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Synthesis, docking studies, in vitro cytotoxicity evaluation and DNA damage mechanism of new tyrosine-based tripeptides

Eray Çalışkan[1] | Alpaslan Kaplan[2] | Güldeniz Şekerci[3] | İrfan Çapan[4] | Suat Tekin[3] | Sultan Erkan[5] | Kenan Koran[2] | Süleyman Sandal[3] | Ahmet O. Görgülü[6]

[1]Department of Chemistry, Faculty of Science and Arts, Bingol University, Bingöl, Türkiye

[2]Department of Chemistry, Faculty of Science, Firat University, Elazig, Türkiye

[3]Physiology Department, Inonu University, Malatya, Türkiye

[4]Department of Material and Material Processing Technologies, Technical Sciences Vocational College, Gazi University, Ankara, Türkiye

[5]Department of Chemistry, Faculty of Science, Cumhuriyet University, Sivas, Türkiye

[6]Department of Chemistry, Faculty of Science, Marmara University, Istanbul, Türkiye

Correspondence
Kenan Koran, Department of Chemistry, Faculty of Science, Firat University, Elazig 23119, Türkiye.
Email: kkoran@firat.edu.tr

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Abstract

Peptides are one of the leading groups of compounds that have been the subject of a great deal of biological research and still continue to attract researchers' attention. In this study, a series of tripeptides based on tyrosine amino acids were synthesized by the triazine method. The cytotoxicity properties of all compounds against human cancer cell lines (MCF-7), ovarian (A2780), prostate (PC-3), and colon cancer cell lines (Caco-2) were determined by the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay method, and % cell viability and logIC50 values of the compounds were calculated. Significant decreases in cell viability were observed in all cells ($p < 0.05$). The comet assay method was used to understand that the compounds that showed a significant decrease in cell viability had this effect through DNA damage. Most of the compounds exhibited cytotoxicity by DNA damage mechanism. Besides, their interactions between investigated molecule groups with PDB ID: 3VHE, 3C0R, 2ZCL, and 2HQ6 target proteins corresponding to cancer cell lines, respectively, were investigated by docking studies. Finally, molecules with high biological activity against biological receptors were determined by ADME analysis.

KEYWORDS
ADME, cytotoxicity, DNA damage, molecular docking, peptides

1 | INTRODUCTION

Cancer is defined as an uncontrolled cell division and invasion of these cells to other tissues, and the formation of tumor mass and metastasis.[1] The discovery of various protein/peptide receptors and related peptides/proteins is expected to take a large share in the future cancer therapeutic market of more effective and selective anticancer drugs.[2–4] Today, many new therapies are being used in cancer treatment, and one of them is peptide-based chemotherapy continues to gain attraction due to the unique properties of peptides with low molecular weight, capable of targeting specific tumor cells, and low toxicity in normal tissues. Peptides have been used as promising therapeutic agents in the treatment of cancer, diabetes, and cardiovascular diseases from the past to the present and continue to attract attention.

Peptides function as structural molecules in tissues such as enzymes, antibodies, neurotransmitters, and hormones that control many physiological processes, from stomach acid separation and carbohydrate metabolism to growth. After the bioactive peptides are released, they can act as regulatory compounds with hormone-like activity. This aspect has been studied since 1979 and many peptides have been found that exhibit various activities such as opiate, antithrombotic or antihypertension activity, and immunomodulation or mineral utilization properties.[5] Research over time has allowed

peptides to be used as effective agents in the treatment of diabetes and cardiovascular diseases. There are about 60 approved peptide drugs on the current market.[6,7] The most important problem of the conventional type of chemotherapy, which is one of the most effective methods used in cancer treatment, is that the desired drug cannot be given to the target cancer cell in the correct amount without affecting the normal cells.[8] Currently, 60 approved peptide drugs in the market and they have a sales volume of around 13 billion dollars.[9–11] A large number of peptides have entered clinical trials so far. For example, some germ-killing peptides belonging to the innate immune system pierce the cell membranes of bacteria and kill them, thereby protecting the organism. Moreover, these germ-killing peptides, which are part of the immune system, are selective and can only kill pathogens, not touching normal cells. It has been found that when tumor cells are targeted to these germicidal peptides, these peptides do not harm normal cells but can only kill tumor cells.[12–20]

In this study, 16 tyrosine-based tripeptides were synthesized using the triazine method, considering molecular docking and possible interactions of compounds against several proteins. Compounds were tested for cell viability by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay against four human cancer cells and the comet assay method was performed to understand whether the active compounds caused cell death through DNA damage. The results revealed that most of the compounds cause cell death through DNA damage.

2 | EXPERIMENTAL SECTION

2.1 | Chemical synthesis

2.1.1 | General information

Amino acids and other chemicals; glycine methyl ester hydrochloride, L-alanine methyl ester hydrochloride, L-valine methyl ester hydrochloride, L-phenylalanine methyl ester hydrochloride, sodium hydroxide, 2-chloro-4,6-dimethoxy-1,3,5-triazine, and *N*-methyl morpholine were purchased from Chem-Impex International and Merck, respectively. Solvents, acetonitrile, methanol (MeOH), chloroform, and *n*-hexane were obtained from Merck Millipore. Dimethyl sulfoxide (DMSO)-d_6 and $CDCl_3$ used as a deuterated solvent for nuclear magnetic resonance (NMR) studies were obtained from Sigma-Aldrich. In cell culture studies, RPMI-1640 medium for cell types (Sigma-Aldrich), Incubator with CO_2 (Panasonic), trypsin–EDTA (Sigma-Aldrich), Microplate reader (BioTEK Spectrophotometer), Inverted Microscope SOIF-XDS, Nuve Brand Autoclave (for sterilization) and biological safety cabinet (Nuve Brand MN-120) were used. An Isolab brand pH meter was used for pH measurements. All compounds are >95% pure by elemental analysis, NMR, and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry spectroscopy techniques.

2.1.2 | General procedure for starting dipeptides

1.0 equivalent of Boc–Tyr–AA–OCH_3[21] was added to a single neck reaction flask containing 40 mL of MeOH and stirred until completely dissolved. After lowering the temperature to 0°C 8% NaOH was slowly added to the reaction flask. The reaction was monitored for reaction formation via thin-layer chromatography (TLC). The pH of the reaction was stabilized around 3 by adding 4 N HCl solution. Solvent of the reaction was removed completely and the residue was dissolved in ethyl acetate. The insoluble part was filtered and solvent dried over $MgSO_4$. After removing of $MgSO_4$ the solvent was removed under reduced pressure. The resulting solid was dissolved in chloroform and precipitated with *n*-hexane. The precipitated solid was filtered off and dried under vacuum.[22]

Synthesis of Boc–Tyr–Gly–OH (TGO): Following the general synthesis method for this compound, 3.76 g (10.67 mmol, 1 eq.) Boc–Tyr–Gly–OCH_3, 40 mL MeOH, NaOH (2 M, 21.34 mmol, 10.67 mL, 2 eq.). Yield: 70% (2.55 g). Fourier-transform infrared (FT-IR) (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3317, 3345; $\nu_{C-H(aromatic)}$, 3016, 2979; $\nu_{C-H(aliphatic)}$, 2859, 2932, $\nu_{C=C}$, 1515, 1596, 1615; $\nu_{C=O}$, 1656 (amide C=O), 1679 (Boc carbamate C=O), 1720 (acid C=O). ^{1}H NMR: 1.01 (9H, s, H^{11}), 2.57–2.63 ve 2.86–2.90 (2H, H^6), 3.71–3.85 (2H, H^{14}), 4.07–4.13 (1H, q, H^7), 6.63 6.65 (2H, d, J = 8.4 Hz, H^2), 6.87–6.85 (1H, d, H^8 [–NH]), 7.05–7.07 (2H, d, J = 8.4 Hz, H^3), 8.22 (1H, H^{13} [–NH]), 9.17 (1H, s, H^5 [–Ph–OH]), 12.61 (1H, s, H^{16} [–COOH]). ^{13}C-APT NMR: 155.70 C^1, 156.14 C^9 115.23 C^2, 078.38 C^{10}, 130.55 C^3, 028.63 C^{11}, 128.75 C^4, 172.74 C^{12}, 037.14 C^6, 041.12 C^{14}, 056.35 C^7, 171.69 C^{15}. Elemental analysis: $C_{16}H_{22}N_2O_6$ (Mw: 338.36 g mol^{-1}); theoretical: C, 56.80; H, 6.55; N, 8.28; experimental: C, 55.83; H, 6.58; N, 8.33.

