, by a stopped-flow technique (errors were in the range of ±5%–10% of the reported values).

an inhibition effect against the hCA I enzyme at concentrations of 100 μM, compounds **2**, **3**, **6**, **9**, **10**, **13**, and **15** showed an inhibition effect against the hCA II enzyme with K_i values in the range of 15.7–65.7 μM. According to the hCA II results, it is understood that the amino acids methionine, phenylalanine, tryptophan, and isoleucine contribute to the hCA II enzyme inhibition. When the structures of the compounds (**2**, **3**, **6**, **9**, **10**, **13**, and **15**) showing inhibitory effects on hCA II were examined, dipeptide–dihydroquinolin-2-one derivatives (**6**, **9**, **10**, and **13**) were found to be more effective than monopeptide–dihydroquinolin-2-one derivatives (**2**, **3**, and **15**). When the Ca I and CA II enzyme inhibition results of the deprotected compounds (14 and 15) were compared with their initial states (I[] and 11), no change was observed for CA I enzyme, whereas an increase in CA II enzyme inhibition of compound 15 was observed. When the structures of the compounds showing the best CA II inhibition are examined, it is seen that the most effective compounds with K_i 15.7 and 23.8 μM (compounds **10** and **13**, respectively) are dipeptide–dihydroquinolin-2-one conjugates containing the amino acids methionine and phenylalanine. The type of protecting group in these compounds also did not make much of a difference to their inhibition properties.

2.2.2 | Antioxidant testing

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant activity of the compounds was determined using the widely used DPPH method.[41] The antioxidant activity results of mono- and dipeptide–dihydroquinolin-2-one compounds are shown in Table 2. From Table 2, it is clear that the newly synthesized mono- and dipeptide–dihydroquinolin-2-one conjugates do not show significant antioxidant activity. Although peptide–dihydroquinolinone conjugates generally do not show significant antioxidant activity, those that show antioxidant

TABLE 2 Antioxidant activities of the synthesized mono- and dipeptide–quinolinone conjugates

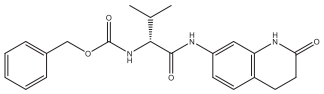
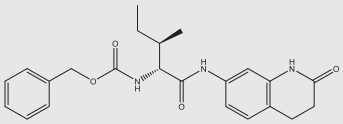
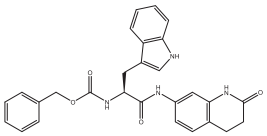
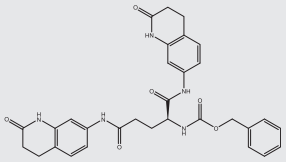
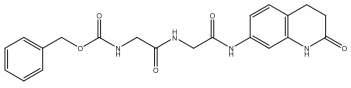
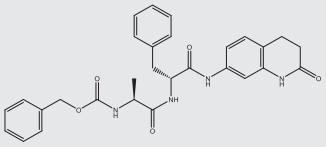
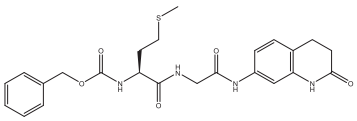
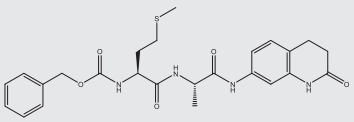
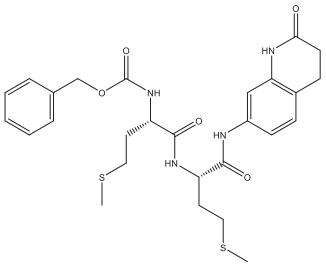
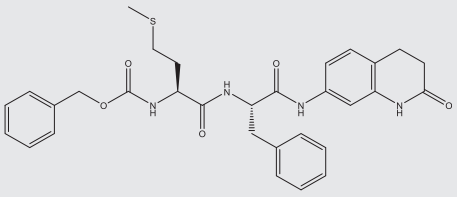
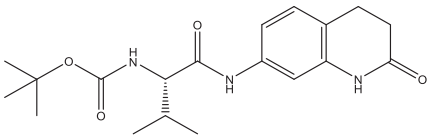
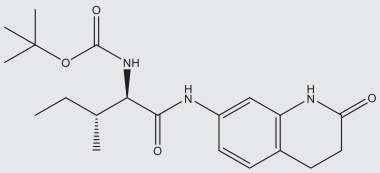
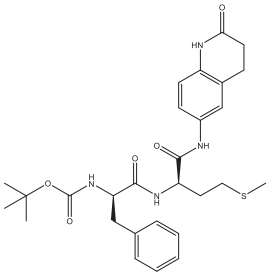
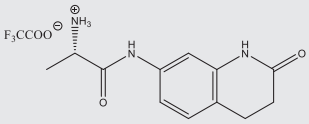
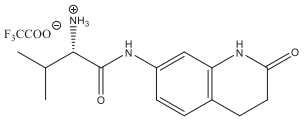
Compound no.		DPPH free radical scavenging activity (%)				
		12.5 μg/ml	25 μg/ml	37.5 μg/ml	62.5 μg/ml	125 μg/ml
1		2.52	1.89	1.93	3.31	3.38
2		2.25	3.02	3.65	3.72	4.86
3		2.20	1.89	1.89	2.83	4.72
4		3.50	4.93	8.42	11.23	19.45
5		2.93	3.34	4.81	7.43	10.85
6		3.50	3.92	4.64	9.22	13.63
7		4.33	4.62	7.84	11.91	16.74
8		4.83	5.25	7.54	11.61	20.62
9		5.23	8.74	11.25	17.91	28.40
10		3.85	4.74	5.24	8.92	12.43

TABLE 2 (Continued)

