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Synthesis, antioxidant and carbonic anhydrase inhibitory properties of monopeptide-anthraquinone conjugates

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Abstract: Novel monopeptide-anthraquinone conjugates (**1**-**16**) were synthesized by the reaction of appropriate N-protected amino acid with 2-hydroxymethylanthraquinone in good or high yields. The structural elucidation of the new compounds was accomplished by ^{1}H NMR, ^{13}C NMR, MS, FT-IR spectroscopy and elemental analysis techniques. The carbonic anhydrase (CA, EC 4.2.1.1) inhibitory activity of the new compounds was determined against two human (h) isoforms, hCA I and hCA II. While three of the sixteen compounds showed moderate in *vitro* carbonic anhydrase inhibitory properties against hCA II with inhibition constants in the micromolar level (43.5, 67.4 and 78.1 µM), they did not show inhibitory activity against hCA I up to 100 µM concentration. The antioxidant abilities of the compounds were determined using the 1,1-diphenyl-2-picrylhydrazil (DPPH) radical scavenging method, ferric ion reducing assay and metal chelation methods. While a small amount of antioxidant activity was observed according to the DPPH and ferric ion reducing power assay methods, none of the compounds showed antioxidant properties according to the metal chelating activity method at the concentrations studied.

Keywords: Anthraquinone derivatives; monopeptide anthraquinone conjugates, carbonic anhydrase inhibition; antioxidant activity.

1. Introduction

Anthraquinone is an anthracene derivative, which is a polynuclear hydrocarbon with three benzene rings linearly fused. Anthraquinone derivatives have been used for about one-third of all organic dye products over the world.[1-4] They are widely used in different fields such as quantum dots in

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semiconducting industry, organic electronic devices applications,[5] photoelectrocatalysis application,[6,7] photo oxidant,[8] organic catalyst,[9] charge-transfer materials[10,11] and sensor technology.[12,13] In addition to their technological importance, anthraquinone derivatives constitute an important class of bioactive compounds for their versatile pharmacological activities such as antimicrobial,[14] antimalarial,[15] antioxidant,[16] anticancer,[17,18] acetylcholinesterase (AChE), butyrylcholinesterase (BChE), β-site amyloid precursor protein cleaving enzyme 1 (BACE1) inhibitors,[19] antiplatelet,[20] anticoagulant,[20] antiviral,[21] anti-Alzheimer's disease[22] and stimulating topoisomerase II-mediated DNA cleavage.[23] They also found in plant-based foods as emodin such as rhubarb, cabbage, beans and buckthorn.[24]

Despite the clinical potential of anthraquinone−amino acid or anthraquninone-peptide conjugates, such conjugates have not been investigated up to now and there are only a few work in the literature.[17,25,26] Therefore, this study was planned to obtain new possible bioactive compounds inspired by the biological importance of anthraquinones in the literature.[27] The new monopeptide-anthraquinone conjugates in this study were obtained from the reaction of the appropriate N-acylbenzotriazole with 2-hydroxymethyl-antraquinone.

Within the framework of this study, 16 new monopeptide-anthraquinone derivatives were synthesized and hCA I and hCA II enzyme inhibition capacities were evaluated using a stopped flow CO_2 hydrase assay.[28] The antioxidant capacities of the compounds were determined using the DPPH radical scavenging, ferric ions reducing power assay and metal chelating activity methods.[29,30,31]

2. Experimental

2.1. Chemical Material and Apparatus

The starting materials and reagents used in the reactions were supplied commercially by Aldrich, Acros, Merck, AFG Bioscience, abcr, Bachem, Alfa Aesar or Fluorochem. Nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) spectra were recorded using a Bruker Avance III 400 or 300 MHz spectrometers (Billerica, Massachusetts , USA) in DMSO-d6. Chemical shifts are reported in parts per million (ppm) and the coupling constants (*J*) are expressed in Hertz (Hz). The assignment of exchangeable protons (OH and NH) was confirmed by the addition of D_2O. Positive or negative-ion electrospray ionization (ESI) mass spectra were recorded on a double-focusing Finnigan MAT 95 instrument (Palo Alto, California, USA) with BE geometry. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F-254 plates. Infrared spectra were recorded with ATR equipment in the range 4000-650 cm^{-1} on a Perkin Elmer Spectrum one FTIR spectrophotometer (USA). Elemental analyses were performed with a LECO CHNS-932 elemental analyzer (Michigan, USA). Melting points were recorded using an Electrothermal-9200 melting point apparatus, and are uncorrected. All starting N-protected monopeptides were prepared according to literature procedures.[32-37]

2.2. Chemistry

*2.2.1. General Synthesis Method of Monopeptide-anthraquinone Conjugates (**1-15**)*

2-Hydroxymethylanthraquinone (1.0 equiv.), 1-(N-protected aminoacyl)benzotriazole (1.0 equiv.) and Et_3N (1.2 equiv.) were reacted in DMF for 3 hours at room temperature [monitored by TLC, methanol (5%) / dichloromethane (95%) mixture]. Then, ice water was added into the reaction mixture to precipitate the crude product. The crude product was crystallized from ethanol.

*(9,10-Dioxo-9,10-dihydroanthracen-2-yl)methyl ((benzyloxy)carbonyl)glycinate (**1**)*: Yellow (82%); m.p.: 150-151 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20-8.17 (m, 4H, Ar-*H*), 7.95-7.87 (m, 3H, Ar-*H*), 7.82 (t, 1H, NH, *J*= 6.0 Hz), 7.37-7.31 (m, 5H, Ar-*H*), 5.36 (s, 2H, CH_2-anthraquinone), 5.08 (s, 2H, PhCH_2O), 3.94 (d, 2H, CONHCH_2, *J*= 4.0 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.7 (*C*O-anthraquinone), 182.6 (*C*O- anthraquinone), 170.6 (NHCH_2*C*O), 157.1 (PhCH_2O*C*O), 143.2, 137.4, 135.1, 135.0, 133.7, 133.5, 133.5, 133.4, 133.0, 128.8, 128.3, 128.2, 127.6, 127.2, 127.2, 126.0 (Ar-*C*), 66.1 (*C*H_2- anthraquinone), 65.5 (Ph*C*H_2OCO), 42.8 (CONH*C*H_2). IR (cm^{-1}): $\nu_{(C=O)ester}$: 1748 cm^{-1}, $\nu_{(C=O)ketone}$: 1692 cm^{-1}, $\nu_{(C=O)carbamate:}$ 1684 cm^{-1}, $\nu_{(N-H)amine}$: 3342 cm^{-1}. Anal. calculated for $C_{25}H_{19}NO_6$: C, 69.92; H, 4.46; N, 3.26. Found: C, 69.10; H, 4.01; N, 3.11. HRMS *m/z* $C_{25}H_{19}NO_6$ $[M+H]^+$ calcd. 430.1,

found 430.1; $[M+NH_4]^+$ calcd. 447.1, found 447.2; $[M+Na]^+$ calcd. 452.0, found 452.1; $[M+K]^+$ calcd. 468.0, found 468.1; $[M-(C_{15}H_9O_2)]^+$ calcd. 221.1, found 220.8.

*(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl ((benzyloxy)carbonyl)-L-alaninate (**2**)*: Cream (94%); m.p.: 172-173 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20-8.17 (m, 4H, Ar-*H*), 7.95-7.85 (m, 4H, Ar-*H*+NH), 7.36-7.29 (m, 5H, Ar-*H*), 5.36 (s, 2H, CH_2-antrakinon), 5.07 (s, 2H, PhCH_2O), 4.28-4.21 (m, 1H, CONHC*H*), 1.36 (d, 3H, CHCH_3, *J*= 8.0 Hz).^{13}C NMR (100 MHz, DMSO-d_6): δ 182.8 (*C*O-anthraquinone), 182.6 (*C*O-anthraquinone), 173.2 (NHCH*C*O), 156.4 (PhCH$_2$O*C*O), 143.4, 137.4, 135.1, 135.0, 133.6, 133.5, 133.4, 132.9, 128.8, 128.3, 128.3, 127.6, 127.2, 127.2, 125.8 (Ar-*C*) 66.0 (*C*H$_2$-anthraquinone), 65.5 (Ph*C*H$_2$OCO), 49.9 (CONH*C*H), 17.3 (CH*C*H$_3$). IR (cm^{-1}): $\nu_{(C=O)ester}$: 1740 cm^{-1}, $\nu_{(C=O)ketone}$: 1688 cm^{-1}, $\nu_{(C=O)carbamate}$: 1673 cm^{-1}, $\nu_{(N-H)amine}$: 3329 cm^{-1}. Anal. calculated for $C_{26}H_{21}NO_6$): C, 70.42; H, 4.77; N, 3.16. Found: C, 70.10; H, 4.12; N, 3.04. HRMS *m/z* $C_{26}H_{21}NO_6$ $[M+H]^+$ calcd. 444.1, found 444.1; $[M+NH_4]^+$ calcd. 461.2, found 461.1; $[M+Na]^+$ calcd. 466.1, found 467.1; $[M+K]^+$ calcd. 482.1, found 482.0; $[M-(C_{15}H_9O_2)]^+$ calcd. 221.1, found 221.0.