Synthesis of Boc–Tyr–Val–OH (TVO): Following the general synthesis method for this compound, 6.72 mmol, 1 eq. Boc–Tyr–Val–OCH_3, 40 mL MeOH, NaOH (2 M, 13.44 mmol, 6.72 mL, 2 eq.). Yield: 71.8%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3425, 3345; $\nu_{C-H(aromatic)}$, 3012; $\nu_{C-H(aliphatic)}$, 2920, 2938, 2971, $\nu_{C=C}$, 1514, 1532, 1594; $\nu_{C=O}$, 1640 (amide C=O), 1661 (Boc carbamate C=O), 1698 (acid C=O). ^{1}H NMR: 0.89–0.91 (6H, dd, H^{18}), 1.32 (9H, s, H^{11}), 2.03–2.12 (1H, m, H^{17}), 2.59–2.66 (1H, H^6), 2.82–2.87 (1H, H^6), 4.12–4.16 (1H, H^{14}), 4.18–4.21 (1H, H^7), 6.64–6.66 (2H, d, J = 8.4 Hz, H^2), 6.91–6.93 (1H, d, H^8 [–NH]), 7.06–7.08 (2H, d, J = 8.4 Hz, H^3), 7.87–7.89 (1H, d, H^{16} [–NH]), 9.19 (1H, s, H^5 [–Ph–OH]), 12.73 (1H, s, H^{16} [–CO–OH]). ^{13}C-APT NMR: 155.73 C^1, 078.48 C^{10}, 115.24 C^2, 028.60 C^{11}, 130.58 C^3, 172.59 C^{12}, 128.66 C^4, 057.36 C^{14}, 056.64 C^{17}, 036.85 C^6, 173.40 C^{15}, 056.45 C^7, 019.51 C^{18}, 156.17 C^9, 018.34 C^{18}. Elemental analysis: $C_{19}H_{28}N_2O_6$ (Mw: 380.44 g mol^{-1}); theoretical: C, 59.99; H, 7.42; N, 7.36; experimental: C, 60.01; H, 7.44; N, 7.39.

Synthesis of Boc–Tyr–Ala–OH (TAO): Following the general synthesis method for this compound, 5.46 mmol Boc–Tyr–Ala–OCH_3, 40 mL MeOH, NaOH (2 M, 10.92 mmol, 5.46 mL, 2 eq.). Yield: 78.0%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3400, 3310, 3212; $\nu_{C-H(aromatic)}$, 3008, 3071; $\nu_{C-H(aliphatic)}$, 2855, 2932, 2979, $\nu_{C=C}$, 1513, 1532, 1596; $\nu_{C=O}$, 1651 (amide C=O), 1688 (Boc

carbamate C=O), 1711 (acid C=O). ^{1}H NMR: 1.26 (3H, d, H^{17}), 1.31 (9H, s, H^{11}), 2.57–2.63 ve 2.86–2.90 (2H, H^{6}), 4.07–4.13 (1H, H^{14}), 4.21–4.27 (1H, H^{7}), 6.624–6.66 (2H, d, J = 8.4 Hz, H^{2}), 6.75–6.77 (1H, d, H^{8} [–NH]), 7.06–7.08 (2H, d, J = 8.4 Hz, H^{3}), 8.15–8.16 (1H, H^{13} [–NH]), 9.15 (1H, s, H^{5} [–Ph–OH]), 12.58 (1H, s, H^{16} [–COOH]). ^{13}C-APT NMR: 155.70 C^{1}, 078.41 C^{10}, 115.24 C^{2}, 028.63 C^{11}, 130.59 C^{3}, 172.18 C^{12}, 128.70 C^{4}, 047.91 C^{14}, 037.05 C^{6}, 174.55 C^{15}, 056.25 C^{7}, 017.74 C^{16}, 156.17 C^{9}. Elemental analysis: $C_{17}H_{24}N_2O_6$ (Mw: 352.39 g mol^{-1}); theoretical: C, 57.94; H, 6.87; N, 7.95; experimental: C, 57.99; H, 6.92; N, 7.91.

Synthesis of Boc–Tyr–Phe–OH (TPO): Following the general synthesis method for this compound, 9.33 mmol Boc–Tyr–Ala–OCH_3, 40 mL MeOH, NaOH (2 M, 18.76 mmol, 9.38 mL, 2 eq.). Yield: 78.0%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3400, 3330, 3294; $\nu_{C-H(aromatic)}$, 3064, 3027, 3084; $\nu_{C-H(aliphatic)}$, 2864, 2929 $\nu_{C=C}$, 1514, 1600, 1616; $\nu_{C=O}$, 1662 (amide C=O), 1682 (Boc carbamate C=O), 1711 (acid C=O). ^{1}H NMR: 1.31 (9H, s, H^{11}), 2.58–2.61 ve 2.78–2.82 (2H, H^{17}), 2.92–2.97 and 3.07–3.11 (2H, H^{6}), 4.05–4.11 (1H, H^{14}), 4.46–4.51 (1H, q, H^{7}), 6.62–6.65 (2H, d, J = 8.4 Hz, H^{2}), 6.68 (1H, d, H^{8} [–NH]), 6.98–7.00 (2H, d, J = 8.4 Hz, H^{3}), 7.20–7.30 (5H, m, H^{19-23}), 7.96–7.98 (1H, H^{13} [–NH]), 9.07 (1H, s, H^{5} [–Ph–OH]), 12.70 (1H, s, H^{16} [–COOH]). ^{13}C-APT NMR: 155.56 C^{1}, 172.31 C^{12}, 115.23 C^{2}, 053.73 C^{14}, 130.53 C^{3}, 173.28 C^{15}, 128.57 C^{4}, 037.08 C^{17}, 037.23 C^{6}, 137.79 C^{18}, 056.50 C^{7}, 129.69 $C^{19,23}$, 156.15 C^{9}, 128.65 $C^{20,22}$, 078.45 C^{10}, 126.93 C^{21}, 028.60 C^{11}. Elemental analysis: $C_{23}H_{28}N_2O_6$ (Mw: 428.49 g mol^{-1}); theoretical: C, 64.47; H, 6.59; N, 6.54; experimental: C, 64.51; H, 6.63; N, 6.58.

2.1.3 | General synthesis procedure of tripeptides

1.0 equivalent Boc–Tyr–Gly–OH, 1.2 equivalent 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT), and 1.0 equivalent amino acid methyl ester hydrochloride compounds were added to the reaction flask at room temperature and acetonitrile was added to stir. 2.5 equivalent *N*-methyl morpholine (NMM) was added dropwise to the resulting suspension and stirred at room temperature. The reaction was stopped by monitoring with TLC (3:2 EtOAc/*n*-hexane) (the longest reaction time was 48 h). The solvent of the reaction mixture was removed at the beginning of the purification step. The residue was dissolved in ethyl acetate and washed with 1 N HCl, 5% $NaHCO_3$, and distilled water. The organic phase was removed and $MgSO_4$ was added. After filtration of $MgSO_4$, ethyl acetate was removed completely under reduced pressure. The residue was dissolved in $CHCl_3$ to precipitate in *n*-hexane. The obtained precipitate was filtered off and the desired product was dried in a vacuum oven. Yields range from 34% to 80%.