Compound no.		DPPH free radical scavenging activity (%) 12.5 µg/ml	25 µg/ml	37.5 µg/ml	62.5 µg/ml	125 µg/ml
11		2.81	2.93	2.94	3.25	3.83
12		2.74	3.13	3.04	3.42	3.85
13		2.20	1.57	1.57	1.57	1.89
14		9.75	10.13	11.47	13.77	18.41
15		8.80	10.01	12.19	13.56	17.15
BHA		61.1	63.0	67.5	71.0	72.4
α-Tocopherol		62.9	63.4	68.4	72.8	74.0

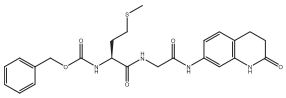
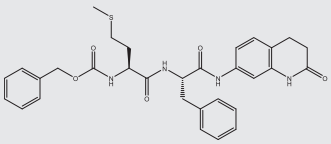
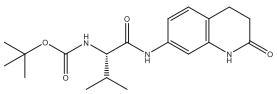
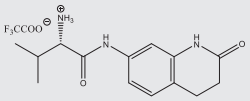
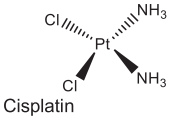
Abbreviations: BHA, butylated hydroxyanisole; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

activity at a concentration of 125 µg/ml by about one-third compared with standard antioxidants are compounds **4**, **8**, **9**, **14**, and **15.** When the structures of the compounds showing relative antioxidant activity are examined, it can be said that L-aspartic acid and methionine amino acids may be responsible for antioxidant activity.

2.2.3 | Cytotoxic activity

The cytotoxicity of some newly synthesized peptide–dihydroquinolinone conjugates on A549 (lung cancer cells) and BEAS-2B (healthy human lung bronchial epithelium cells) cells was determined, and the IC_{50} values of the compounds are presented in Table 3 with the standard drug cisplatin. The compounds tested were generally more effective on the A549 than on BEAS-2B cells. As shown in Table 3, compounds **7**, **11**, and **15** had more cytotoxic effects on A549 cells compared with BEAS-2B cells. Compound **7** showed more cytotoxicity on A549 cells after 48 and 72 h incubation. While the IC_{50} values of compound **7** on A549 cells for 48 and 72 h were 26.87 and 9.98 µg/ml, respectively, IC_{50} values on BEAS-2B cells were >100 µg/ml. Moreover, the IC_{50} values of compound **15** on A549 and BEAS-2B cells for 72 h were found to be 13.94 and 60.95 µg/ml, respectively. When compounds **11** and **15** are compared, it can be seen that protection group removal did not have a positive effect on the cytotoxic effect at 24 and 48 h incubation times, but increased cytotoxicity at 72 h. On the contrary, when compounds **11** and **15** were compared in terms of their effects on healthy cells, it was observed that removal of the protecting group did not contribute positively to cytotoxicity. The tested compounds (**7**, **10**, **11**, and **15**) showed better cytotoxicity against A549 cell lines than the standard drug cisplatin during the first 24-h incubation period and less cytotoxicity in healthy cells (BEAS-2B) at 48 and 72 h incubation times.

TABLE 3 IC_{50} (μg/ml) values of some peptide–dihydroquinolinone conjugates on A549 and BEAS-2B cells

		A549			BEAS-2B		
		Time (h)					
Compound no.		24	48	72	24	48	72
7		1.74	26.87	9.98	1.93	>100	>100
10		1.90	>100	>100	1.96	>100	>100
11		1.60	29.89	>100	1.75	>100	>100
15		1.80	40.96	13.94	1.94	48.52	60.95
Cisplatin	 Cisplatin	4.44	2.72	2.56	3.17	2.60	2.53

2.2.4 | Antimicrobial activity

The antimicrobial activity of 16 new peptide–dihydroquinolinone conjugates against bacteria and yeasts was tested using microdilution and disc diffusion methods. However, these newly synthesized compounds **1**–**15** had no antimicrobial activity against any of the microorganisms used according to these methods.

3 | CONCLUSIONS

In this study, 15 new peptide–dihydroquinolinone conjugates were synthesized under benzotriazole-mediated mild reaction conditions and their structures were determined using spectrometric and analytical methods. Among them, compounds **2**, **3**, **6**, **10**, **13**, and **15** showed an inhibition effect against hCA II in the range of 15.7–65.7 μM. None of the compounds showed inhibition against the hCA I enzyme at a concentration of 100 μM. The antioxidant properties of the synthesized compounds were examined using the DPPH method in the concentration range of 12.5–125 μg/ml, but the compounds were found to have weak antioxidant activity. Compound **7**, the methionine–dihydroquinolinone derivative with the Cbz protecting group, was found to be most effective among the two benzyloxycarbonyls, a *tert*-butoxycarbonyl protecting group and deprotected peptide–dihydroquinolinone conjugates tested for cytotoxic activity. The IC_{50} values of compound **7** on A549 cells for 48 and 72 h were 26.87 and 9.979 μg/ml, respectively, and IC_{50} values on BEAS-2B cells were >100 μg/ml. None of the tested compounds showed antimicrobial activity in the concentration range (800–1.56 μg/ml) studied.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