*(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl ((benzyloxy)carbonyl)-L-phenylalaninate (**3**)*: Cream (78%);m.p.: 142-143 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.22-8.16 (m, 4H, Ar-*H*), 7.97-7.93 (m, 3H, Ar-*H*), 7.78 (d, 1H, NH, *J*= 8.0 Hz), 7.34-7.21 (m, 10H, Ar-*H*), 5.34 (s, 2H, CH_2-anthraquinone), 5.06-4.98 (m, 2H, PhCH_2O), 4.45-4.39 (m, 1H, CONHC*H*), 3.15 and 3.11 dd, 1H, CHCH_2Ph, *J*= 4 and 8 Hz), 2.99 and 2.96 (dd, 1H, CHCH_2Ph, *J*= 8 and 12 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.7 (*C*O-anthraquinone), 182.6 (*C*O-anthraquinone), 172.2 (NHCH*C*O), 156.5 (PhCH$_2$O*C*O), 143.2, 137.7, 137.3, 135.1, 135.0, 133.5, 133.5, 133.0, 129.6, 128.8, 128.8, 128.2, 128.1, 127.6, 127.3, 127.2, 127.0, 126.0 (Ar-*C*) 66.0 (*C*H$_2$-anthraquinone), 65.7 (Ph*C*H$_2$OCO), 56.1 (CONH*C*H), 36.9 (CH*C*H$_2$Ph). IR (cm^{-1}): $\nu_{(C=O)ester}$: 1725 cm^{-1}, $\nu_{(C=O)ketone}$: 1709 cm^{-1}, $\nu_{(C=O)carbamate}$: 1668 cm^{-1}, $\nu_{(N-H)amine}$: 3360 cm^{-1}. Anal. calculated for $C_{32}H_{25}NO_6$: C, 73.98; H, 4.85; N, 2.70. Found: C, 73.12; H, 4.74; N, 2.75. HRMS *m/z* $C_{32}H_{25}NO_6$ $[M+H]^+$ calcd. 520.2, found 520.1; $[M+NH_4]^+$ calcd. 537.2, found 537.2; $[M+Na]^+$ calcd. 542.2, found 542.1; $[M+K]^+$ calcd. 558.1, found 558.1; $[M-(C_{15}H_9O_2)]^+$ calcd. 221.1, found 221.0.

*(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl ((benzyloxy)carbonyl)-L-valinate (**4**)*: Cream (90%); m.p.: 134-135 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20-8.17 (m, 4H, Ar-*H*), 7.94-7.82 (m, 4H, Ar-*H*+NH), 7.38-7.25 (m, 5H, Ar-*H*), 5.40-5.32 (m, 2H, CH_2- anthraquinone), 5.11-5.04 (m, 2H, PhCH_2O), 4.08-4.04 (m, 1H, OCONHC*H*), 2.17-2.08 (m, 1H, C*H*(CH$_3$)$_2$), 0.92 (d, 6H, CH(CH_3)$_2$, *J*= 8.0 Hz).^{13}C NMR (100 MHz, DMSO-d_6): δ 182.7 (*C*O- anthraquinone), 182.6 (*C*O- anthraquinone), 172.2 (NHCH*C*O), 157.0 (PhCH$_2$O*C*O), 143.3, 137.4, 135.1, 135.0, 133.7, 133.5, 133.4, 133.0, 128.8, 128.3, 128.3, 127.6, 127.2, 127.2, 126.0 (Ar-*C*), 66.1 (*C*H$_2$- anthraquinone), 65.5 (Ph*C*H$_2$OCO), 60.4 (CONH*C*H), 30.1 (*C*H(CH$_3$)$_2$), 19.5 and 18.7 (CH(*C*H$_3$)$_2$). IR (cm^{-1}): $\nu_{(C=O)ester}$: 1725 cm^{-1}, $\nu_{(C=O)ketone}$: 1711 cm^{-1}, $\nu_{(C=O)carbamate}$: 1668 cm^{-1}, $\nu_{(N-H)amine}$: 3370 cm^{-1}. Anal. calculated for $C_{28}H_{25}NO_6$: C, 71.33; H, 5.34; N, 2.97. Found: C, 71.12; H, 5.76; N, 3.08. HRMS *m/z* $C_{28}H_{25}NO_6$ $[M+H]^+$ calcd. 472.2, found 472.1; $[M+NH_4]^+$ calcd. 489.2, found 489.1; $[M+Na]^+$ calcd. 494.2, found 494.1; $[M+K]^+$ calcd. 510.1, found 511.2; $[M-(C_{15}H_9O_2)]^+$ calcd. 221.1, found 221.0.

*(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl ((benzyloxy)carbonyl)-L-leucinate (**5**)*: Cream (73%); m.p.: 103-104 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.22-8.18 (m, 4H, Ar-*H*), 7.95-7.86 (m, 4H, Ar-*H*+NH), 7.36-7.21 (m, 5H, Ar-*H*), 5.40-5.32 (m, 2H, CH_2- anthraquinone), 5.11-5.03 (m, 2H, PhCH_2O), 4.22-4.16 (m, 1H, OCONHC*H*), 1.72-1.52 (m, 3H, CHCH_2C*H*(CH$_3$)$_2$), 0.92-0.87 (m, 6H, CH(CH_3)$_2$).^{13}C NMR (100 MHz, DMSO-d_6): δ 182.8 (*C*O- anthraquinone), 182.6 (*C*O- anthraquinone), 173.1 (NHCH*C*O), 156.7 (PhCH$_2$O*C*O), 143.4, 137.4, 135.1, 135.0, 133.6, 133.5, 133.0, 128.8, 128.3, 128.2, 127.6, 127.3, 127.22, 125.8 (Ar-*C*), 66.1 (*C*H$_2$- anthraquinone), 65.5 (Ph*C*H$_2$OCO), 52.9 (CONH*C*H), 39.9 (CH*C*H$_2$CH(CH$_3$)$_2$), 24.8 (CHCH$_2$*C*H(CH$_3$)$_2$), 23.2 and 21.7 (CHCH$_2$CH(*C*H$_3$)$_2$). IR (cm^{-1}): $\nu_{(C=O)ester}$: 1722 cm^{-1}, $\nu_{(C=O)ketone}$: 1711 cm^{-1}, $\nu_{(C=O)carbamate}$: 1671 cm^{-1}, $\nu_{(N-H)amine}$: 3370 cm^{-1}. Anal. calculated for $C_{29}H_{27}NO_6$: C, 71.74; H, 5.61; N, 2.88. Found: C, 71.23; H, 5.93; N, 2.93. HRMS *m/z* $C_{29}H_{27}NO_6$ $[M+H]^+$ calcd. 486.2, found 486.1; $[M+NH_4]^+$ calcd. 503.2, found 504.2; $[M+Na]^+$ calcd. 508.2, found 508.1; $[M+K]^+$ calcd. 524.2, found 525.1; $[M-(C_{15}H_9O_2)]^+$ calcd. 221.1, found 221.0.

*(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl ((benzyloxy)carbonyl)-L-methioninate (**6**):* Cream (91%); m.p.: 153-154 ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.21-8.17 (m, 4H, Ar-*H*), 7.95-7.91 (m, 3H, Ar-*H*), 7.87 (d, 1H, NH, *J*= 8.0 Hz), 7.36-7.29 (m, 5H, Ar-*H*), 5.40-5.33 (m, 2H, C*H*$_2$- anthraquinone), 5.11-5.04 (m, 2H, PhC*H*$_2$O), 4.38-4.31 (m, 1H, OCONHC*H*), 2.61-2.52 (m, 2H, CHCH$_2$C*H*$_2$SCH$_3$), 2.04 (s, 3H, CHCH$_2$CH$_2$SC*H*$_3$), 2.03-1.95 (m, 2H, CHC*H*$_2$CH$_2$SCH$_3$).^{13}C NMR (100 MHz, DMSO-d_6): δ 182.7 (*C*O- anthraquinone), 182.6 (*C*O- anthraquinone), 172.5 (NHCH*C*O), 156.7 (PhCH$_2$O*C*O), 143.3, 137.3, 135.1, 135.0, 133.6, 133.4, 133.0, 128.8, 128.3, 128.3, 127.6, 127.2, 127.2, 125.9 (Ar-*C*), 66.1 (*C*H$_2$- anthraquinone), 65.7 (Ph*C*H$_2$OCO), 53.3 (CONH*C*H), 30.6 (CHCH$_2$*C*H$_2$SCH$_3$), 30.0 (CHCH$_2$CH$_2$S*C*H$_3$), 14.9 (CH*C*H$_2$CH$_2$SCH$_3$). IR (cm^{-1}): $\nu_{(C=O)ester}$: 1742 cm^{-1}, $\nu_{(C=O)ketone}$: 1687 cm^{-1}, $\nu_{(C=O)carbamate}$: 1673 cm^{-1}, $\nu_{(N-H)amine}$: 3327 cm^{-1}. Anal. calculated for $C_{28}H_{25}NO_6S$: C, 66.78; H, 5.00; N, 2.78; S, 6.37. Found: C, 65.68; H, 4.59; N, 2.81; S, 6.14. HRMS *m/z* $C_{28}H_{25}NO_6S$ $[M+H]^+$ calcd. 504.1, found 504.1; $[M+Na]^+$ calcd. 526.1, found 526.1; $[M+K]^+$ calcd. 542.1, found 542.0; $[M-(C_{15}H_9O_2)]^+$ calcd. 221.1, found 220.9.