Synthesis of Boc–Tyr–Gly–Gly–OCH_3 (TGG): Following the general synthesis method for this compound, 1 g (2.96 mmol, 1.0 eq.) Boc–Tyr–Gly–OH, 0.623 g (3.55 mmol, 1.2 eq.) CDMT, 0.371 mg (2.96 mmol, 1.0 eq.) of glycine methyl ester hydrochloride (Gly–OCH_3) and 0.747 g (7.39 mmol, 812.33 μmL) NMM were used. Yield: 58% (0.7 g). FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3242, 3280, 3311, 3402; $\nu_{C-H(aromatic)}$, 3062, 3092; $\nu_{C-H(aliphatic)}$, 2853, 2936, 2953, 2983; $\nu_{C=C}$, 1517, 1543, 1583; $\nu_{C=O}$, 1610 (amide C=O), 1638 (amide C=O), 1679 (Boc carbamate C=O), 1743 (C=O). ^{1}H NMR: 1.31 (9H, s, H^{11}), 2.57–2.63 (2H, H^{6}), 2.85–2.90 (1H, H^{7}), 3.63 (3H, s, H^{19}), 3.79–3.91 (2H, H^{14}), 4.05–4.11 and 4.30–4.37 (1H, H^{17}), 6.63–6.65 (2H, d, J = 8.4 Hz, H^{2}), 6.84–6.86 (1H, d, H^{8} [–NH]), 7.03–7.05 (2H, d, J = 8.4 Hz, H^{3}), 7.97 (1H, H^{16} [–NH]), 8.22–8.25 (1H, d, H^{13} [–NH]), 9.15 (1H, s, H^{5} [–Ph–OH]). ^{13}C-APT NMR: 155.74 C^{1}, 115.25 C^{2}, 130.56 C^{3}, 128.66 C^{4}, 036.97 C^{6}, 056.43 C^{7}, 156.16 C^{9}, 078.50 C^{10}, 028.61 C^{11}, 071.90 C^{12}, 040.98 $C^{14,17}$, 173.13 C^{15}, 170.60 C^{18}, 052.16 C^{19}. MALDI-TOF: Mw: 409.44 g mol^{-1} (theoretical); **[M+K]:** 445.569 *m/z*, **[M–((CH$_3$)$_3$)]:** 367.347 *m/z*, **[M–(C(CH$_3$)$_3$)–OCH$_3$]:** 323.521 *m/z* (experimental). Elemental analysis: **$C_{19}H_{27}N_3O_7$** (Mw: 409.44 g mol^{-1}); theoretical: C, 55.74; H, 6.65; N, 10.26; experimental: C, 55.79; H, 6.67; N, 10.28.

Synthesis of Boc–Tyr–Gly–Ala–OCH_3 (TGA): Following the general synthesis method for this compound, 1.34 g (3.96 mmol) **Boc–Tyr–Gly–OH**, 0.765 g (4.36 mmol) CDMT, 0.553 g (3.96 mmol) alanine methyl ester hydrochloride **(Ala–OCH$_3$)** and (9.90 mmol, 1.09 μmL) NMM were used. Yield: 28%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3233, 3277, 3292; $\nu_{C-H(aromatic)}$, 3073; $\nu_{C-H(aliphatic)}$, 2933, 2954, 2979; $\nu_{C=C}$, 1515, 1529, 1594; $\nu_{C=O}$, 1615 (amide C=O), 1650 (amide C=O), 1683 (Boc carbamate C=O), 1739 (C=O). ^{1}H NMR: 1.29–1.31 (d, J = 7.2 Hz, H^{20}), 1.32 (9H, s, H^{11}), 2.59–2.71 (1H, m, H^{6}), 2.87–2.92 (1H, m, H^{6}), 3.64–3.65 (3H, s, J = 3.2 Hz, H^{19}), 3.75–3.74 (2H, d, J = 5.6 Hz, H14), 4.07–4.11 (1H, m, H^{7}), 4.26–4.36 (1H, m, H^{17}), 6.40–6.66 (2H, d, J = 8.4 Hz, H^{2}), 6.73–6.80 (1H, d, H^{8} [–NH]), 7.03–7.05 (2H, d, J = 8.4 Hz, H^{3}), 7.84–8.25 (2H, H^{13} ve H^{16} [–NH]), 9.08 (1H, s, H^{5} [–Ph–OH]). ^{13}C-APT NMR: 155.21 C^{1}, 115.32 C^{2}, 130.51 C^{3}, 128.63 C^{4}, 037.06 C^{6}, 056.55 C^{7}, 156.21 C^{9}, 078.60 C^{10}, 028.63 C^{11}, 172.50 C^{12}, 042.23 C^{14}, 173.29 C^{15}, 047.99 C^{17}, 169.03 C^{18}, 052.32 C^{19}, 017.54 C^{20}. Elemental analysis: $C_{20}H_{29}N_3O_7$ (Mw: 423.47 g mol^{-1}); theoretical: C, 56.73; H, 6.90; N, 9.92; experimental: C, 56.78; H, 6.93; N, 9.99.

Synthesis of Boc–Tyr–Val–Val–OCH_3 (TVV): Following the general synthesis method for this compound, 2.0 g (5.26 mmol) **Boc–Tyr–Val–OH**, 1.02 g (5.78 mmol) CDMT, 0.88 g (5.26 mmol) valine methyl ester hydrochloride **(Val–OCH$_3$)** and (13.14 mmol, 920 μmL) NMM were used. Yield: 60%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3268, 3291; $\nu_{C-H(aromatic)}$, 3014, 3073; $\nu_{C-H(aliphatic)}$, 2876, 2935, 2965; $\nu_{C=C}$, 1515, 1544, 1594; $\nu_{C=O}$, 1615 (amide C=O), 1645 (amide C=O), 1690 (Boc carbamate C=O), 1741 (C=O). ^{1}H NMR: 0.84–0.92 (12H,m, $H^{21,22,24,25}$), 1.32 (9H, s, H^{11}), 1.92–2.09 (2H, m, $H^{20,}$ H^{23}), 2.59–2.71 (1H, H^{6}), 2.82–2.86 (1H, H^{6}), 3.62 (3H, s, H^{19}), 4.07–4.16 (2H, m, $H^{14,17}$), 4.33–4.37 (1H, m, H^{7}), 6.63–6.65 (2H, d, J = 8.4 Hz, H^{2}), 6.94–6.96 (1H, d, H^{8} [–NH]), 7.02–7.04 (2H, d, J = 8.4 Hz, H^{3}), 7.67–6.69 (1H, d, H^{13} [–NH]), 8.24–8.25 (1H, H^{16} [–NH]), 9.17 (1H, s, H^{5} [–Ph–OH]). ^{13}C-APT NMR: 156.18 C^{1}, 115.26 C^{2}, 130.51 C^{3}, 128.63 C^{4}, 036.85 C^{6}, 078.55 C^{10}, 028.58 C^{11}, 171.21 C^{12}, 057.99 C^{14}, 171.75 C^{15}, 057.29 C^{17}, 172.02 C^{18}, 052.02 C^{19}, 031.65 C^{20}, 019.48 C^{21}, 019.35 C^{22}, 030.09 C^{23}, 018.73 C^{24}, 018.42 C^{25}. Elemental analysis: $C_{25}H_{39}N_3O_7$ (Mw: 483.60 g mol^{-1}); theoretical: C, 60.83; H, 7.96; N, 8.51; experimental: C, 60.86; H, 7.99; N, 8.85.