All solvents and reagents were purchased from Sigma-Aldrich, Acros, Novabiochem, Merck, Isolab, and Alkomed. All reactions were synthesized under normal atmospheric conditions. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra (see the Supporting Information) were recorded using a Bruker Advance III 400 or 300 MHz spectrometer in dimethyl sulfoxide (DMSO)-d_6. The assignment of exchangeable protons (O*H* and N*H*) was confirmed by the addition of D_2O. Positive-ion electrospray ionization (ESI) mass spectra were recorded on a double-focusing Finnigan MAT 95 instrument with BE geometry. Infrared spectra were recorded with the ATR equipment in the range 4000–650 cm^{-1} on a PerkinElmer Spectrum one FTIR spectrophotometer. Micro-analyses were performed using a LECO CHNS-932 elemental analyzer. Melting points were recorded in a capillary tube using an Electrothermal-9200 melting point apparatus, and are uncorrected. All dihydroquinolinone–peptide derivatives synthesized by microwave heating were obtained in a microwave synthesis instrument manufactured by Milestone Start S. All initial

N-protected mono- and dipeptides used in this study were prepared according to literature methods.[42,43]

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

4.1.2 | General procedure for the synthesis of quinolinone–peptide conjugates **1**–**13**

A mixture of the appropriate N-protected aminoacylbenzotriazole and equivalent amounts of 7-amino-3,4-dihydroquinolin-2(1*H*)-one was subjected to microwave irradiation (100 W, 70°C) for 1 h in anhydrous tetrahydrofuran. After the completion of the reaction, all volatiles were removed by a rotavapor and the resulting crude product was crystallized from methanol.

*Benzyl {(R)-3-methyl-1-oxo-1-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)amino]butan-2-yl}carbamate (**1**)*

White solid (78%); m.p. 225–226°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.13 (s, 1H, NH_{lactam}), 10.01 (s, 1H, N*H*), 7.49 (d, 1H, N*H*, *J* = 8 Hz), 7.38–7.06 (m, 7H, Ar–*H*), 5.05 (s, 2H, CH_2Ph), 3.99 (t, 1H, C*H*NH, *J* = 8 Hz), 2.81 (t, 2H, COCH_2CH$_2$, *J* = 8 Hz), 2.42 (t, 2H, COCH$_2$CH_2, *J* = 8 Hz), 2.01–1.97 (m, 1H, C*H*(CH$_3$)$_2$), 0.90 (d, 6H, CH_3, *J* = 8 Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 171.7 (NHCOCH$_2$), 171.0 (NH–*C*=O), 156.7 (COOCH$_2$Ph), 138.9, 138.2, 137.5, 128.8, 128.3, 128.2, 128.1, 119.0, 113.3, 106.7 (Ar–*C*), 65.9 (OCH$_2$Ph), 61.4 (*C*HNH), 31.1 (COCH_2CH$_2$), 30.7 (COCH$_2$CH_2), 24.7 (*C*H(CH$_3$)$_2$), 19.6 and 18.9 (CH$_3$)$_2$CH). Elemental analysis: $C_{22}H_{25}N_3O_4$ required C, 66.82; H, 6.37; N, 10.63; found C, 66.72; H, 6.35; N, 10.59. HRMS *m/z* for $C_{22}H_{25}N_3O_4$ [M+H]$^+$ calcd. 396.2, found 396.2; [M+Na]$^+$ calcd. 418.2, found 418.1; [M+Cl]$^-$ calcd. 430.2, found 430.1.

*Benzyl {(2R,3R)-3-methyl-1-oxo-1-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)amino]pentan-2-yl}carbamate (**2**)*

Beige solid (91%); m.p. 142–143°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.13 (s, 1H, NH_{lactam}), 10.03 (s, 1H, N*H*), 7.52 (d, 1H, N*H*, *J* = 12 Hz), 7.37–7.06 (m, 8H, Ar–*H*), 5.04 (s, 2H, CH_2NH), 4.04–4.00 (m, 1H, NHC*H*), 2.81 (t, 2H, COCH_2CH$_2$, *J* = 8 Hz), 2.42 (t, 2H, COCH$_2$CH_2, *J* = 8 Hz), 1.78–1.76 (m, 1H, C*H*CH$_3$), 1.51–1.49 and 1.20–1.16 (2 m, 2H, CH$_3$CH_2), 0.85–0.81 (m, 6H, CH_3CH$_2$ + CH_3). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 171.0 (COCH$_2$), 170.8 (*C*OCH), 156.6 (*C*OOCH$_2$Ph), 138.9, 138.2, 137.5, 128.8, 128.4, 128.2, 120.8, 199.0, 113.4, 106.8 (Ar–*C*), 65.8 (OCH$_2$Ph), 60.2 (*C*HNH), 36.7 (COCH$_2$CH$_2$), 31.1 (COCH$_2$CH$_2$), 25.0 (*C*HCH$_3$), 24.8 (CH$_3$CH$_2$), 15.7 (CH$_2$CH$_3$), 11.2 (CH$_3$CH). Elemental analysis: $C_{23}H_{27}N_3O_4$ required C, 67.46; H, 6.65; N, 10.26; found C, 67. 02; H, 6.51; N, 9.97. HRMS *m/z* for $C_{23}H_{27}N_3O_4$ [M+H]$^+$ calcd. 410.2, found 410.1; [M+Na]$^+$ calcd. 432.2, found 432.1; [M+Cl]$^-$ calcd. 444.2, found 444.1.