*(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl ((benzyloxy)carbonyl)-L-tryptophanate (**7**)*: Yellow (70%); m.p.: 168-169 ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.89 (s, 1H, indole-N*H*), 8.22-8.11 (m, 4H, Ar-*H*), 7.97-7.93 (m, 3H, Ar-*H* + NH), 7.63-7.60 (m, 1H, Ar-*H*), 7.54 (d, 1H, Ar-*H*, *J*= 8.0 Hz), 7.36-7.19 (m, 7H, Ar-*H*), 7.09-6.97 (m, 2H, Ar-*H*), 5.30 (s, 2H, C*H*$_2$- anthraquinone), 5.07-4.98 (m, 2H, PhC*H*$_2$O), 4.47-4.42 (m, 1H, OCONHC*H*), 3.27-3.22 (m, 1H, (3-indolyl)C*H*$_2$CH), 3.15-3.09 (m, 1H, (3-indolyl)C*H*$_2$CH).^{13}C NMR (100 MHz, DMSO-d_6): δ 182.8 (*C*O- anthraquinone), 182.6 (*C*O- anthraquinone), 172.6 (NHCH*C*O), 156.5 (PhCH$_2$O*C*O), 143.2, 137.3, 136.6, 135.1, 135.0, 133.5, 133.4, 132.9, 128.8, 128.3, 128.2, 127.5, 127.5, 127.3, 127.2, 125.9, 124.4, 121.5, 119.0, 118.5, 112.0, 109.9 (Ar-*C*), 66.0 (*C*H$_2$- anthraquinone), 65.5 (Ph*C*H$_2$OCO), 55.7 (CONH*C*H), 27.4 ((3-indolyl)*C*H$_2$CH). IR (cm^{-1}): $\nu_{(C=O)ester}$: 1740 cm^{-1}, $\nu_{(C=O)ketone}$: 1699 cm^{-1}, $\nu_{(C=O)carbamate}$: 1669 cm^{-1}, $\nu_{(N-H)amine}$: 3373,3337 cm^{-1}. Anal. calculated for $C_{34}H_{26}N_2O_6$: C, 73.11; H, 4.69; N, 5.02. Found: C, 73.43; H, 4.41; N, 5.03. HRMS *m/z* $C_{34}H_{26}N_2O_6$ $[M+H]^+$ calcd. 559.2, found 559.1; $[M+NH_4]^+$ calcd. 576.2, found 576.1; $[M+Na]^+$ calcd. 581.2, found 581.1; $[M+K]^+$ calcd. 579.1, found 579.1; $[M-H]^-$ calcd. 557.2, found 557.2.

*(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (tert-butoxycarbonyl)-L-alaninate (**8**)*: Cream (71%); m.p.: 164-165 ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.17-8.13 (m, 4H, Ar-*H*), 7.94-7.92 (m, 3H, Ar-*H* + NH), 7.43 (d, 1H, Ar-*H*, *J*= 8.0 Hz), 5.38-5.28 (m, 2H, C*H*$_2$- anthraquinone), 4.18-4.11 (m, 1H, OCONHC*H*), 1.38 (s, 9H, OC(C*H*$_3$)$_3$), 1.32 (d, 3H, CHC*H*$_3$, *J*= 8.0 Hz).^{13}C NMR (100 MHz, DMSO-d_6): δ 182.7 (*C*O- anthraquinone), 182.5 (*C*O- anthraquinone), 173.4 (NHCH*C*O), 155.8 ((CH$_3$)$_3$CO*C*O), 143.5, 135.1, 135.0, 133.5, 133.4, 132.9, 127.5, 127.2, 127.2, 125.8 (Ar-*C*), 78.7 ((CH$_3$)$_3$*C*OCO), 65.4 (*C*H$_2$- anthraquinone), 49.6 (CONH*C*H), 28.6 (OC(*C*H$_3$)$_3$), 17.3 (CH*C*H$_3$). IR (cm^{-1}): $\nu_{(C=O)ester}$: 1747 cm^{-1}, $\nu_{(C=O)ketone}$: 1687 cm^{-1}, $\nu_{(C=O)carbamate}$: 1673 cm^{-1}, $\nu_{(N-H)amine}$: 3364 cm^{-1}. Anal. calculated for $C_{23}H_{23}NO_6$: C, 67.47; H, 5.66; N, 3.42. Found: C, 66.90; H, 5.14; N, 3.26. HRMS *m/z* $C_{23}H_{23}NO_6$ $[M+Na]^+$ calcd. 432.2, found 432.1; $[M+K]^+$ calcd. 448.1, found 448.2; $[M-(C_{15}H_9O_2)]^+$ calcd. 221.1, found 221.0.

*(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (tert-butoxycarbonyl)-L-phenylalaninate (**9**)*: Yellow (90%); m.p.: 130-131 ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.21-8.15 (m, 4H, Ar-*H*), 7.95-7.91 (m, 2H, Ar-*H*), 7.78 (d, 1H, NH, *J*= 8.0 Hz), 7.47 (d, 1H, Ar-*H*, *J*= 8.0 Hz), 7.31-7.19 (m, 5H, Ar-*H*), 5.32 (s, 2H, C*H*$_2$- anthraquinone), 4.33-4.27 (m, 1H, OCONHC*H*), 3.09-3.04 (m, 1H, CHC*H*$_2$Ph), 2.98-2.92 (m, 1H, CHC*H*$_2$Ph), 1.33 (s, 9H, OC(C*H*$_3$)$_3$).^{13}C NMR (100 MHz, DMSO-d_6): δ 182.7 (*C*O-anthraquinone), 182.6 (*C*O- anthraquinone), 172.5 (NHCH*C*O), 155.9 ((CH$_3$)$_3$CO*C*O), 143.2, 137.9, 135.0, 133.6, 133.5, 133.5, 133.4, 132.9, 129.6, 128.7, 127.5, 127.3, 127.2, 127.0, 126.0 (Ar-*C*), 78.9 ((CH$_3$)$_3$*C*OCO), 65.5 (*C*H$_2$- anthraquinone), 55.9 (CONH*C*H), 36.8 (CH*C*H$_2$Ph), 28.6 (OC(*C*H$_3$)$_3$). IR (cm^{-1}): $\nu_{(C=O)ester}$: 1728 cm^{-1}, $\nu_{(C=O)ketone}$: 1688 cm^{-1}, $\nu_{(C=O)carbamate}$: 1676 cm^{-1}, $\nu_{(N-H)amine}$: 3341 cm^{-1}. Anal. calculated for $C_{29}H_{27}NO_6$: C, 71.74; H, 5.61; N, 2.88. Found: C, 71.42; H, 5.94; N, 3.09. HRMS *m/z* $C_{29}H_{27}NO_6$ $[M+H]^+$ calcd. 486.2, found 586.1; $[M+NH_4]^+$ calcd. 503.2, found 503.2; $[M+Na]^+$ calcd.

508.2, found 508.2; $[M+HCOOH]^+$ calcd. 531.2, found 531.2; $[M-(C_{15}H_9O_2)]^+$ calcd. 221.1, found 221.0.

(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (tert-butoxycarbonyl)-L-valinate (***10***): White (62%); m.p.: 136-137 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.22-8.18 (m, 4H, Ar-*H*), 7.96-7.89 (m, 3H, Ar-*H* + NH), 7.33 (d, 1H, Ar-*H*, *J*= 8.0 Hz), 5.40-5.31 (m, 2H, CH_2- anthraquinone), 3.97-3.93 (m, 1H, OCONHC*H*), 2.12-2.02 (m, 1H, C*H*$(CH_3)_2$), 1.39 (s, 9H, OC(CH_3)$_3$), 0.90 (d, 6H, CH(CH_3)$_2$, *J*= 8.0 Hz).^{13}C NMR (100 MHz, DMSO-d_6): δ 182.7 (*C*O- anthraquinone), 182.6 (*C*O- anthraquinone), 172.4 (NHCH*C*O), 156.3 ($(CH_3)_3COCO$), 143.4, 135.1, 135.1, 133.7, 133.6, 133.5, 133.0, 127.5, 127.3, 127.2, 126.1 (Ar-*C*), 78.8 ($(CH_3)_3COCO$), 65.3 (*C*H$_2$- anthraquinone), 60.1 (CONH*C*H), 30.0 (*C*H$(CH_3)_2$), 28.6 (OC(*C*H$_3$)$_3$), 19.5 and 18.9 (CH(*C*H$_3$)$_2$). IR (cm^{-1}): $\nu_{(C=O)ester}$: 1736 cm^{-1}, $\nu_{(C=O)carbamate}$: 1674 cm^{-1}, $\nu_{(C=O)ketone}$: 1687 cm^{-1}, $\nu_{(N-H)amine}$: 3348 cm^{-1}. Anal. calculated for $C_{25}H_{27}NO_6$: C, 68.64; H, 6.22; N, 3.20. Found: C, 68.38; H, 6.83; N, 3.21. HRMS *m/z* $C_{25}H_{27}NO_6$ $[M+Na]^+$ calcd. 460.1, found 460.2; $[M+K]^+$ calcd. 476.1, found 476.2; $[M-(C_{15}H_9O_2)]^+$ calcd. 221.1, found 221.0.