Synthesis of Boc–Tyr–Val–Gly–OCH_3 (TVG): Following the general synthesis method for this compound, 1.45 g (3.81 mmol) **Boc–Tyr–Val–OH**, 0.74 g (4.19 mmol) CDMT, 0.48 g (3.81 mmol) glycine methyl ester hydrochloride **(Gly–OCH_3)** and (9.53 mmol, 920 μmL) NMM were used. Yield: 30%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3305, 3396, 3472; $\nu_{C-H(aromatic)}$, 3078; $\nu_{C-H(aliphatic)}$, 2851, 2875, 2933; $\nu_{C=C}$, 1516, 1540, 1595; $\nu_{C=O}$, 1619 (amide C=O), 1642 (amide C=O), 1687 (Boc carbamate C=O), 1741 (C=O). ^{1}H NMR: 0.87–0.91 (6H, H^{21}), 1.33 (9H, s, H^{11}), 1.95–2.04 (1H, m, H^{20}), 2.63–2.69 (1H, m, H^6), 2.85–2.90 (1H, m, H^6), 3.64 (3H, s, H^{19}), 3.79–3.93 (2H, m, H^{17}), 4.11–4.16 (1H, m, H^{14}), 4.22–4.26 (1H, m, H^7), 6.64–6.66 (2H, d, J = 8.4 Hz, H^2), 6.86–6.88 (1H, d, H^8 [–NH]), 7.02–7.04 (2H, d, J = 8.4 Hz, H^3), 7.59–7.61 (1H, d, H^{16} [–NH]), 8.30–8.33 (1H, t, H^{13} [–NH]), 9.08 (1H, s, H^5 [–Ph–OH]). ^{13}C-APT NMR: 155.71 C^1, 115.31 C^2, 130.51 C^3, 036.81 C^6, 056.58 C^7, 156.19 C^9, 078.64 C^{10}, 028.51 C^{11}, 171.77 C^{12}, 057.69 C^{14}, 172.05 C^{15}, 040.99 C^{17}, 170.53 C^{18}. Elemental analysis: $C_{22}H_{33}N_3O_7$ (Mw: 451.52 g mol^{-1}); theoretical: C, 58.52; H, 7.37; N, 9.31; experimental: C, 58.58; H, 7.39; N, 9.33.

Synthesis of Boc–Tyr–Val–Ala–OCH_3 (TVA): Following the general synthesis method for this compound, 2.5 g (6.57 mmol) **Boc–Tyr–Val–OH**, 1.27 g (7.23 mmol) CDMT, 0.92 g (6.57 mmol) L-alanine methyl ester hydrochloride **(Ala–OCH_3)** and (16.43 mmol, 1.81 mL) NMM were used. Yield: 49%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3283, 3312, 3432; $\nu_{C-H(aromatic)}$, 3070; $\nu_{C-H(aliphatic)}$, 2875, 2932, 2960; $\nu_{C=C}$, 1514, 1526, 1595; $\nu_{C=O}$, 1616 (amide C=O), 1642 (amide C=O), 1687 (Boc carbamate C=O), 1735 (C=O). ^{1}H NMR: 0.85–091 (6H, m, H^{22}), 1.29–1.31 (3H, d, J = 7.2 Hz, H^{18}), 1.33 (9H, s, H^{11}), 1.92–2.00 (1H, m, H^{21}), 2.60–2.66 (1H, m, H^6), 2.83–2.87 (1H, m, H^6), 3.62 (3H, s, H^{19}), 4.08–4.20 (1H, m, H^{17}), 4.21–4.30 (2H, m, H^7 ve H^{14}), 6.63–6.65 (2H, d, J = 8.4 Hz, H^2), 6.93–6.95 (1H, d, H^8 [–NH]), 7.02–7.04 (2H, d, J = 8.4 Hz, H^3), 7.61–7.63 (1H, d, H^{16} [–NH]), 8.45–8.47 (1H, d, H^{13} [–NH]), 9.15 (1H, s, H^5 [–Ph–OH]). ^{13}C-APT NMR: 155.70 C^1, 115.25 C^2, 130.50 C^3, 128.62 C^4, 036.87 C^6, 056.66 C^7, 156.18C^9, 078.57 C^{10}, 028.38 C^{11}, 171.17 C^{12}, 048.00 C^1, 171.99 C^{15}, 057.28 C^{17}, 017.20 C^{18}, 173.31 C^{19}, 052.22 C^{20}, 031.72 C^{21}, 018.39 C^{22}, 019.43 C^{22}. Elemental analysis: $C_{23}H_{35}N_3O_7$ (Mw: 465.55 g mol^{-1}); theoretical: C, 59.34; H, 7.58; N, 9.03; experimental: C, 59.38; H, 7.63; N, 9.07.

Synthesis of Boc–Tyr–Val–Phe–OCH_3 (TVP): Following the general synthesis method for this compound, 2.5 g (6.57 mmol) **Boc–Tyr–Val–OH**, 1.27 g (7.23 mmol) CDMT, 0.92 g (6.57 mmol) L-phenylalanine methyl ester hydrochloride **(Ala–OCH_3)** and (16.43 mmol, 1.81 mL) NMM were used. Yield: 49%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3272, 3312, 3428; $\nu_{C-H(aromatic)}$, 3027, 3065, 3084; $\nu_{C-H(aliphatic)}$, 2872, 2931, 2966; $\nu_{C=C}$, 1514, 1530, 1597; $\nu_{C=O}$, 1617 (amide C=O), 1646 (amide C=O), 1691 (Boc carbamate C=O), 1729 (C=O). ^{1}H NMR: 0.81–0.86 (6H, m, H^{28}), 1.31 (9H, s, H^{11}), 1.92–1.97 (1H, m, H^{27}), 2.58–2.64 (1H, H^6), 2.78–2.82 (1H, H^6), 2.95–3.06 (2H, H^{20}), 3.57 (3H, s, H^{19}), 4.07–4.12 (1H, m, H^{14}), 4.23–4.27 (1H, m, H^{17}), 4.46–4.52 (1H, m, H^7), 6.64–6.66 (2H, d, J = 8.4 Hz, H^2), 6.94–6.96 (1H, d, H^8 [–NH]), 7.02–7.04 (2H, d, J = 8.4 Hz, H^3), 7.18–7.29 (5H, m, H^{22-26}), 7.59–7.61 (1H, H^{16} [–NH]), 8.47–8.49 (1H, d, H^{13} [–NH]), 9.15 (1H, s, H^5 [–Ph–OH]). ^{13}C-APT NMR: 155.73 C^1, 115.26 C^2, 130.50 C^3, 137.48 C^4, 036.82 C^6, 056.60 C^7, 156.16 C^9, 078.53 C^{10}, 028.59 C^{11}, 171.42 C^{12}, 053.96 C^{14}, 172.21 C^{15}, 057.34 C^{17}, 172.04 C^{18}, 052.17 C^{19}, 036.97 C^{20}, 137.48 C^{21}, 128.71 $C^{22,26}$, 129.40 $C^{23,25}$, 127.00 C^{24}. Elemental analysis: $C_{29}H_{39}N_3O_7$ (Mw: 541.65 g mol^{-1}); theoretical: C, 64.31; H, 7.26; N, 7.76; experimental: C, 64.35; H, 7.31; N, 7.79.