*Benzyl {(S)-3-(1H-indol-3-yl)-1-oxo-1-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)amino]propan-2-yl}carbamate (**3**)*

Yellow solid (89%); m.p. 119–120°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.58 (s, 1H, NH_{indole}), 10.15 (s, 1H, NH_{lactam}), 10.13 (s, 1H, N*H*), 7.70 (d, 1H, N*H*, *J* = 8 Hz), 7.70 (d, 1H, Ar–*H*, *J* = 8 Hz), 7.36–6.97 (m, 1H, Ar–*H*), 5.01–4.94 (m, 1H, CH_2O), 5.07–4.84 (m, 2H, Ar–*H*), 4.50–4.44 (s, H, NHC*H*), 3.13–3.12 and 3.03–3.01 (2 m, 2H, C$H_{2\text{-diasterotopic}}$), 2.82 (t, 2H, COC$H_2CH_2$, *J* = 8 Hz), 2.43 (t, 2H, COCH$_2$CH_2, *J* = 8 Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 171.0 (CH$_2$CONH), 170.8 (NHCOCH), 156.6 (COOCH$_2$Ph), 138.9, 138.3, 137.4, 136.5, 128.8, 128.2, 128.1, 127.7, 124.4, 121.4, 119.1, 119.0, 118.7, 113.6, 111.8, 110.3, 107.0 (Ar–*C*), 65.8 (OCH$_2$Ph), 56.5 (*C*HNH), 31.1 (CH$_{2\text{-indole}}$), 28.3 (COCH$_2$CH$_2$), 24.8 (COCH$_2$CH$_2$). Elemental analysis: $C_{28}H_{26}N_4O_4$ required C, 69.70; H, 5.43; N, 11.61; found C, 69. 43; H, 5.41; N, 11.37. HRMS *m/z* for $C_{28}H_{26}N_4O_4$ [M+Na]$^+$ calcd. 506.1, found 505.2; [M+K]$^+$ calcd. 521.2, found 523.2.

*Benzyl {(S)-1,5-dioxo-1,5-bis[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)amino]pentan-2-yl}carbamate (**4**)*

Beige solid (87%); m.p. 211–212°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.20 and 10.18 (2 s, 2H, NH_{lactam}), 10.15 and 9.94 (2 s, 2H, NH_{amide}), 7.94 (d, 1H, N*H*, *J* = 4 Hz), 7.65 (m, 4H, Ar–*H*), 7.49 (s, 1H, Ar–*H*), 7.40 (m, 2H, Ar–*H*), 7.39–7.33 (m, 2H, Ar–*H*), 7.32–7.25 (s, 1H, Ar–*H*), 7.22–7.04 (s, 1H, Ar–*H*), 5.06 (s, 2H, OCH_2Ph), 4.34–4.03 (m, 1H, C*H*NH), 2.80 (t, 4H, COCH_2CH$_2$, *J* = 8 Hz), 2.50–2.29 (m, 6H, COCH$_2$CH_2 and COCH_2CH$_2$CH), 2.10–2.05 and 2.04–1.92 (2 m, 2H, COCH$_2$CH_2CH). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 170.9 and 170.8 (COCH$_2$), 170.2 and 170.0 (CONH), 156.4 (COOCH$_2$Ph), 148.3, 138.9, 138.8, 138.7, 138.3, 137.4, 128.8, 128.3, 128.1, 119.0, 118.6, 113.2, 108.4, 106.8, 106.6, 101.3 (Ar–*C*), 65.9 (CH$_2$Ph), 55.4 (CHNH), 33.2 (COCH$_2$CH$_2$), 31.7 (COCH$_2$CH$_2$), 31.1 (CHCH$_2$), 28.0 (COCH$_2$CH$_2$), 24.7 (COCH$_2$CH$_2$), 24.5 (COCH$_2$CH$_2$CH). Elemental analysis: $C_{31}H_{31}N_5O_6$ required C, 65.37; H, 5.49; N, 12.30; found C, 65. 12; H, 5.46; N, 11.97. HRMS *m/z* for $C_{31}H_{31}N_5O_6$ [M+H]$^+$ calcd. 570.2, found 570.2; [M+Na]$^+$ calcd. 592.2, found 592.1; [M−H]$^-$ calcd. 568.2, found 568.2; [M+Cl]$^-$ calcd. 604.2, found 604.1.

*Benzyl [2-oxo-2-({2-oxo-2-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)amino]ethyl}amino)ethyl]carbamate (**5**)*

White solid (74%); m.p. 235–236°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.14 (s, 1H, NH_{lactam}), 9.85 (s, 1H, N*H*), 8.22 (t, 1H, N*H*, *J* = 6 Hz), 7.56 (t, 1H, N*H*, *J* = 6 Hz), 7.38–7.07 (m, 8 H, Ar–*H*), 5.06 (s, 2H, CH_2Ph), 3.89 (d, 2H, CH_2NH, *J* = 6 Hz), 3.66 (d, 2H, CH_2NH, *J* = 6 Hz), 2.81 (t, 2H, COCH_2CH$_2$, *J* = 8 Hz), 2.42 (t, 2H, COCH$_2$CH_2, *J* = 8 Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 170.7 (COCH$_2$CH$_2$), 170.0 (COCH$_2$), 167.8 (COCH$_2$), 157.8 (COOCH$_2$Ph), 138.9, 138.2, 137.4, 128.8, 128.3, 128.2, 118.9, 113.3, 106.7 (Ar–*C*), 66.0 (CH$_2$Ph), 44.0 (CHNH), 43.0 (CHNH), 31.1 (OCH$_2$CH$_2$), 24.7 (COCHCH$_2$). Elemental analysis: $C_{21}H_{22}N_4O_5$ required C, 61.45; H, 5.40; N, 13.65; found C, 61. 42; H, 5.38; N, 13.59.