(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (tert-butoxycarbonyl)-L-alloisoleucinate (***11***): White (72%); m.p.: 113-114 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.21-8.17 (m, 4H, Ar-*H*), 7.95-7.88 (m, 3H, Ar-*H* + NH), 7.33 (d, 1H, Ar-*H*, *J*= 8.0 Hz), 5.40-5.30 (m, 2H, CH_2-anthraquinone), 4.02-3.98 (m, 1H, OCONHC*H*), 1.86-1.79 (m, 1H, CHC*H*$(CH_3)CH_2CH_3$), 1.39 (s, 9H, OC(CH_3)$_3$), 1.28-1.18 (m, 2H, CHCH(CH_3)CH_2CH$_3$), 0.87-0.81 (m, 6H, CHCH(CH_3)CH$_2$CH_3).^{13}C NMR (100 MHz, DMSO-d_6): δ 182.7 (*C*O-anthraquinone), 182.6 (*C*O-anthraquinone), 172.4 (NHCH*C*O), 156.2 ($(CH_3)_3COCO$), 143.4, 135.1, 135.0, 133.7, 133.5, 133.5, 133.0, 127.5, 127.2, 127.2, 126.1 (Ar-*C*), 78.8 ($(CH_3)_3COCO$), 65.3 (*C*H$_2$-anthraquinone), 58.9 (CONH*C*H), 36.4 (CHC*H*$(CH_3)CH_2CH_3$), 28.6 (OC(CH_3)$_3$), 25.4 (CHCH(CH_3)*C*H$_2$CH$_3$), 16.0 (CHCH(*C*H$_3$)CH$_2$CH$_3$), 11.6 (CHCH(CH_3)CH$_2$*C*H$_3$). IR (cm^{-1}): $\nu_{(C=O)ester}$: 1736 cm^{-1}, $\nu_{(C=O)ketone}$: 1682 cm^{-1}, $\nu_{(C=O)carbamate}$: 1674 cm^{-1}, $\nu_{(N-H)amine}$: 3351 cm^{-1}. Anal. calculated for $C_{26}H_{29}NO_6$: C, 69.16; H, 6.47; N, 3.10. Found: C, 69.96; H, 6.50; N, 2.85. HRMS *m/z* $C_{26}H_{29}NO_6$ $[M+Na]^+$ calcd. 474.2, found 474.1; $[M+K]^+$ calcd. 490.1, found 490.2; $[M-(C_{15}H_9O_2)]^+$ calcd. 221.1, found 221.0.

(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (tert-butoxycarbonyl)-L-methioninate (***12***): Cream (89%); m.p.: 117-118 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.23-8.20 (m, 4H, Ar-*H*), 7.96-7.89 (m, 3H, Ar-*H* + NH), 7.45 (d, 1H, Ar-*H*, *J*= 8.0 Hz), 5.41-5.32 (m, 2H, CH_2- anthraquinone), 4.25-4.20 (m, 1H, OCONHC*H*), 2.57-2.51 (m, 2H, $CHCH_2CH_2SCH_3$), 2.04 (s, 3H, $CHCH_2CH_2SCH_3$), 1.99-1.92 (m, 2H, $CHCH_2CH_2SCH_3$), 1.39 (s, 9H, OC(CH_3)$_3$).^{13}C NMR (100 MHz, DMSO-d_6): δ 182.8 (*C*O-anthraquinone), 182.6 (*C*O- anthraquinone), 172.7 (NHCH*C*O), 156.1 ($(CH_3)_3COCO$), 143.4, 135.1, 133.6, 133.5, 133.0, 127.5, 127.3, 127.2, 126.0 (Ar-*C*), 78.9 ($(CH_3)_3COCO$), 65.6 (*C*H$_2$- anthraquinone), 53.1 (CONH*C*H), 30.6 ($CHCH_2CH_2SCH_3$), 30.1 ($CHCH_2CH_2SCH_3$), 28.6 (OC(*C*H$_3$)$_3$), 15.0 ($CHCH_2CH_2SCH_3$). IR (cm^{-1}): $\nu_{(C=O)ester}$: 1733 cm^{-1}, $\nu_{(C=O)ketone}$: 1688 cm^{-1}, $\nu_{(C=O)carbamate}$: 1674 cm^{-1}, $\nu_{(N-H)amine}$: 3348 cm^{-1}. Anal. calculated for $C_{25}H_{27}NO_6S$: C, 63.95; H, 5.80; N, 2.98; S, 6.83. Found: C, 63.98; H, 5.27; N, 3.09; S, 6.67. HRMS *m/z* $C_{25}H_{27}NO_6S$ $[M+Na]^+$ calcd. 492.2, found 492.1; $[M+K]^+$ calcd. 508.1, found 508.1; $[M-(C_{15}H_9O_2)]^+$ calcd. 221.1, found 221.0.

(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (tert-butoxycarbonyl)-L-tryptophanate (***13***):Yellow (75%); m.p.: 154-155 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.87 (s, 1H, indole-N*H*), 8.23-8.12 (m, 4H, Ar-*H*), 7.97-7.92 (m, 2H, Ar-*H* + NH), 7.64 (d, 1H, Ar-*H*, *J*= 8.0 Hz), 7.52 (d, 1H, Ar-*H*, *J*= 8.0 Hz), 7.41 (d, 1H, Ar-*H*, *J*= 8.0 Hz), 7.34 (d, 1H, Ar-*H*, *J*= 8.0 Hz), 7.18 (bs, 1H, Ar-*H*), 7.08-6.98 (m, 2H, Ar-*H*), 5.29 (s, 2H, CH_2-anthraquinone), 4.37-4.31 (m, 1H, OCONHC*H*), 3.22-3.17 (m, 1H, (3-indolyl)CH_2CH), 3.12-3.06 (m, 1H, (3-indolyl)CH_2CH), 1.35 (s, 9H, OC(CH_3)$_3$).^{13}C NMR (100 MHz, DMSO-d_6): δ 182.7 (*C*O- anthraquinone), 182.6 (*C*O- anthraquinone), 172.8 (NHCH*C*O), 155.9 ($(CH_3)_3COCO$), 143.3, 136.6, 135.1, 133.5, 133.5, 133.4, 132.9, 127.5, 127.4, 127.3, 127.2, 126.0,

124.3, 121.4, 118.9, 118.5, 111.9, 110.0 (Ar-*C*), 78.8 ((CH$_3$)$_3$*C*OCO), 65.4 (*C*H$_2$- anthraquinone), 55.4 (CONH*C*H), 28.6 (OC(*C*H$_3$)$_3$), 27.3 ((3-indolyl)*C*H$_2$CH). IR (cm^{-1}): $\nu_{(C=O)ester}$: 1748cm^{-1}, $\nu_{(C=O)ketone}$: 1689 cm^{-1}, $\nu_{(C=O)carbamate}$: 1676 cm^{-1}, $\nu_{(N-H)amine}$: 3397,3350 cm^{-1}. Anal. calculated for $C_{31}H_{28}N_2O_6$: C, 70.98; H, 5.38; N, 5.34. Found: C, 70.35; H, 5.45; N, 5.78. HRMS *m/z* $C_{31}H_{28}N_2O_6$ [M+Na]$^+$ calcd. 547.2, found 547.1; [M+K]$^+$ calcd. 563.2, found 563.1; [M-H]$^-$ calcd. 523.2, found 523.1; [M+Cl]$^-$ calcd. 559.2, found 559.1; [M-($C_{15}H_9O_2$)]$^+$ calcd. 221.1, found 221.1.

*(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (((9H-fluoren-9-yl)methoxy)carbonyl)glycinate (**14**):* Yellow (88%); m.p.: 180-181 ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.18-8.11 (m, 4H, Ar-*H*), 7.92-7.80 (m, 6H, Ar-*H* + NH), 7.67 (d, 2H, Ar-*H*, *J*= 8.0 Hz), 7.39-7.27 (m, 4H, Ar-*H*), 5.34 (s, 2H, C*H*$_2$- anthraquinone), 4.32 (d, 2H, CHC*H*$_2$OCO, *J*= 8.0 Hz), 4.18 (t, 1H, C*H*CH$_2$OCO, *J*= 6.0 Hz), 3.93 (d, 2H, OCONHC*H*$_2$, *J*= 8.0 Hz).^{13}C NMR (100 MHz, DMSO-d_6): δ 182.6 (*C*O- anthraquinone), 182.5 (*C*O- anthraquinone), 170.6 (NHCH$_2$*C*O), 157.1 (CHCH$_2$O*C*O), 144.2, 143.1, 141.1, 135.0, 135.0, 133.8, 133.5, 133.4, 132.9, 128.0, 127.5, 127.2, 127.2, 126.1, 125.6, 120.5 (Ar-*C*), 66.3 (*C*H$_2$- anthraquinone), 65.6 (CH*C*H$_2$OCO), 47.0 (*C*HCH$_2$OCO), 42.8 (OCONH*C*H$_2$). IR (cm^{-1}): $\nu_{(C=O)ester}$: 1765 cm^{-1}, $\nu_{(C=O)ketone}$: 1704cm^{-1}, $\nu_{(C=O)carbamate}$: 1673 cm^{-1}, $\nu_{(N-H)amine}$: 3343 cm^{-1}. Anal. calculated for $C_{32}H_{23}NO_6$: C, 74.27; H, 4.48; N, 2.71. Found: C, 74.03; H, 4.05; N, 2.57. HRMS *m/z* $C_{32}H_{23}NO_6$ [M+H]$^+$ calcd. 518.2, found 518.1; [M+NH$_4$]$^+$ calcd. 535.2, found 535.1; [M+Na]$^+$ calcd. 540.1, found 540.1; [M+K]$^+$ calcd. 556.2, found 556.1; [M-($C_{15}H_9O_2$)]$^+$ calcd. 221.1, found 221.0.