Synthesis of Boc–Tyr–Val–Met–OCH_3 (TVM): Following the general synthesis method for this compound, 1.45 g (3.81 mmol) **Boc–Tyr–Val–OH**, 0.74 g (4.19 mmol) CDMT, 0.76 g (3.81 mmol) L-methionine methyl ester hydrochloride **(Met–OCH_3)** and (9.53 mmol, 1.05 μL) NMM were used. Yield: 34%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3216, 3287; $\nu_{C-H(aromatic)}$, 3070; $\nu_{C-H(aliphatic)}$, 2873, 2917, 2932; $\nu_{C=C}$, 1515, 1539, 1595; $\nu_{C=O}$, 1616 (amide C=O), 1645 (amide C=O), 1687 (Boc carbamate C=O), 1741 (C=O). ^{1}H NMR: 0.87–0.91 (6H, m, $H^{23,24}$), 1.33 (9H, s, H^{11}), 1.85–1.93 (1H, m, H^{20}), 1.93–2.02 (1H, m, H^{20}), 2.05 (3H, s, H^{22}), 2.62–2.68 (1H, H^6), 2.84–2.88(1H, H^6), 3.64 (3H, s, H^{19}), 4.12–4.14 (1H, m, H^{14}), 4.23–4.27 (1H, m, H^{17}), 4.37–4.43 (1H, m, H^7), 6.64–6.66 (2H, d, J = 8.4 Hz, H^2), 6.83–6.84 (1H, d, H^8 [–NH]), 7.02–7.04 (2H, d, J = 8.4 Hz, H^3), 7.60–7.61 (1H, H^{16} [–NH]), 8.32–8.34 (1H, d, H^{13} [–NH]), 9.06 (1H, s, H^5 [–Ph–OH]). ^{13}C-APT NMR: 155.68 C^1, 115.32 C^2, 115.32 C^2,130.48 C^3, 128.63 C^4, 036.89 C^6, 056.59 C^7, 156.20 C^9, 078.60 C^{10}, 028.61 C^{11}, 172.03 C^{12}, 057.58 C^{14}, 171.55 C^{15}, 052.28 C^{17}, 172.42 C^{18}, 051.43 C^{19}, 030.98 C^{20}, 030.00 C^{21}, 015.07 C^{22}, 019.47 C^{23}, 018.39 C^{24}. Elemental analysis: $C_{25}H_{39}N_3O_7S$ (Mw: 525.66 g mol^{-1}); theoretical: C, 57.12; H, 7.48; N, 7.99; experimental: C, 57.14; H, 7.51; N, 8.02.

Synthesis of Boc–Tyr–Ala–Ala–CH_3 (TAA): Following the general synthesis method for this compound, 1.56 g (4.43 mmol) **Boc–Tyr–Ala–OH**, 0.85 g (4.87 mmol) CDMT, 0.62 g (4.43 mmol) L-alanine methyl ester hydrochloride **(Ala–OCH_3)** and (11.07 mmol, 1.22 mL) NMM were used. Yield: 47%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3218, 3295; $\nu_{C-H(aromatic)}$, 3075; $\nu_{C-H(aliphatic)}$, 2933, 2953, 2979; $\nu_{C=C}$, 1515, 1541, 1595; $\nu_{C=O}$, 1616 (amide C=O), 1650 (amide C=O), 1686 (Boc carbamate C=O), 1741 (C=O). ^{1}H NMR: 1.23–1.25 (3H, H^{20}), 1.29–1.31 (3H, H^{21}), 1.32 (9H, s, H^{11}), 2.56–2.65 (1H, H^6), 2.86–2.89 (1H, H^6), 3.64 (3H, s, H^{19}), 4.07–4.11 (1H, m, H^{14}), 4.28–4.31 (1H, m, H^{17}), 4.32–4.36(1H, m, H^7), 6.64–6.66 (2H, d, J = 8.4 Hz, H^2), 6.73–6.75 (1H, d, H^8 [–NH]), 7.03–7.05 (2H, d, J = 8.4 Hz, H^3), 7.84–7.86 (1H, H^{16} [–NH]), 8.23–8.24 (1H, d, H^{13} [–NH]), 9.07 (1H, s, H^5 [–Ph–OH]). ^{13}C-APT NMR: 155.74 C^1, 115.29 C^2, 130.51 C^3, 128.63 C^4, 037.06 C^6, 056.41 C^7, 156.21 C^9, 078.56 C^{10}, 028.62 C^{11}, 171.77 C^{12}, 047.99 C^{14}, 172.46 C^{15}, 048.17 C^{17}, 173.29 C^{18}, 052.29 C^{19}, 017.35 C^{20}, 017.50 C^{21}. Elemental analysis: $C_{21}H_{31}N_3O_7$ (Mw: 437.49 g mol^{-1}); theoretical: C, 57.65; H, 7.14; N, 9.60; experimental: C, 57.61; H, 7.18; N, 9.64.

Synthesis of Boc–Tyr–Ala–Gly–OCH_3 (TAG): Following the general synthesis method for this compound, 0.48 g (1.36 mmol) **Boc–Tyr–Ala–OH**, (1.50 mmol) CDMT, (1.35 mmol) glycine methyl ester hydrochloride **(Gly–OCH_3)** and (3.41 mmol, 374.40 μmL) NMM were used. Yield: 55%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3241, 3304, 3392; $\nu_{C-H(aromatic)}$, 3009, 3077; $\nu_{C-H(aliphatic)}$, 2853, 2933, 2977; $\nu_{C=C}$, 1515, 1527, 1593; $\nu_{C=O}$, 1615 (amide C=O), 1627 (amide C=O), 1751 (C=O). ^{1}H NMR: 1.23–1.24 (3H, d, H^{17}), 1.31 (9H, s, H^{11}), 2.62–2.68

ve 2.85–2.89 (2H, H^6), 3.63 (3H, s, H^{20}), 3.80–3.91 (2H, H^{18}), 4.04–4.10 (1H, H^{14}), 4.29–4.35 (1H, H^7), 6.63–6.65 (2H, d, J = 8.4 Hz, H^2), 6.88–6.90 (1H, d, H^8 [–NH]), 7.03–7.05 (2H, d, J = 8.4 Hz, H^3), 8.26–8.29 (1H, t, H^{16} [–NH]), 8.00–8.02 (1H, d, H^{13} [–NH]), 9.18 (1H, s, H^5 [–Ph–OH]). ^{13}C-APT NMR: 155.74 C^1, 028.61 C^{11}, 115.24 C^2, 173.15 C^{12}, 130.57 C^3, 048.28 C^{14}, 128.68 C^4, 171.93 C15, 036.95 C^6, 018.91 C17, 056.44 C^7, 040.97 C^{18}, 156.15 C^9,170.63 C^{19}, 078.49 C^{10}, 052.18 C^{20}. Elemental analysis: $C_{27}H_{35}N_3O_7$ (Mw: 423.47 g mol^{-1}); theoretical: C, 56.73; H, 6.90; N, 9.92; experimental: C, 56.77; H, 6.96; N, 9.95.

Synthesis of Boc–Tyr–Ala–Val–OCH_3 (TAV): Following the general synthesis method for this compound, 0.48 g (1.36 mmol) **Boc–Tyr–Ala–OH**, (1.50 mmol) CDMT, (1.36 mmol) L-valine methyl ester hydrochloride **(Val–OCH_3)** and (3.41 mmol, 374.40 μmL) NMM were used. Yield: 60%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3277, 3299, 3376; $\nu_{C-H(aromatic)}$, 3015, 3072; $\nu_{C-H(aliphatic)}$, 2855, 2874, 2933; $\nu_{C=C}$, 1515, 1530, 1595; $\nu_{C=O}$, 1647 (amide C=O), 1691, 1714, 1739 (C=O). ^{1}H NMR: 0.86–0.89 (6H, $H^{22,23}$), 1.22–1.23 (3H, d, H^{17}), 1.30 (9H, s, H^{11}), 2.62–2.68 and 2.85–2.89 (1H, m, H^{21}), 2.02–2.07 (1H, m, H^{21}), 2.55–2.61 ve 2ik.83–2.87 (2H, H^6), 3.64 (3H, s, H^{20}), 4.03–4-08 (1H, H^{18}), 4.17–4.21 (1H, H^{14}), 4.3–4.35 (1H, H^7), 6.63–6.65 (2H, d, J = 8.4 Hz, H^2), 6.86–6.88 (1H, d, H^8 [–NH]), 7.03–7.05 (2H, d, J = 8.4 Hz, H^3), 7.98–8.00 (1H, d, H^{16} [–NH]), 8.15–8.17 (1H, d, H^{13} [–NH]), 9.17 (1H, s, H^5 [–Ph–OH]). ^{13}C-APT NMR: 155.73 C^1, 048.19 C^{14}, 115.23 C^2, 172.33 C^{15}, 130.55 C^3, 019.38 C^{17}, 128.69 C^4, 057.73 C^{18}, 036.95 C^6, 171.98 C^{19}, 056.44 C^7, 052.18 C^{20}, 156.16 C^9, 030.40 C^{21}, 078.44 C^{10}, 018.78 C^{22}, 028.59 C^{11}, 018.56 C^{23}, 173.03 C^{12}. Elemental analysis: $C_{23}H_{35}N_3O_7$ (Mw: 465.55 g mol^{-1}); theoretical: C, 56.34; H, 7.58; N, 9.03; experimental: C, 56.38; H, 7.63; N, 8.99.