*Benzyl [(S)-1-oxo-1-({(R)-1-oxo-1-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)amino]-3-phenylpropan-2-yl}amino)propan-2-yl]carbamate (**6**)*

White solid (62%); m.p. 178–179°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.18 (s, 1H, NH_{lactam}), 10.00 (s, 1H, N*H*), 8.13 (d, 1H, N*H*, *J* = 8 Hz), 7.41–7.13 (m, 13 H, Ar–*H* + N*H*), 5.08 (d, 2H, CH_2Ph), 4.71 (t, 1H,

CHNH, J = 6 Hz), 4.10 (t, 1H, CHNH, J = 6 Hz), 3.12–3.00 and 2.97–2.94 (2 m, 2H, CH_2Ph), 2.86 (t, 2H, $COCH_2CH_2$, J = 8 Hz), 2.47 (t, 2H, $COCH_2CH_2$, J = 8 Hz), 1.20 (d, 3H, CH_3, J = 8 Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 172.8 ($COCH_2$), 170.7 (COCH), 170.1 (COCH), 156.1 ($COOCH_2$Ph), 138.9, 138.1, 137.9, 137.4, 129.7, 128.8, 128.5, 128.3, 128.2, 128.1, 126.9, 119.2, 113.5, 108.9 (Ar–C), 65.9 (CH_2Ph), 54.9 (CHNH), 50.6 (CHNH), 38.1 ($CHCH_2$Ph), 31.0 ($COCH_2CH_2$), 24.7 ($COCH_2CH_2$), 18.5 ($CHCH_3$). Elemental analysis: $C_{29}H_{30}N_4O_5$ required C, 67.69; H, 5.88; N, 10.89; found C, 67.54; H, 5.83; N, 10.62. HRMS m/z for $C_{29}H_{30}N_4O_5$ $[M+H]^+$ calcd. 515.2, found 515.2; $[M+Cl]^-$ calcd. 549.2, found 549.1.

*Benzyl [(S)-4-(methylthio)-1-oxo-1-({2-oxo-2-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)amino]ethyl}amino)butan-2-yl]-carbamate (**7**)*

Beige solid (75%); m.p. 158–159°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.13 (s, 1H, NH_{lactam}), 9.82 (s, 1H, NH), 8.28 (t, 1H, NH, J = 6 Hz), 7.92 (bs, 1H, Ar–H), 7.62 (d, 1H, NH, J = 8 Hz), 7.38–7.31 (m, 5H, Ar–H), 7.26 (s, 1H, Ar–H), 7.08 (s, 2H, Ar–H), 5.04 (s, 2H, CH_2Ph), 4.17–4.13 (m, 1H, CHNH), 3.91–3.85 (m, 2H, CH_2NH), 2.81 (t, 2H, $COCH_2CH_2$, J = 8 Hz), 2.44–2.40 (m, 4H, $COCH_2CH_2$), 2.05 (s, 3H, CH_3), 1.96–1.92 and 1.86–1.81 (2 m, 2H, CH_2CH_2S). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 170.4 ($NHCOCH_2$), 170.3 (COCH), 167.8 (COCH), 156.4 ($COOCH_2$Ph), 148.2, 139.0, 137.3, 128.8, 128.2, 118.8, 113.2, 108.3, 106.4, 101.4 (Ar–C), 63.4 (CH_2Ph), 54.3 (CHNH), 43.0 (CHNH), 31.0 ($CHCH_2$), 30.1 ($COCH_2CH_2$), 24.8 ($COCHCH_2$), 15.6 (CH_2CH_2), 15.1 (CH_3). Elemental analysis: $C_{24}H_{28}N_4O_5S$ required C, 59.49; H, 5.82; N, 11.56; S, 6.62; found C, 59. 32; H, 5.81; N, 11.47; S, 6.51. HRMS m/z for $C_{24}H_{28}N_4O_5S$ $[M+H]^+$ calcd. 485.2, found 485.1; $[M+Cl]^-$ calcd. 519.2, found 519.1.

*Benzyl [(S)-4-(methylthio)-1-oxo-1-({(S)-1-oxo-1-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)amino]propan-2-yl}amino)butan-2-yl]-carbamate (**8**)*

Beige solid (72%); m.p. 202–203°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H, NH_{lactam}), 9.97 (s, 1H NH), 8.21 (d, 1H, N–H, J = 8 Hz), 7.63–7.11 (m, 9H, Ar–H + NH), 5.09 (s, 2H, CH_2Ph), 4.47–4.42 (m, 1H, CHNH), 4.21–4.17 (m, 1H, CHNH), 2.85 (t, 2H, $COCH_2CH_2$, J = 8 Hz), 2.46 (t, 2H, $COCH_2CH_2$, J = 8 Hz), 2.09 (s, 3H, CH_3), 1.94–1.85 (2 m, 2H, $CHCH_2CH_2$), 1.36–1.33 (m, 3H, $CHCH_3$). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 171.3 ($COCH_2$), 170.7 (COCH), 156.4 ($COOCH_2$Ph), 138.9, 138.4, 137.4, 128.8, 128.2, 118.9, 113.2, 106.6 (Ar–C), 65.9 (CH_2Ph), 54.7 (CHNH), 54.2 (CHNH), 49.4 (CH_2CH_2), 31.3 ($COCH_2CH_2$), 30.1 ($COCHCH_2$), 24.7 (SCH_3), 18.5 (CH_2CH_2S), 15.1 ($CHCH_3$). Elemental analysis: $C_{25}H_{30}N_4O_5S$ required C, 60.22; H, 6.07; N, 11.24; S, 6.43; found C, 59. 97; H, 6.06; N, 11.22; S, 6.23. HRMS m/z for $C_{25}H_{30}N_4O_5S$ $[M+H]^+$ calcd. 499.2, found 499.1; $[M+Na]^+$ calcd. 521.2, found 521.1; $[M+Cl]^-$ calcd. 533.2, found 533.1.