*(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl(((9H-fluoren-9-yl)methoxy)carbonyl)-L-phenylalaninate (**15**):* Yellow (%78); m.p.: 198-199 ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.19-8.03 (m, 5H, Ar-*H*), 7.93 (bs, 2H, Ar-*H* + NH), 7.77 (d, 2H, Ar-*H*, *J*= 8.0 Hz), 7.59 (t, 2H, Ar-*H*, *J*= 8.0 Hz), 7.36-7.22 (m, 10H, Ar-*H*), 5.33 (s, 2H, C*H*$_2$- anthraquinone), 4.43-4.28 (m, 2H, CHC*H*$_2$OCO), 4.19-4.08 (m, 2H, C*H*CH$_2$OCONHC*H*), 3.18-3.13 (m, 1H, CHC*H*$_2$Ph), 3.03-2.97 (m, 1H, CHC*H*$_2$Ph).^{13}C NMR (100 MHz, DMSO-d_6): δ 182.6 (*C*O- anthraquinone), 182.5 (*C*O- anthraquinone), 172.1 (NHCH*C*O), 156.5 (CHCH$_2$O*C*O), 144.2, 144.1, 143.0, 141.1, 137.9, 135.0, 133.7, 133.5, 133.4, 132.9, 129.6, 128.6, 128.0, 127.4, 127.2, 127.2, 127.0, 126.1, 125.6, 120.5 (Ar-*C*), 66.2 (*C*H$_2$- anthraquinone), 65.7 (CH*C*H$_2$OCO), 56.1 (CONH*C*H), 46.9 (*C*HCH$_2$OCO), 36.7 (CH*C*H$_2$Ph). IR (cm^{-1}): $\nu_{(C=O)ester}$: 1742 cm^{-1}, $\nu_{(C=O)ketone}$: 1702 cm^{-1}, $\nu_{(C=O)carbamate}$: 1677 cm^{-1}, $\nu_{(N-H)amine}$: 3314 cm^{-1}. Anal. calculated for $C_{39}H_{29}NO_6$: C, 77.09; H, 4.81; N, 2.31. Found: C, 77.61; H, 4.55; N, 2.00. HRMS *m/z* $C_{39}H_{29}NO_6$ [M+H]$^+$ calcd. 608.2, found 608.2; [M+NH$_4$]$^+$ calcd. 625.2, found 625.1; [[M+K]$^+$ calcd. 646.2, found 646.1; [M-($C_{15}H_9O_2$)]$^+$ calcd. 221.1, found 221.0.

*Synthesis of (S)-1-((9,10-dioxo-9,10-dihydroanthracen-2-yl)methoxy)-1-oxo-3-phenylpropan-2-aminium 2,2,2-trifluoroacetate (**16**)*

(9,10-Dioxo-9,10-dihydroanthracen-2-yl)methyl (tert-butoxycarbonyl)-L-phenylalaninate (**9**) (0.15 mmol) was dissolved in TFA /DCM mixture (1.5 / 1.5 mL) and stirred for 2 hours at room temperature according to the literature method.[38] 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. Cream solid (89%); m.p. 167-168 ºC; ^{1}H NMR (400 MHz, DMSO) δ 8.65 (s, 3H, N*H*$^+_3$), 8.23-8.15 (m, 4H, Ar-*H*), 7.97-7.94 (m, 2H, Ar-*H*), 7.71 (d, 1H, Ar-*H*, *J*= 8 Hz), 7.33-7.24 (m, 5H, Ar-*H*), 5.41-5.33 (m, 2H, OC*H*$_2$-anthraquinone), 4.48 (t, 1H, C*H*CH$_2$Ph, *J* = 8 Hz), 3.24-3.19 and 3.14-3.09 (2m, 2H, CHC*H*$_2$Ph). ^{13}C NMR (101 MHz, DMSO) δ 182.7 (*C*O-anthraquinone), 182.6 (*C*O-anthraquinone), 169.4 (CH*C*OO), 168.5 (*C*OCH), 158.6 (F$_3$C*C*O, q, $^2J_{(C-F)=}$ 30 Hz), 142.1, 135.2, 135.1, 135.0, 134.1, 133.5, 133.5, 133.2, 129.8, 129.1, 127.8, 127.5, 127.3, 126.6 (Ar-*C*), 117.6 (q, *C*F$_3$CO, $^1J_{(C-F)}$= 290 Hz) (Ar-*C*), 66.6 (*C*H$_2$-anthraquinone), 53.7 (CONH*C*H), 36.6 (*C*H$_2$Ph). Elemental analysis: $C_{26}H_{20}F_3NO_6$ required C, 62.53; H, 4.04; N, 2.80; found C, 62.48; H, 4.04; N, 2.78.

2.3. CA Inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity by using method of Khalifah.[28,39] Phenol red (at a concentration of 0.2mM) 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 the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7–17mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5%-10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled–deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow the formation of the E-I complex. The inhibition constants were obtained by non-linear least-square methods using PRISM (www.graphpad.com), and non-linear least squares methods, values representing the mean of at least three different determinations, as described earlier by us. [40-45]

2.4.Antioxidant Activity

2.4.1. Radical Scavenging Activity Using DPPH method

Antioxidant activity was determined based on the ability of the antioxidants to act as radical scavengers towards the stable free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH). As detailed by Yang, Guo, and Yuan (2008),[29] 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 equation

$$[1 – (As\text{-}Ao)/Ab]x100$$

whereby As was the absorbance reading for samples containing antioxidant, Ao was the absorbance of the antioxidant in pure ethanol and Ab corresponded to the absorbance of the DPPH solution.

2.4.2. Ferric Ions Reducing Power Assay

The reducing power of the compounds and standards was determined as described by Oyaizu.[30] In brief, different concentrations of compounds (5.88, 14.70 and 29.41 μg/mL) in 1 mL ethanol were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide and incubated at 50°C for 20 min. At the end of the incubation, 2.5 mL of 10% trichloroacetic acid solution was added and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm against blank that contained distilled water and phosphate buffer. The reducing power tests were run in triplicate. Increase in absorbance of the reaction mixture indicated the reducing power of the samples/standards. α-tocopherol, BHT or BHA was used as a positive control.

2.4.3. Metal Chelating Activity

Metal chelating activity was measured spectrophotometrically, by the method of Carter (1971).[31] 50 μL of 2.0 mM $FeCl_2$ is added to different concentrations of the compounds dissolved in ethanol. After 10 min of incubation at room temperature (25 °C), 200 μL of 5.0 mM ferrozine solution is added to this mixture. The mixture is again incubated for 25 min at room temperature (25 °C) and then the absorbance is measured at 562 nm using a spectrophotometer against blank (3.75 mL ethanol, 50 μL $FeCl_2$). The solution of 3.75 mL ethanol, 50 μL $FeCl_2$, and 200 μL ferrozine is used as a control. Percentage of inhibition is calculated using the formula:

$$\text{Metal chelating activity} = (A_{control} - A_{sample/standard})/A_{control} \times 100$$

Where $A_{control}$ is the absorbance of control reaction (without analysed sample extract), and A_{sample}/ $A_{standard}$ is the absorbance of the analysed sample/standard. The values are presented as the mean of three measurements. Ethylenediaminetetraacetic acid (EDTA) was used as a positive control.