Synthesis of Boc–Tyr–Ala–Phe–OCH_3 (TAP): Following the general synthesis method for this compound, 0.48 g (1.36 mmol) **Boc–Tyr–Ala–OH**, 263.07 mg (1.50 mmol) CDMT, 293.78 (1.36 mmol) L-phenylalanine methyl ester hydrochloride **(Phe–OCH_3)** and (3.41 mmol, 374.40 μmL) NMM were used. Yield: 60%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3242, 3280, 3311; $\nu_{C-H(aromatic)}$, 3062, 3092; $\nu_{C-H(aliphatic)}$, 2853, 2936, 2953; $\nu_{C=C}$, 1517, 1543, 1583; $\nu_{C=O}$, 1610 (amide C=O), 1638, 1679, 1743 (C=O). ^{1}H NMR: 1.19–1.20 (3H, d, H^{17}), 1.30 (9H, s, H^{11}), 2.57–2.60 and 2.81–2.85 (2H, H^6), 2.92–2.98 ve 3.01–3.06 (2H, H^{21}), 3.59 (3H, s, H^{20}), 4.03–4.09 (1H, H^{14}), 4.31–4.34 (1H, H^{18}), 4.45–4.50 (1H, H^7), 6.63–6.65 (2H, d, J = 8.4 Hz, H^2), 6.83–6.85 (1H, d, H^8 [–NH]), 7.02–7.04 (2H, d, J = 8.4 Hz, H^3), 7.21–7.28 (5H, m, H^{23-27}), 7.89–7.91 (1H, d, H^{16} [–NH]), 8.32–8.34 (1H, d, H^{13}[–NH]), 9.14 (1H, s, H^5 [–Ph–OH]). ^{13}C-APT NMR: 155.71 C^1, 171.85 C^{15}, 115.24 C^2, 018.90 C^{17},129.50 C^3, 054.05 C^{18}, 128.70 C^4,172.72 C^{19}, 037.02 C^6, 052.30 C^{20}, 056.38 C^7, 037.02 C^{21}, 156.16 C^9,137.46 C^{22}, 078.47 C^{10}, 130.53 $C^{23,27}$, 028.60 C^{11}, 128.73 $C^{24,26}$ 172.19 C^{12}, 127.04 C^{25}, 048.19 C^{14}. MALDI-TOF: Mw: 513.59 g mol^{-1} (theoretical); **[M]:** 513.767 *m/z*, **[M+Na]:** 535.904 *m/z*, **[M–OCH_3]:** 481.706 *m/z*, **[M-Boc]:** 429.538 *m/z* (experimental). Elemental analysis: $C_{27}H_{35}N_3O_7$ (Mw: 513.59 g mol^{-1}); theoretical: C, 63.14; H, 6.87; N, 8.18; experimental: C, 63.18; H, 6.93; N, 8.20.

Synthesis of Boc–Tyr–Phe–Phe–OCH_3 (TPP): Following the general synthesis method for this compound, 2.0 g (4.67 mmol) **Boc–Tyr–Phe–OH**, 0.90 g (7.23 mmol) CDMT, 1.01 g (4.67 mmol) L-phenylalanine methyl ester hydrochloride **(Phe–OCH_3)** and (11.67 mmol, 1.28 mL) NMM were used. Yield: 47%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3299, 3338, 3435; $\nu_{C-H(aromatic)}$, 3030, 3065; $\nu_{C-H(aliphatic)}$, 2931, 2952, 2969; $\nu_{C=C}$, 1522, 1538, 1595; $\nu_{C=O}$, 1648 (amide C=O), 1668, 1684, 1727 (C=O). ^{1}H NMR: 1.29 (9H, s, H^{11}), 2.65–2.70 (1H, H^6), 2.75–2.81 (1H, H^6), 2.93–2.98 (2H, H^{20}), 3.00–3.07 (2H, H^{27}), 3.58 (3H, s, H^{19}), 3.97–4.07 (1H, m, H^{17}), 4.47–4.53 (1H, m, H^{14}), 4.56–4.62 (1H, m, H^7), 6.60–6.62 (2H, d, J = 8.4 Hz, H^2), 6.93–6.95 (2H, d, J = 8.4 Hz, H^3), 6.99–7.01 (1H, H^{16} [–NH]), 7.19–7.30 (10H, m, $H^{21-26/28-33}$), 7.89–7.91 (1H, d, H^8 [–NH]), 8.57–8.59 (1H, d, H^{13} [–NH]), 9.16 (1H, s, H^5 [–Ph–OH]). ^{13}C NMR: 155.50 C^1, 078.50 C^{10}, 115.22 C^2, 028.59 C^{11}, 130.47 C^3, 171.51 C^{12}, 128.45 C^4, 054.06 C^{14}, 038.29 C^6, 171.84 C^{15}, 056.60 C^7, 172.12 C^{18}, 156.12 C^9, 052.32 C19, 137.87 C^{21}, 137.39 C^{28}, 129.79 $C^{30,32}$,129.48 $C^{23,25}$, 128.76 $C^{29,33}$,128.45 $C^{22,26}$, 127.06 C^{31}, 126.73 C^{24}, 053.66 C^{17}, 037.24 C^{27}, 037.09 C^{20}. Elemental analysis: $C_{33}H_{39}N_3O_7$ (Mw: 589.69 g mol^{-1}); theoretical: C, 67.22; H, 6.67; N, 7.13; experimental: C, 67.25; H, 6.71; N, 7.16.

Synthesis of Boc–Tyr–Phe–Gly–OCH_3 (TPG): Following the general synthesis method for this compound, 0.4 g (0.934 mmol) **Boc–Tyr–Phe–OH**, 180.29 mg (1.03 mmol) CDMT, 117 mg (0.934 mmol) glycine methyl ester hydrochloride **(Gly–OCH_3)** and (2.33 mmol, 256 μmL) NMM were used. Yield: 64%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3135, 3343, 3371; $\nu_{C-H(aromatic)}$, 3011, 3038, 3052; $\nu_{C-H(aliphatic)}$, 2852, 2934, 2983; $\nu_{C=C}$, 1514, 1559, 1597; $\nu_{C=O}$, 1658 (amide C=O), 1721, 1754 (C=O). ^{1}H NMR: 1.30 (9H, s, H^{11}), 2.53–2.56 ve 270–2.75 (2H, H^6), 4.56–4.62 (1H, H^7), 2.80–2.86 ve 3.02–3.06 (2H, H^{20}), 3.64 (3H, s, H^{19}), 3.85–3.90 (2H, H^{17}), 4.00–4.05 (1H, H^{14}), 6.61–6.63 (2H, d, J = 8.4 Hz, H^2), 6.77–6.80 (1H, d, H^8 [–NH]), 8.43–8.44 (1H, d, H^{13} [–NH]), 6.93–6.95 (2H, d, J = 8.4 Hz, H^3), 7.18–7.27 (5H, m, H^{22-26}), 7.94–7.96 (1H, d, H^{16} [–NH]), 9.13 (1H, s, H^5 [–Ph–OH]). ^{13}C NMR: 155.53 C^1, 053.86 C^{14}, 115.25 C^2, 171.94 C^{15}, 130.49 C^3, 041.07 C^{17}, 128.65 C^4, 170.55 C^{18}, 037.21 C^6, 052.19 C^{19}, 056.66 C^7, 038.21 C^{20},156.14 C^9, 138.00 C^{21}, 078.57 C^{10}, 128.50 $C^{22,26}$, 028.60 C^{11}, 129.77 $C^{23,25}$, 171.94 C^{12}, 126.75 C^{24}. MALDI-TOF: Mw: 499.56 g mol^{-1} (theoretical); **[M+Na]:** 521.934 *m/z*, **[M–((CH$_3$)$_3$)]:** 443.631 *m/z*, **[M–((CH$_3$)$_3$)–(CH$_3$)]:** 457.693 *m/z*, **[M-Boc]:** 401.450 *m/z* (experimental). Elemental analysis: $C_{26}H_{33}N_3O_7$ (Mw: 499.56 g mol^{-1}); theoretical: C, 62.51; H, 6.66; N, 8.41; experimental: C, 62.55; H, 6.69; N, 8.39.