*Benzyl [(S)-4-(methylthio)-1-({(S)-4-(methylthio)-1-oxo-1-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)amino]butan-2-yl}amino)-1-oxobutan-2-yl]carbamate (**9**)*

Beige solid (72%); m.p. 202–203°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.13 (s, 1H, NH_{lactam}), 9.98 (s, 1H, NH), 8.19 (d, 1H, NH, J = 8 Hz), 7.57–7.09 (m, 9H, Ar–H + NH), 5.04 (s, 2H, CH_2Ph), 4.47–4.46 (m, 1H, CHNH), 4.17–4.12 (m, 1H, CHNH), 2.81 (t, 2H, $COCH_2$, J = 8 Hz), 2.44–2.06 (m, 6H, $COCH_2CH_2 + CH_2SCH_3$), 2.03 (s, 6H, CH_3), 1.91–1.80 (m, 4H, CH_2CH_2S). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 172.1 ($COCH_2$), 170.8 (COCH), 170.3 (COCH), 156.5 ($COOCH_2$Ph), 138.9, 138.2, 137.4, 128.8, 128.3, 128.2, 119.1, 113.4, 108.4, 106.8 (Ar–C), 65.9 (CH_2Ph), 54.3 (CHNH), 53.2 (CHNH), 32.4 ($COCH_2CH_2$), 32.1 ($COCHCH_2$), 31.7 ($CHCH_2CH_2$), 31.1 ($CHCH_2CH_2$), 30.1 (SCH_3), 24.8 (SCH_3), 15.2 (CH_2CH_2S), 15.1 (CH_2CH_2S). Elemental analysis: $C_{27}H_{34}N_4O_5S_2$ required C, 58.04; H, 6.13; N, 10.03; S, 11.48; found C, 57. 92; H, 6.01; N, 9.93; S, 11.39. HRMS m/z for $C_{27}H_{34}N_4O_5S_2$ $[M+H]^+$ calcd. 559.2, found 559.2; $[M+Cl]^-$ calcd. 553.2, found 553.1.

*Benzyl [(S)-4-(methylthio)-1-oxo-1-{[(S)-1-oxo-1-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)amino]-3-phenylpropan-2-yl}amino)butan-2-yl]carbamate (**10**)*

Beige solid (67%); m.p. 216–217°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H, NH_{lactam}), 10.04 (s, 1H, NH), 8.19 (d, 1H, NH, J = 8 Hz), 7.56 (d, 1H, NH, J = 8 Hz), 7.42–7.14 (m, 13 H, Ar–H), 5.09 (d, 2H, CH_2Ph, J = 4 Hz), 4.73–4.72 (m, 1H, CHNH), 4.17–4.11 (m, 1H, CHNH), 3.12–3.09 and 2.99–2.94 (2 m, 2H, CH_2Ph), 2.86 (t, 2H, $COCH_2CH_2$, J = 8 Hz), 2.49–2.41 (m, 4H, $COCH_2CH_2$, and SCH_2), 2.06 (s, 3H, CH_3), 1.87–1.78 (m, 2H, SCH_2CH_2). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 171.8 ($NHCOCH_2$), 170.7 (COCH), 170.1 (COCH), 156.4 ($COOCH_2$Ph), 139.0, 138.2, 137.8, 137.4, 129.7, 128.8, 128.5, 128.3, 128.2, 126.9, 119.2, 113.4, 106.9 (Ar–C), 66.0 (CH_2Ph), 55.0 (CHNH), 54.4 (CHNH), 38.1 ($CHCH_2$), 32.2 ($COCH_2CH_2$), 31.0 ($COCH_2CH_2$), 30.0 (SCH_2), 24.8 (SCH_3), 15.1 (SCH_2CH_2). Elemental analysis: $C_{31}H_{34}N_4O_5S$ required C, 64.79; H, 5.96; N, 9.75; S, 5.58; found C, 64.72; H, 5.91; N, 9.67; S, 5.51. HRMS m/z for $C_{31}H_{34}N_4O_5S$ $[M+H]^+$ calcd. 575.2, found 575.2; $[M+Na]^+$ calcd. 597.2, found 597.2; $[M+Cl]^-$ calcd. 609.2, found 609.2.

tert-*Butyl (S)-{3-methyl-1-oxo-1-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)amino]butan-2-yl}carbamate (**11**)*

White solid (84%); m.p. 217–218°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H, NH_{lactam}), 9.92 (s, 1H, NH), 7.24 (s, 1H, Ar–H), 7.23–7.06 (m, 2H, Ar–H), 6.85 (d, 1H, NH, J = 12 Hz), 3.92–3.88 (m, 1H, CHNH), 2.80 (t, 2H, $COCH_2CH_2$, J = 8 Hz), 2.40 (t, 2H, $COCH_2CH_2$, J = 8 Hz), 1.99–1.92 (m, 1H, $CH(CH_3)_2$), 1.39 (s, 9H, $(CH_3)_3C$), 0.89 (d, 6H, $(CH_3)_2CH$). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 171.0 ($NHCOCH_2$), 170.7 (CHCONH), 155.9 ($COOCH_2$Ph), 138.9, 138.2, 128.2, 118.9, 113.3, 106.7 (Ar–C), 78.4 ($CO(CH_3)_3$), 60.9 (CHNH), 31.1 ($COCH_2CH_2$), 30.8 ($COCH_2CH_2$), 28.7 [$(CH_3)_2CHCH$], 24.8 [$CH(CH_3)_3$], 19.6 and 19.0 [$(CH_3)_2CH$]. Elemental analysis: $C_{19}H_{27}N_3O_4$ required C, 63.14; H, 7.53; N, 11.63; found C, 62. 72; H, 7.47; N, 11.52.

tert-*Butyl {(2R,3R)-3-methyl-1-oxo-1-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)amino]pentan-2-yl}carbamate (**12**)*