3. Results and Discussion

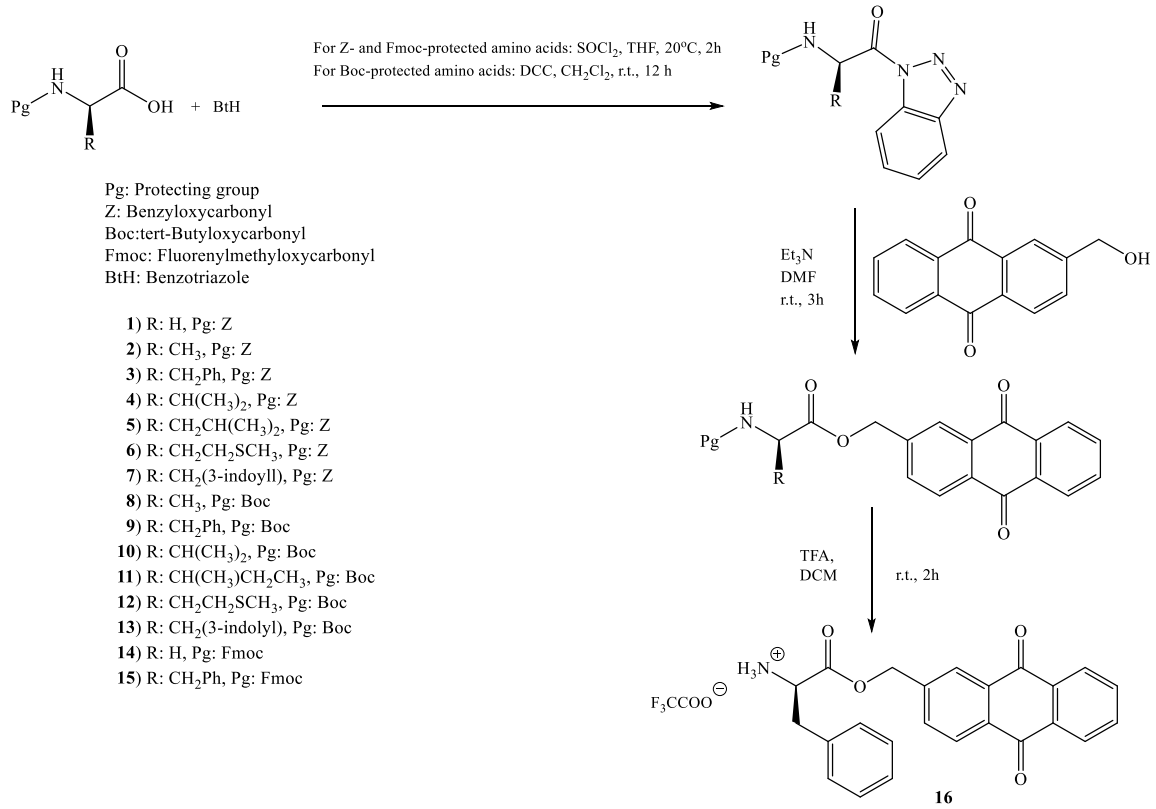
3.1. Chemistry

Anthraquinones, consisting of a tricyclic planar system, exhibit a wide range of important pharmaceutical and technological properties depending on their substituents.[4] Despite their important pharmaceutical and technological properties, monopeptide-antraquinone derivatives have not been synthesized up to now. As far as we know, there are only a few reports about the synthesis of anthraquinone-containing peptide derivatives in the literatur.[17,26,46] To fill this gap in the literature, we planned and synthesized a series of monopeptide-anthraquinone conjugates from the reaction of 2-hydroxymethyl-anthraquinone with 1-(N-protected aminoacyl)benzotriazole in DMF (N,N-dimethylformamide) in the presence of Et_3N. The synthesis of the N-protected monopeptide-anthraquinone conjugates **1**-**16** is summarized in the Scheme 1. Kewmp and Reczek mentioned 2-oxymethyleneanthraquinone (Maq) as a protecting group in peptide synthesis in their study,[47] although they shared information about compound **1**, but since it appeared as new in the SciFinder database search, all information about this compound is included in this article.

All the synthesized monopeptide-anthraquinone conjugates were crystalline solids, air stable and had sharp melting points. The structures of N-protected monopeptide-anthraquinone conjugates (**1**-**16**) were elucidated by ^{1}H NMR, ^{13}C NMR, IR, mass and elemental analyses. While the characteristic methylene resonances of the 2-hydroxymethylanthraquinone moiety of the monopeptide-anthraquinone derivatives for compounds **1**-**3**, **7**, **9**, **13**-**16** were observed at 5.29-5.36 ppm region as singlet peak, for compounds **4**-**6**, **8**, **10**-**12** were observed as multiplet due to chiral neighboring effects of the asymmetric carbons of the amino acids in the ^{1}H NMR spectra. Similar peak splitting was observed for the methylene peak of the benzyloxycarbonyl-protecting group of compounds **3**, **4**, **5**, **6** and **7**. For compound **1**, the NH proton signal was observed at 7.82 ppm as the triplet peak, while those of compounds **3**, **6**, and **9** were observed as a doublet peaks in the range of 7.78-7.87 ppm as expected in the ^{1}H NMR spectra. All other NH peaks resonated with some aromatic protons in the region of 7.97-7.80 ppm, while that of compound **15** was observed as a large single peak at 7.93 ppm. In Compound **9**, the single peak of the *tert*-butoxy group of about 1.33 ppm was disappeared in compounds **16** where the Boc protecting group was removed.

Similarly, the quaternary carbon peak around 78,9 ppm and the carbamate carbonyl peak around 155.9 ppm seen in the ^{13}C NMR spectra of the compounds **9** disappeared in compounds **16**. After removing the Boc protecting group, the free amino group formed was converted to trifluoroacetate salt by taking a proton from TFA. This was confirmed by a broad singlet peak corresponding to 3 protons at 8.65 ppm in the proton NMR spectra of compounds **16** and their element analysis result. Similar results were obatined in our previous study[27]. In compounds **16**, carbon peaks belonging to the trifluoroacetate group were seen as quartet in the carbon 13 spectra at around 158 and 117 ppm. All NH protons were confirmed by deuterium exchange by D_2O. Carbonyl resonances of the anthraquinone moiety for monopeptide-anthraquinone conjugates were observed around 182.8-182.5 ppm. Carbonyl resonances of the ester carbonyl and carbamate carbonyl for monopeptide-anthraquinone conjugates were observed around 174.4-170.6 and 157.1-155.8 ppm, respectively. All other aliphatic and aromatic protons and carbons peaks of monopeptide-antarquinone conjugates are seen in the expected places and confirm the proposed structures, as can be seen from the spectra given in the supporting information files, which are not explained here individually. IR spectra of mono peptide-anthraquinone conjugates, **1**-**15**, showed characteristic ester, ketone and carbamate carbonyl peaks around between 1749-1722 cm^{-1}, 1711-1682 cm^{-1}, 1684-1613 cm^{-1}, respectively. While no peak of carbamate carbonyl was observed in the IR spectrum of compound **16**, other carbonyls were seen at 1745, 1672 and 1645 cm^{-1}. While NH

vibrations were observed between 3374-3314 cm^{-1} in the IR spectra of compounds **1-15**, this peak was observed at 3159 cm^{-1} ppm in compound **16** as NH_3. When the mass spectra of the compounds (**1-15**) were examined, fragmentation compatible with the targeted structures and molecular ion peaks were clearly observed.



Pg: Protecting group
Z: Benzyloxycarbonyl
Boc:tert-Butyloxycarbonyl
Fmoc: Fluorenylmethyloxycarbonyl
BtH: Benzotriazole

1) R: H, Pg: Z
2) R: CH_3, Pg: Z
3) R: CH_2Ph, Pg: Z
4) R: $CH(CH_3)_2$, Pg: Z
5) R: $CH_2CH(CH_3)_2$, Pg: Z
6) R: $CH_2CH_2SCH_3$, Pg: Z
7) R: CH_2(3-indoyll), Pg: Z
8) R: CH_3, Pg: Boc
9) R: CH_2Ph, Pg: Boc
10) R: $CH(CH_3)_2$, Pg: Boc
11) R: $CH(CH_3)CH_2CH_3$, Pg: Boc
12) R: $CH_2CH_2SCH_3$, Pg: Boc
13) R: CH_2(3-indolyl), Pg: Boc
14) R: H, Pg: Fmoc
15) R: CH_2Ph, Pg: Fmoc

Scheme 1. Synthetic pathways of N-protected monopeptide-anthraquinone conjugates, **1-16**

3.2. Carbonic Anhydrase Inhibition

All the synthesized monopeptide-anthraquinone conjugates have been evaluated by means of a stopped flow CO_2 hydrase assa[28] to test their inhibitory potency against four human (h) CA isoforms (hCA I and hCA II). Inhibition data are reported in Table 1, along with those referred to acetazolamide (AAZ), used as standard sulfonamide inhibitor. As seen in Table 1, none of the compounds showed inhibition below Ki 100µM concentrations against hCA I enzyme. However, compounds **7**, **13** and **15** showed considerable inhibition against CA II with Ki values in the low µM levels 43.5, 78.2 and 67.4, respectively. When the structures of compounds effective against hCA II are examined, it is understood that compounds **7** and **13**, which contain a tryptophan amino acid moiety, play an important role in inhibition. The inhibition effect in compound **15** that shows inhibition by Ki 67.4 M against hCA II is thought to be effective together with the phenylalanine and Fmoc (fluorenylmethyloxycarbonyl) group. In order to see the effect of amino acid protecting group in monopeptide-anthraquinone conjugates on CA I and CA II enzyme inhibitions, the protecting group of compound **9** was removed. When the CA I and CA II enzyme inhibition results of the deprotected compound **16** were compared with the compound **9**, there was no change in the CA I and CA II enzyme inhibition results.

Table 1. Inhibition data of hCA I and hCA II, with compounds **1-16** and the standard sulfonamide inhibitor acetazolamide (**AAZ**) by a stopped flow CO_2 hydrase assay

	K_I (μM)*	
Compound	**hCA I**	**hCA II**
1	>100	>100
2	>100	>100
3	>100	>100
4	>100	>100
5	>100	>100
6	>100	>100
7	>100	43.5
8	>100	>100
9	>100	>100
10	>100	>100
11	>100	>100
12	>100	>100
13	>100	78.2
14	>100	>100
15	>100	67.4
16	>100	>100
AAZ	0.25	0.012

* Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5-10 % of the reported values).