Synthesis of Boc–Tyr–Phe–Val–OCH_3 (TPV): Following the general synthesis method for this compound, 1.0 g (2.33 mmol) **Boc–Tyr–Phe–OH**, 450.73 mg (2.57 mmol) CDMT, (2.33 mmol) L-valine methyl ester hydrochloride **(Val–OCH_3)** and (5.83 mmol, 641 μmL) NMM were used. Yield: 80%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3241, 3300, 3340, 3388; $\nu_{C-H(aromatic)}$, 3029, 3069, 2969; $\nu_{C-H(aliphatic)}$, 2849, 2977, 2922; $\nu_{C=C}$, 1514, 1534, 1593; $\nu_{C=O}$, 1649 (amide C=O), 1659, 1686, 1740 (C=O). ^{1}H NMR: 0.86–0.90 (6H, m, $H^{21,22}$), 1.29 (9H, s, H^{11}), 2.03–2.05 (1H, H^{20}), 2.53–2.56 ve

2.68–2.73 (2H, H^{6}), 2.78–2.84 ve 2.98–3.03 (2H, H^{23}), 3.63 (3H, s, H^{19}), 3.96–4.02 (1H, H^{17}), 4.17–4.20 (1H, H^{14}), 4.65–4.70 (1H, H^{7}), 6.59–6.61 (2H, d, J = 8.4 Hz, H^{2}), 6.80–6.83 (1H, d, H^{8} [–NH]), 6.93–6.95 (2H, d, J = 8.4 Hz, H^{3}), 7.18–7.27 (5H, H^{25-29}), 7.93–7.95 (1H, d, H^{16} [–NH]), 8.33–8.36 (1H, d, H^{13} [–NH]), 9.17 (1H, s, H^{5} [–Ph–OH]). ^{13}C NMR: 155.48 C^{1}, 053.61 C^{14}, 115.21 C^{2}, 171.97 C^{15}, 128.44 C^{3}, 057.85 C^{17}, 128.52 C^{4}, 171.79 C^{18}, 038.20 C^{6}, 052.21 C^{19}, 056.67 C^{7}, 030.39 C^{20}, 156.14 C^{9}, 019.39 C^{21}, 078.51 C^{10}, 018.66 C^{22}, 028.57 C^{11}, 037.15 C^{23}, 172.21 C^{12}, 137.88 C^{24}, 130.48 $C^{25.29}$, 129.81 $C^{26,28}$. Elemental analysis: $C_{29}H_{39}N_3O_7$ (Mw: 541.65 g mol^{-1}); theoretical: C, 64.31; H, 7.26; N, 7.76; experimental: C, 64.35; H, 7.30; N, 7.79.

Synthesis of Boc–Tyr–Phe–Ala–OCH_3 (TPA): Following the general synthesis method for this compound, 1.0 g (2.33 mmol) **Boc–Tyr–Phe–OH**, 450.73 mg (2.57 mmol) CDMT, 325.75 mg (2.33 mmol) L-alanine methyl ester hydrochloride **(Ala–OCH_3)** and (5.83 mmol, 641 µmL) NMM were used. Yield: 79%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3229, 3296, 3404; $\nu_{C-H(aromatic)}$, 3065, 3030, 2979; $\nu_{C-H(aliphatic)}$, 2853, 2931; $\nu_{C=C}$, 1515, 1593, 1615; $\nu_{C=O}$, 1646 (amide C=O), 1689, 1714, 1742 (C=O). ^{1}H NMR: 1.29 (3H, d, H^{19}), 1.31 (9H, s, H^{11}), 2.55–2.58 ve 2.72–2.76 (2H, H^{6}), 2.80–2.86 ve 3.02–3.06 (2H, H^{21}), 3.63 (3H, s, H^{20}), 3.89–3.94 (1H, H^{14}), 4.27–4.34 (1H, H^{17}), 4.57–4.62 (1H, H^{7}), 6.61–6.63 (3H, m, H^{8} [–NH], H^{2}), 6.93–6.95 (2H, d, J = 8.4 Hz, H^{3}), 7.18–7.27 (5H, m, H^{23-27}), 7.80–7.82 (1H, d, H^{16} [–NH]), 8.38–8.39 (1H, d, H^{13} [–NH]), 9.03 (1H, s, H^{5} (–Ph–OH)). ^{13}C NMR: 155.48 C^{1}, 171.23 C^{15}, 115.29 C^{2}, 048.06 C^{17}, 128.43 C^{3}, 173.17 C^{18}, 128.50 C^{4}, 017.39 C^{19}, 038.30 C^{6}, 052.29 C^{20}, 053.72 C^{7}, 033.81 C^{21}, 156.20 C^{9}, 137.96 C^{22}, 078.60 C^{10}, 129.77 $C^{23,27}$, 028.60 C^{11}, 130.43 $C^{24,26}$, 171.82 C^{12}, 126.69 C^{25}, 055.47 C^{14}. MALDI-TOF: Mw: 513.59 g mol^{-1} (theoretical); **[M+Na]:** 536.313 *m/z*, **[M+K]:** 553.044 *m/z*, **[M–(CH_3)$_3$]:** 457.623 *m/z*, **[M–OCH_3]:** 481.871 *m/z* (experimental). Elemental analysis: $C_{27}H_{35}N_3O_7$ (Mw: 513.59 g mol^{-1}); theoretical: 63.14; H, 6.87; N, 8.18; experimental: 63.17; H, 6.92; N, 8.21.

Synthesis of Boc–Tyr–Phe–Leu–OCH_3 (TPL): Following the general synthesis method for this compound, 1.0 g (2.33 mmol) **Boc–Tyr–Phe–OH**, 450.73 mg (2.57 mmol) CDMT, (2.33 mmol) L-leucine methyl ester hydrochloride **(Leu–OCH_3)** and (5.83 mmol, 641 µmL) NMM were used. Yield: 65%. FT-IR (ATR, cm^{-1}): ν_{N-H}, ν_{OH} 3280, 3316, 3392; $\nu_{C-H(aromatic)}$, 3029, 3065, 2955; $\nu_{C-H(aliphatic)}$, 2870, 2932; $\nu_{C=C}$, 1502, 1597, 1616; $\nu_{C=O}$, 1647 (amide C=O), 1689, 1723, 1743 (C=O). ^{1}H NMR: 155.48 C^{1}, 171.55 C^{15}, 115.22 C^{2}, 050.67 C^{17}, 128.46 C^{3}, 173.17 C^{18},128.53 C^{4}, 052.35 C^{19}, 037.14 C^{6}, 038.22 C^{20},056.69 C^{7}, 024.62 C^{21},156.15 C^{9}, 021.70 C^{22},078.52 C^{10}, 023.24 C23, 028.57 C^{11}, 037.14 C24, 171.91 C^{12}, 137.88 C^{25}, 053.65 C^{14}, 130.47 $C^{26.30}$, 129.80 $C^{27,29}$. Elemental analysis: $C_{30}H_{41}N_3O_7$ (Mw: 555.67 g mol^{-1}); theoretical: C, 64.85; H, 7.44; N, 7.56; experimental: C, 64.89; H, 7.47; N, 7.60.