White solid (83%); m.p. 199–200°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H, NH_{lactam}), 9.93 (s, 1H, NH), 7.24 (s, 1H, Ar–H), 7.24–7.06 (m, 2H, Ar–H), 6.89 (d, 1H, NH, J = 8 Hz), 3.94 (t, 1H, CHCHNH,

J = 8 Hz), 2.80 (t, 2H, $COCH_2CH_2$, J = 8 Hz), 2.42 (t, 2H, $COCH_2CH_2$, J = 8 Hz), 1.74–1.45 and 1.47–1.45 (m, 2H, CH_3CH_2CH), 1.39 (s, 9H, $(CH_3)_3C$), 1.16–1.13 (m, 1H, CH_3CH_2CH), 0.85–0.81 (m, 6H, $(CH_3)_2CH$). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 171.1 ($NHCOCH_2$), 170.7 (CHNHCO), 155.9 ($COOC(CH_3)_3$), 138.9, 138.2, 128.2, 118.9, 113.3, 106.7 (Ar–C), 78.5 ($CO(CH_3)_3$), 59.8 (CHNH), 36.8 ($COCH_2CH_2$), 31.1 ($COCH_2CH_2$), 28.7 ($CHCH_2CH_3$), 25.0 ($C(CH_3)_3$), 24.8 ($CHCH_2CH_3$), 15.8 (CH_3), 11.2 (CH_3). Elemental analysis: $C_{20}H_{29}N_3O_4$ required C, 63.98; H, 7.79; N, 11.19; found C, 62.72; H, 7.77; N, 11.07. HRMS m/z for $C_{20}H_{29}N_3O_4$ $[M+Na]^+$ calcd. 398.2, found 398.1; $[M-H]^-$ calcd. 374.2, found 374.1; $[M+Cl]^-$ calcd. 410.2, found 410.1.

*tert-Butyl [(R)-1-{[(R)-4-(methylthio)-1-oxo-1-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)amino]butan-2-yl}amino)-1-oxo-3-phenylpropan-2-yl]carbamate (**13**)*

White solid (80%); m.p. 197–198°C; ^1H NMR (400 MHz, DMSO-d_6) δ 10.19 (s, 1H, NH_{lactam}), 10.06 (s, 1H, NH), 8.21 (d, 1H, NH, J = 8 Hz), 7.36–7.02 (m, 8H, Ar–H + NH), 4.59–5.56 (m, 1H, CHNH), 4.30–4.24 (m, 1H, CHNH), 3.07–3.03 (m, 1H, CH_2Ph), 2.88–2.85 (m, 2H, $COCH_2CH_2$ + CH_2Ph), 2.50–2.46 (m, 4H, $COCH_2CH_2$ + $CHCH_2CH_2S$), 2.12 (s, 5H,CH_3), 2.04–1.95 (m, 2H, $CHCH_2CH_2S$), 1.36 (s, 9H, $OC(CH_3)_3$). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 172.3 ($NHCOCH_2$), 170.8 (COCH), 170.2 (COCH), 155.8 ($COOC(CH_3)_3$), 138.9, 138.6, 138.2, 129.7, 128.5, 128.2, 126.6, 119.2, 113.5, 106.9 (Ar–C), 78.6 ($OCH(CH_3)_3$), 56.2 (CHNH), 53.2 (CHNH), 32.6 ($COCH_2CH_2$), 31.1 ($CHCH_2$), 30.9 ($CHCH_2CH_2S$), 29.9 ($COCH_2CH_2$), 28.6 (SCH_3), 24.8 ($CHCH_2CH_2S$), 15.1 ($OCH(CH_3)_3$). Elemental analysis: $C_{28}H_{36}N_4O_5S$ required C, 62.20; H, 6.71; N, 10.36; S, 5.93; found C, 62. 12; H, 6.70; N, 10.27; S, 5.83.

4.1.3 | General procedure to remove the Boc protecting group

An appropriate Boc protected compound (0.15 mmol) was dissolved in a TFA/dichloromethane mixture (1.5/1.5 ml) and stirred for 2 h at room temperature according to the literature method.[37] The reaction mixture was concentrated at reduced pressure, and the excess of diethyl ether was added to afford the desired cream powder. This powder was filtered and air-dried.

*(R)-1-Oxo-1-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)amino]propan-2-aminium 2,2,2-trifluoroacetate (**14**)*

Cream solid (86%); m.p. 239–240°C; ^1H NMR (400 MHz, DMSO) δ 10.46 (s, 1H, NH_{lactam}), 10.22 (s, 1H, NH), 8.29 (s, 3H, NH_3^+), 7.23 (s, 1H, Ar–H), 7.12 (s, 1H, Ar–H), 4.06–3.97 (m, 1H, CH_3CH), 2.82 (t, 2H, $COCH_2CH_2$, J = 8 Hz), 2.43 (t, 2H, $COCH_2CH_2$, J = 7.5 Hz (t, J = 78 Hz), 1.44 (d, 3H, CH_3, J = 7.0 Hz). ^{13}C NMR (101 MHz, DMSO) δ 170.8 ($NHCOCH_2$), 168.5 (COCH), 158.8 (F_3CCO, q, $^2J_{(C-F)}$ = 30 Hz), 139.1, 137.6, 128.5, 119.7, 117.6 (q, CF_3CO, $^1J_{(C-F)}$ = 296 Hz), 113.4, 106.9 (Ar–C), 49.4 ($CHCH_3$), 31.0 ($COCH_2CH_2$), 24.8 ($COCH_2CH_2$), 17.6 (CH_3). Elemental analysis: $C_{14}H_{16}F_3N_3O_4$ required C, 48.12; H, 4.64; N, 12.10; found C, 48.02 12; H, 4.63; N, 11.98.