3.3. Antioxidant Activity

3.3.1. DPPH Radical Scavenging Activity

The antioxidant activities of the monopeptide-anthraquinone conjugates were determined by considering the radical scavenging capacity of the antioxidants against the stable free radical, 1,1-diphenyl-2-picrylhydrazil (DPPH).[29] Antioxidant testing results are reported in Table 2, along with those referred to BHA, BHT and α-tocopherol, commonly used as standard antioxidants. When the antioxidant activities of the compounds were examined, it was seen that the compounds showing the best antioxidant activity were in the tryptophan (compounds **7** and **13**), methionine (compounds **6** and **12**) and valine (compounds **4** and **10**) amino acids and anthraquinone conjugates. When the obtained antioxidant activities are evaluated in terms of protecting groups in monopeptide-anthraquinone conjugates, it can be said that in general, Fmoc (compound **15**) and Boc (compound **8**-**13**) protecting groups contribute more to antioxidant activity compared to Z (compounds **1**-**7**). In order to see the effect of amino acid protecting group in monopeptide-anthraquinone conjugates on antioxidant activity, the protecting group of compound **9** was removed. When the antioxidant results of compound **16**, obtained by removing the protecting group, were compared with compound **9**, a significant increase was observed in the antioxidant properties of compound **16**. In our previous study, we reached a similar conclusion regarding antioxidant activity.[48]

Table 2. Antioxidant activities of the synthesized monopeptide-athraquinone derivatives according to radical Scavenging method

Compound	DPPH Free Radical Scavenging Activity, %*				
	12.5 µM	25 µM	37.5 µM	62.5 µM	125 µM
1	3.908	6.255	9.382	9.382	9.382
2	3.785	2.270	4.543	5.299	4.453
3	4.303	6.660	8.525	11.444	12.849
4	9.231	13.818	13.335	19.828	32.124
5	8.168	9.627	10.332	12.084	13.186
6	2.677	14.673	27.346	27.307	37.285
7	15.408	20.476	30.599	39.341	45.962
8	13.133	14.748	15.626	17.190	22.656
9	6.915	8.135	8.996	14.022	14.204
10	13.050	15.353	19.176	26.822	39.904
11	9.668	10.844	11.824	15.616	26.773
12	13.587	17.163	19.309	27.835	36.402
13	17.284	26.885	32.608	42.249	55.668
14	3.242	3.654	3.674	3.890	4.126
15	8.289	16.579	18.236	26.527	36.474
16	8.795	10.133	13.193	26.575	37.151
BHA	86.233	86.221	86.222	88.145	88.114
BHT	65.657	73.869	75.654	75.987	78.181
α-Tocopherol	87.242	88.343	89.125	90.343	90. 357

* Mean from 3 different assays (errors were in the range of ± 0.3-1.0 % of the reported values)

3.3.2. Ferric Ions Reducing Power Assay

Antioxidant results of the ferric ion reducing power of the compounds are given in Table 3 together with the standard antioxidants BHA, BHT and alpha tocopherol. When the values in Table 3 were compared with the antioxidant values of the standard compounds, it was determined that monopeptide-anthraquinone conjugates had weak antioxidant capacity. Although weak, compounds **7** and **13** containing tryptophan amino acid, **11** compounds containing isoleucine amino acid and **16** compounds containing phenylalanine amino acid from which the protecting group was removed showed the best antioxidant activity.

3.3.3. Metal Chelating Activity

None of the compounds showed antioxidant activity according to the metal chelation method made according to the Carter method.

Table 3. Antioxidant activities of the synthesized monopeptide-athraquinone derivatives according to ferric ions reducing power

Compound	Ferric ions reducing power (Absorbance, 700 nm)		
	5.88 µg/mL	**14.70 µg/mL**	**29.41 µg/mL**
1	0.094	0.104	0.114
2	0.081	0.093	0.097
3	0.094	0.097	0.111
4	0.094	0.099	0.107
5	0.096	0.095	0.095
6	0.070	0.075	0.091
7	0.069	0.091	0.141
8	0.094	0.096	0.098
9	0.076	0.079	0.094
10	0.073	0.097	0.105
11	0.095	0.114	0.161
12	0.090	0.094	0.114
13	0.085	0.097	0.156
14	0.093	0.095	0.116
15	0.079	0.090	0.095
16	0.094	0.095	0.124
BHA	0.464	1.296	1.827
BHT	0.257	0.491	0.844
α-Tocopherol	0.220	0.456	0.779

4. Conclusion

In conclusion, we prepared sixteen new ester-structured monopeptide-anthraquinone conjugates using the benzotriazole methodology. To prepare these compounds, first the carbonyl carbon of the amino acid with the protecting group was activated with benzotriazole, an easily leaving group, and then esterified with 2-hydroxymethylanthraquinone. Human carbonic anhydrase hCA I and hCA II enzyme inhibition and antioxidant capacities of the compound were evaluated by the stopped-flow and DPPH methods, respectively. However, only three compounds (**7**, **13** and **15**) showed some inhibition properties against hCA II enzymes and none of the compounds showed inhibitory properties against hCA I enzyme. The antioxidant capacities of the compounds were determined by DPPH, ferric ion reducing assay and metal chelation methods. When the antioxidant capacities of the compounds were compared with the results of the standard antioxidants BHA, BHT and α-tocopherol, a small amount of antioxidant activity was observed according to the DPPH and ferric ion reducing power test methods, while none of the compounds showed antioxidant properties according to the metal chelating activity method using ethylenediaminetetracetic acid as a standard at the concentrations studied.

Disclosure statement

The author(s) confirm that this article content has no conflict of interest.

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Supporting Information

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References

[1] Park, J.; Park, S.; Park, J. Synthesis of new dye compounds based on anthraquinone moiety for color filter colorants. *Mol. Cryst. Liq. Cryst.* **2013**, *579*, 110-114.
[2] Kaim, W.; Lahiri, G.K. The coordination potential of indigo, anthraquinone and related redox-active dyes. *Coord. Chem. Rev.* **2019**, *393*, 1-8.
[3] Langdon-Jones, E.E.; Pope, S.J.A. The coordination chemistry of substituted anthraquinones: Developments and applications. *Coord. Chem. Rev*. **2014**, *269*, 32-53.
[4] Peixoto, D.; Figueiredo, M.; Malta, G.; Roma-Rodrigues, C.; Baptista, P.V.; Fernandes, A.R.; Barroso, S.; Carvalho, A.L.; Afonso, C.A.M.; Ferreira, L.M.; Paula S. Branco, P.S. Synthesis, cytotoxicity evaluation in human cell lines and in vitro DNA interaction of a hetero-arylidene-9(10H)-anthrone. *Eur. J. Org. Chem.* **2018**, 545-549.
[5] Dibble, D.; Kurakake, R.; Wardrip, A.G.; Bartlett, A.; Lopez, R.; Linares, J.A.; Firstman, M.; Schmidt, A.M.; Umerani, M.J.; Gorodetsky, A.A. Aza-Diels-Alder approach to diquinolineanthracene and polydiquinolineanthracene derivatives. *Org. Lett*. **2018**, *20*, 502-505.
[6] Qiao, M.; Liu, B.; Zhao, X.; Gong, Y.; Wang, Y.; Cao, W. Formation of oxygenated polycyclic aromatic hydrocarbons by photoelectrocatalysis using TiO_2 nanotubes. *RSC Adv.* **2017**, *7*, 51678-51686.
[7] Bardagi, J.I.; Ghosh, I.; Schmalzbauer, M.; Ghosh, T.; König, B. Anthraquinones as photoredox catalysts for the reductive activation of aryl halides. *Eur. J. Org. Chem*. **2018**, 34-40.
[8] Bartels, P.L.; Stodola, J.L.; Burgers, P.M.J.; Barton, J.K. A redox role for the [$4Fe_4S$] cluster of yeast DNA polymerase δ. *J. Am. Chem. Soc.* **2017**, *139*, 18339-18348.
[9] Inagawa, H.; Uchida, S.; Yamaguchi, E.; Itoh, A. Metal-free oxidative amidation of aromatic aldehydes using an anthraquinone-based organophotocatalyst. *Asian J. Org. Chem.* **2019**, *8*, 1411-1414.
[10] Petroselli, M.; Mosca, S.; Martí-Rujas, J.; Comelli.; Cametti, M. Mixed stacked charge-transfer π-organic materials based on anthracenyl boronic acid. *Eur. J. Org. Chem.* **2017**, 7190-7194.
[11] Hussein, Y.H.A.; Anderson, N.; Lian, T.T.; Abdou, I.M.; Strekowski, L.; Timoshchuk, V.A.; Vaghefi, M.M.; Thomas L. Netzel, T.L. Solvent and linker influences on AQ .--/dA .+ charge-transfer state energetics and dynamics in anthraquinonyl-linker-deoxyadenosine conjugates. *J. Phys. Chem. A* **2006**, *110*, 4320-8.
[12] Khankaew, S.; Mills, A.; Yusufu, D.; Wellsb, N.; Hodgenb, S.; Boonsupthipc, W.; Suppakula, P. Multifunctional anthraquinone-based sensors: UV, O_2 and time. *Sensors Actuators B. Chem.* **2017**, *238*, 76-82.
[13] Liu, W.; Dong, H.; Zhang, L.; Tian, Y. Development of an efficient biosensor for the In vivo monitoring of Cu+ and pH in the brain: Rational design and synthesis of recognition molecules. *Angew. Chem. Int. Ed.* **2017**, *56*, 16328-16332.
[14] Xiang, W.; Song, Q.S.; Zhang, H.J.; Guo, S.P. Antimicrobial anthraquinones from Morinda angustifolia. *Fitoterapia* **2008**, *79*, 501-504.
[15] Bringmann, G.; Mutanyatta-Comar, J.; Maksimenka, K.; Wanjohi, J.M.; Heydenreich, M.; Brun, R.; Müller, W.E.G.; Peter, M.G.; Jacob O. Midiwo, J.O.; Yenesew, A. Joziknipholones A and B: The first dimeric phenylanthraquinones, from the roots of Bulbine frutescens. *Chem. A Eur. J*. **2008**, *14*, 1420-1429.

[16] Mishra, S.K.; Tiwari, S.; Shrivastava, A.; Srivastava, S.; Boudh, G.K.; Chourasia , S.K.; Chaturvedi, U.; Mir , S.S.; Saxena, A.K.; Bhatia, G.; Lakshmi, V. Antidyslipidemic effect and antioxidant activity of anthraquinone derivatives from Rheum emodi rhizomes in dyslipidemic rats. *J. Nat. Med.* **2014**, *68*, 363-371.

[17] Hsin, L.W.; Wang, H.P.; Kao, P.H.; Lee, O.; Chen, W.R.; Chen, H.W.; Guh, J.H.; Chan, Y.L.; His, C.P.; Yang, M.S.; Tsai-Kun Lic, .K.; Lee, C.H. Synthesis, DNA binding, and cytotoxicity of 1,4- bis(2-amino-ethylamino)anthraquinone-amino acid conjugates. *Bioorg. Med. Chem*. **2008**, *16*, 1006-1014.

[18] Yan, Y.; Su, X.; Liang, Y.; Zhang, J.; Shi, C.; Lu, Y.; Gu, L.; Fu, L. Emodin azide methyl anthraquinone derivative triggers mitochondrial- dependent cell apoptosis involving in caspase-8-mediated Bid cleavage. *Mol. Cancer. Ther.* **2008**, *7*, 1688-1697.

[19] Lee, Y.K.; Bang, H.; Oh, J.B.; Whang, W.K. Bioassay-guided isolated compounds from morinda officinalis inhibit Alzheimer's disease pathologies. *Molecules* **2017**, *22*, Article number 1638, 1-12.

[20] Seo, E. J.; Ngoc, T. M.; Lee, S. M.; Kim, Y. S.; Jung, Y. S. Chrysophanol-8-O-glucoside, an anthraquinone derivative in rhubarb, has antiplatelet and anticoagulant activities. *J. Pharmacol. Sci*. **2012**, *118*, 245–54..

[21] Barnard, D.L.; Huffman, J. H.; Morris, J.L.B.; Wood, S.G.; Hughes, B.G.; Sidwell, R. W. Evaluation of the antiviral activity of anthraquinones, anthrones and anthraquinone derivatives against human cytomegalovirus. *Antiviral Res*. **1992**, *17*, 63–77.

[22] Campora, M.; Francesconi, V.; Schenone, S.; Tasso, B.; Tonelli, M. Journey on naphthoquinone and anthraquinone derivatives: New insights in alzheimer's disease. *Pharmaceuticals*. **2021**, *14*, 1–33..

[23] Routier, S.; Cotelle, N.; Catteau, J. P.; Bernier, J. L.; Waring, M.J.; Riou, J.F.; Bailly, C. Salen-anthraquinone conjugates. Synthesis, DNA-binding and cleaving properties, effects on topoisomerases and cytotoxicity. *Bioorg. Med. Chem.* **1996**, *4*, 1185–96.

[24] Evans, L.W.; Bender, A.; Burnett, L.; Godoy, L.; Shen, Y.; Staten, D.; Zhou, T.; Angermann, J.E.; Ferguson, B.S. Emodin and emodin-rich rhubarb inhibits histone deacetylase (HDAC) activity and cardiac myocyte hypertrophy. *J. Nutr. Biochem.* **2020**, *79*, Article Number: 108339.

[25] Luo, M.; Cui, Z.; Huang, H.; Song, X.; Sun, A.; Dang, Y.; Lu, L.; JJu, J. Amino acid conjugated anthraquinones from the marine-derived fungus penicillium sp. SCSIO sof101. *J. Nat. Prod.* **2017**, *80*, 1668-1673.

[26] Zagotto, G.; Sissi, C.; Lucatello, L.; Pivetta, C.; Cadamuro, S.A.; Fox, K.R.; Neidle, S.; Palumbo, M. Aminoacyl-anthraquinone conjugates as telomerase inhibitors: Synthesis, biophysical and biological evaluation. *J. Med. Chem*. **2008**, *51*, 5566-5574.

[27] Yuan, C.; He, Q.; Song, S.; Zhang, X.; Miao, Z.; Yang, C. One Pot and Metal-Free Approach to 3-(2-Hydroxybenzoyl)-1-aza- anthraquinones. *Molecules* **2019**, *24*, Article Number: 3017.

[28] Khalifah, R.G. The Carbon dioxide hydration activity of carbonic anhydrase. *J. Biol. Chem.* **1971**, *246(8)*, 2561-2573.

[29] Yang, J.; Guo, J.; Yuan, J. In vitro antioxidant properties of rutin. LWT - *Food Sci. Technol.* **2008**, *41*, 1060-1066.

[30] Oyaizu, M. Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese J. Nutr. Diet.* **1986**, *44*, 307–315.

[31] Carter, P. Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). *Anal. Biochem.* **1971**, *40*, 450-458.

[32] El-Khatib, M.; Jauregui, L.; Tala, S.R.; Khelashvili, L.; Katritzky, A.R. Solution-phase synthesis of chiral O-acyl isodipeptides. *Medchemcomm*. **2011**, *2*, 1087-1092.

[33] Küçükbay, F.Z.; Küçükbay, H.; Tanc, M.; Supuran, C.T. Synthesis and carbonic anhydrase inhibitory properties of amino acid-coumarin/quinolinone conjugates incorporating glycine, alanine and phenylalanine moieties. *J. Enzyme. Inhib. Med. Chem.* **2016**, *31*, 198-202.

[34] Küçükbay, F.Z.; Küçükbay, H.; Tanc, M.; Supuran, C.T. Synthesis and carbonic anhydrase I, II, IV and XII inhibitory properties of N- protected amino acid – sulfonamide conjugates. *J. Enzyme Inhib. Med. Chem.* **2016**, *31*, 1476-1483.

[35] Buğday, N.; Küçükbay, F.Z.; Apohan, E.; Küçükbay, H.; Serindağ, A.; Yeşilada, Ö. Synthesis and evaluation of novel benzimidazole conjugates incorporating amino acids and dipeptide moieties. *Lett. Org. Chem*. **2017**, *14*, 198-206.

[36] Küçükbay, H.; Buğday, N.; Küçükbay, F. Z.; Berrino, E.; Bartolucci, G.; Prete, S.D.; Capasso, C.; Supuran.; C.T. Synthesis and carbonic anhydrase inhibitory properties of novel 4-(2-aminoethyl)benzenesulfonamide-dipeptide conjugates. *Bioorg. Chem.* **2019**, *83*, 414-423.

[37] Katritzky, A .R.; Singh, S. A.; Haase, D. N.; Yoshioka, M. N-(Fmoc-α-aminoacyl)benzotriazoles: versatile synthetic reagents from proteinogenic amino acids. *Arkivoc* **2009**, *(viii)*, 47-56.

[38] Merrifield, B. Solid phase synthesis. *Science* **1986**, 232, 341-347.

[39] Khalifah, R. G. Edsall, J. Carbon dioxide hydration activity of carbonic anhydrase: kinetics of alkylated

anhydrases B and C from humans (metalloenzymes-isoenzymes-active sites-mechanism). *Proc. Natl. Acad. Sci. USA* **1972**, *69*, 172-176.

[40] Mincione, F.; Starnotti, M.; Menabuoni, L.; Scozzafava, A.; Casini, A.; Supuran, C.T. arbonic anhydrase inhibitors: 4-Sulfamoyl-benzenecarboxamides and 4-chloro-3-sulfamoyl-benzenecarboxamides with strong topical antiglaucoma properties. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1787-1791.

[41] Prete, S. D.; Vullo, D.; Luca, D. V.; AlOthman, Z.; Osman, S .M.; Supuran, C. T.; Capasso, C.. Biochemical characterization of recombinant β-carbonic anhydrase (PgiCAb) identified in the genome of the oral pathogenic bacterium Porphyromonas gingivalis. *J. Enzyme Inhib. Med. Chem.* **2015,** *30*, 366-370.

[42] Supuran, C. T.; Scozzafava, A. Carbonic anhydrases as targets for medicinal chemistry. *Bioorg. Med. Chem*. **2007**, *15*, 4336-4350.

[43] Maresca, A.; Vullo, D.; Scozzafava, A.; Manole, G.; Supuran, C. T. Inhibition of the β-class carbonic anhydrases from Mycobacterium tuberculosis with carboxylic acids. *J. Enzyme Inhib. Med. Chem*. **2014**, *28*, 392-396.

[44] Scozzafava, A.; Passaponti, M.; Supuran, C. T.; Gülçin, I. Carbonic anhydrase inhibitors: Guaiacol and catechol derivatives effectively inhibit certain human carbonic anhydrase isoenzymes (hCA I, II, IX and XII). *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 586-591.

[45] Küçükbay, H.; Buğday, N.; Küçükbay, F. Z.; Angeli, A.; Bartolucci, G.; Supuran, C. T.;Synthesis and human carbonic anhydrase I, II, VA, and XII inhibition with novel amino acid–sulphonamide conjugates *J. Enzyme Inhib. Med. Chem.* **2020**, *35*, 489-497.

[46] Bogdanowicz, R.; Sawczak, M.; Niedzialkowski, P.; Zieba, P.; Finke, B.; Ryl, J.; Ossowski, T. Direct amination of boron-doped diamond by plasma polymerized allylamine film. *Phys. Status Solidi A.* **2014**, *211*, 2319-327.

[47] Kemp, D. S.; Reczek, J. New protective groups for peptide synthesis--III the maq ester group mild reductive cleavage of 2-acyloxymethyleneanthraquinones. *Tetrahedron Lett*. **1977**, *18*, 1031–1034.

[48] Küçükbay, H.; Gönül, Z.; Küçükbay, F. Z.; Tekin, Z.; Angeli, A.; Bartolucci, G.; Supuran, C. T.; Tatlıcı, E.; Apohan, E.; Yeşilada, Ö. Synthesis of new 7-amino-3,4-dihydroquinolin-2(1*H*)-one-peptide derivatives and their carbonic anhydrase enzyme inhibition, antioxidant and cytotoxic activities. *Arch. Pharm. (Weinheim).* **2021,** *(July), Article Number: e2100122.*