*(S)-3-Methyl-1-oxo-1-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)-amino]butan-2-aminium 2,2,2-trifluoroacetate (**15**)*

Cream solid (72%); m.p. 81–82°C; ^1H NMR (400 MHz, DMSO) δ 10.49 (s, 1H, NH_{lactam}), 10.20 (s, 1H, NH), 8.31 (s, 3H, NH_3^+), 7.23–7.16 (m, 2H, Ar–H), 6.73 (s, 1H, Ar–H), 3.80–3.73 (m, 1H, NHCH), 2.84 (t, 2H, $COCH_2CH_2$, J = 8 Hz), 2.44 (t, 2H, $COCH_2CH_2$, J = 78 Hz (t, J = 78 Hz), 2.20–2.12 (m, 1H, $[(CH_3)_2CH]$), 0.98 (d, 6H, $[(CH_3)_2CH$, J = 4.0 Hz). ^{13}C NMR (101 MHz, DMSO) δ 170.8 ($NHCOCH_2$), 167.2 (COCH), 159.4 (F_3CCO, q, $^2J_{(C-F)}$ = 30 Hz), 139.7, 134.6, 129.2, 121.3, 117.7 (q, CF_3CO, $^1J_{(C-F)}$ = 256.7 Hz), 115.0, 108.3 (Ar–C), 58.3 (CHNH), 30.8 ($COCH_2CH_2$), 30.4 ($COCH_2CH_2$), 24.7 $[CHCH(CH_3)_2]$, 18.8 and 18.2 $[CHCH(CH_3)_2]$. Elemental analysis: $C_{14}H_{16}F_3N_3O_4$ required C, 48.12; H, 4.64; N, 12.10; found C, 48.02 12; H, 4.63; N, 11.98.

4.2 | Biological assays

4.2.1 | CA Inhibition

An Applied Photophysics Stopped-Flow instrument was used for assaying the CA-catalyzed CO_2 hydration activity using the method of Khalifah.[44] Phenol red (at a concentration of 0.2 mM) has been used as an 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 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 to determine 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–deionized water, and dilutions up to 0.01 nM were performed thereafter with the assay buffer. Inhibitor and enzyme solutions were pre-incubated together for 15 min at room temperature before the assay, 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.[45–47]

4.2.2 | Antioxidant testing

DPPH radical scavenging activity

Antioxidant activities of the compounds were determined based on the ability of the antioxidants to act as radical scavengers toward the stable free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH). As detailed by Yang et al.,[41] 1 ml of antioxidant solution (solubilized in ethanol) was added to 3 ml of a 0.1 mM ethanolic solution of DPPH. After

30 min at ambient temperature in darkness, absorbance readings were taken at 517 nm. Inhibition (%) was calculated using the following equation:

$$[1 - (A_s - A_o)/A_b] \times 100,$$

where A_s is the absorbance reading for samples containing the antioxidant, A_o is the absorbance of the antioxidant in pure methanol, and A_b corresponds to the absorbance of the DPPH solution.

4.2.3 | Cytotoxic activity

Stock solutions of the peptide–dihydroquinolinone conjugates were prepared in DMSO and then, further dilutions were made with a fresh culture medium. The concentration of DMSO in the final culture medium was <0.1%. A549 and BEAS-2B cell lines were plated in 96-well plates (5×10^3 cells/well) for 24 h. After the cells attached to the surface of the plate, the tested peptide–dihydroquinolinone conjugates were added to the wells to obtain the final concentrations in the range of 0–100 μg/ml. Then, the cells were incubated at 37°C in a humidified incubator with 5% CO_2 for 24, 48, and 72 h. Their cytotoxic effects against the cells were determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) cell proliferation assay according to the methodology previously described by our group.[48] Twelve wells were used for every concentration, and IC_{50} values (μg/ml) were defined as the compound concentrations reducing absorbance to 50% of the control values.

4.2.4 | Antimicrobial activity

Microdilution and disc diffusion methods were used to test the antimicrobial activity of the newly synthesized quinoline compounds against microorganisms. The microorganisms were bacteria, Gram-negative bacterium *Escherichia coli* ATCC 25922, Gram-positive bacterium *Staphylococcus aureus* ATCC 29213, and yeasts *Candida albicans* ATCC 90028 *and Candida tropicalis*. These bacteria and yeasts were grown on nutrient agar and Sabouraud dextrose agar media at 37°C for 24 h. Then, suspensions of the cultures were prepared based on McFarland standard. The quinoline compounds were dissolved in DMSO. Minimum inhibitory concentrations (MICs) of the compounds were investigated using the microdilution method. Twofold serial dilutions of the compounds were prepared in 96-well plates containing Mueller–Hinton broth and RPMI for bacteria and yeasts, respectively. Then, 100 μl from the suspension of cultures prepared based on McFarland standard was added to each well and incubated for 24 and 48 h at 37°C for bacteria and yeasts, respectively. MIC is the lowest concentration of the compounds preventing the growth of the microorganisms. The antimicrobial potential of these compounds was also tested using the disc diffusion method. In this application, Mueller–Hinton agar and Sabouraud dextrose agar were used for bacteria and yeasts, respectively. The suspensions of the microbial cultures at 0.5 McFarland were spread on agar plates using cotton swabs. Then, the filter paper discs containing 30 μl compounds were placed on agar surfaces and these cultures were incubated for 24 and 48 h at 37°C for bacteria and yeasts, respectively. The diameter of the inhibition of growth zones shows the antimicrobial activity of the compounds.[49,50]

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CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interests.

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