2.2 | Cytotoxicity studies

% Changes in cell viability rates of 1, 5, 25, 50, and 100 µM concentrations for compounds were determined by the MTT assay method.[23–29] In this method, the MTT dye is absorbed by living cells, and the reaction is catalyzed by mitochondrial succinate dehydrogenase, degrading the tetrazolium ring to a blue-violet colored, water-insoluble formazan. Formazan formation indicates that only active mitochondria are found in living cells. Although this is considered as an indicator of cell viability, the value determined is associated with the number of living cells. The absorbance values from the solvent and agent-treated wells were proportioned to the control absorbance value and considered as percent viability.[30] Human breast cancer cell line (MCF-7), ovarian cancer cell line (A2780), human prostate cancer cell line (PC-3), and human colon cancer cell line (Caco-2) were selected as cell types. The solutions of the substances in DMSO were used in cell cultures, and the effects of the substances against DMSO were statistically analyzed and compared with other results. Solutions were prepared with a DMSO ratio below 1%. The same amounts of solvent (DMSO) with five different concentrations of the tested compounds were added to the wells containing the cells and incubated at 37°C for 24 h in a CO_2 incubator. Cell viability was determined after incubations using 0.4% tryphan blue in a hemocytometer. For statistical analysis, compliance with normal distribution was evaluated with the Shapiro–Wilk test using the IBM SPSS Statistics 22.0 (Windows) package program. Intergroup comparisons of quantitative variables were measured with the Kruskal–Wallis H test. When the significant statistical difference was determined between the groups, multiple comparisons were made with the Mann–Whitney U test with Bonferroni correction. Data were presented as mean ± standard deviation, and $p < 0.05$ value was considered statistically significant. LogIC50 values were calculated according to the MTT results using the GraphPad Prism 6 program.

2.3 | Genotoxicity studies

DNA damage studies of tripeptide compounds in human breast (MCF-7), ovarian (A2780), prostate (PC-3), and colon (Caco-2) cancer cell lines were performed at the highest concentration. Tail length (TL), tail density (TI), olive tail intensity (OTI), head diameter (head length), and head intensity parameters were determined and the presence and rate of DNA damage were determined with the changes in these parameters and are presented in the Supporting Information.

2.4 | Molecular docking and ADME (Absorption, distribution, metabolism, and excretion)

Docking studies were performed with Schrödinger Maestro (version 12.2) to obtain possible binding poses between the studied molecules and the target biological receptors.[31] Ligands were optimized with OPLSe and prepared with the LigPrep-Epic module.[32] Integrated modeling program, applied chemical theory (IMPACT).[33] Four different receptor proteins representing human cancer cell lines

(MCF-7), ovarian (A2780), prostate (PC-3), and colon cancer cell lines (Caco-2) are PDB ID: 3VHE,[34] 3C0R,[35] 2ZCL,[36] and 2HQ6,[37] respectively, and were obtained from the PDB database (http://www.rcsb.org/pdb). Water molecules were removed, and molecular docking was performed using GLIDE at pH 7.0 ± 2.0. Afterward, Absorption, distribution, metabolism, excretion and toxicity (ADME/T) analysis was performed for the designed compounds. ADME/T was performed with QikProp at Schrödinger.[38]

3 | RESULTS AND DISCUSSION

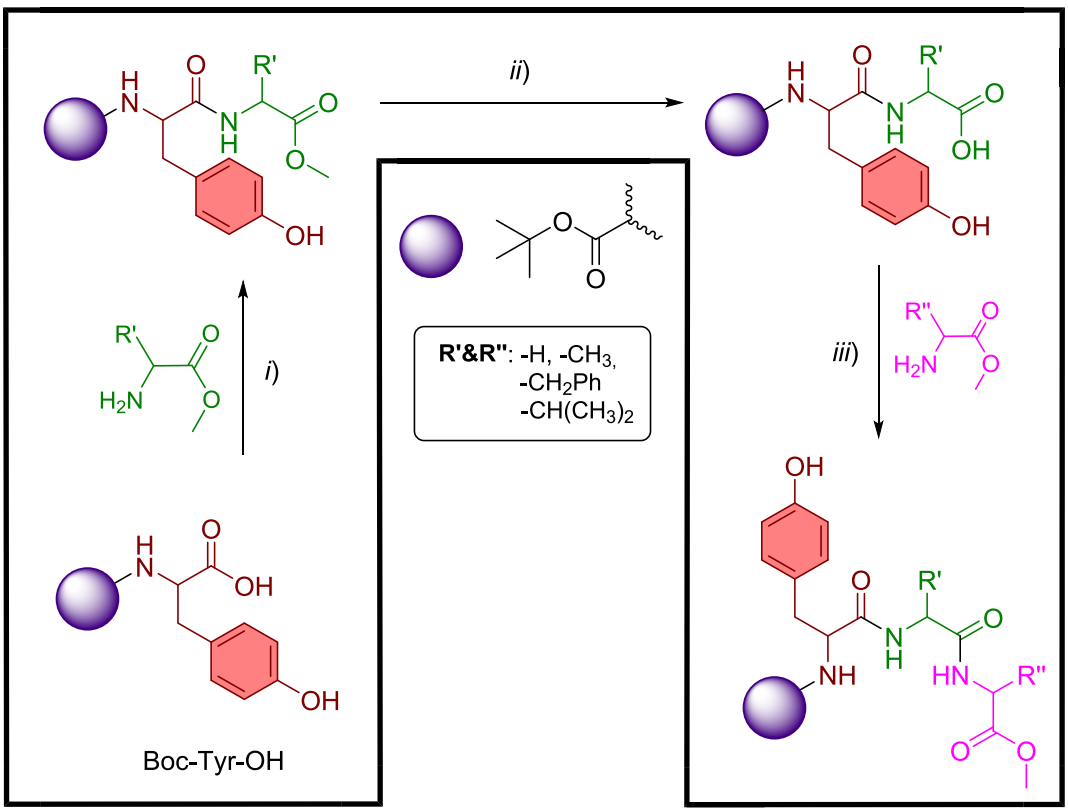
3.1 | Chemistry

Tyrosine **(Boc-Tyr-OH)** with free amine group protected carboxyl group as starting compound, Glycine **(NH_2–Gly–OCH_3.HCl)**, alanine **(NH_2–Ala–OCH_3.HCl)**, valine **(NH_2–Val–OCH_3.HCl)**, and phenylalanine **(NH_2–Phe–OCH_3.HCl)** methyl ester hydrochloride forms interacted with the CDMT method and four dipeptide compounds with methyl ester groups **(Boc–Tyr–Gly–OCH_3, Boc–Tyr–Val–OCH_3, Boc–Tyr–Ala–OCH_3,** and **Boc–Tyr–Phe–OCH_3)** were obtained. The ester groups of methyl ester dipeptide compounds were converted into carboxyl groups by adding 8% NaOH solution in acetonitrile to obtain free dipeptides. In the next step, each dipeptide reacted with glycine **(NH_2–Gly–OCH_3.HCl)**, L-alanine **(NH_2–Ala–OCH_3.HCl)**, L-valine **(NH_2–Val–OCH_3.HCl)**, L-phenylalanine **(NH_2–Phe–OCH_3.HCl)** in acetonitrile at room temperature using the CDMT method to obtain 16 tripeptides (Scheme 1).

Due to the structural similarities of all synthesized compounds, only one dipeptide **(Boc–Tyr–Val–OH)** and tripeptide's **(Boc–Tyr–Phe–Phe–OMe)** characterization was discussed in detail. Detailed spectroscopic data are given in the Supporting Information (Figure 1).

Singlet –OCH_3 ester proton peaks of three protons at 3.64 ppm of the Boc–Tyr–Val–OCH_3 compound are not observed in the ^{1}H-NMR of the Boc–Tyr–Val–OH compound. Similarly, the –OCH_3 ester carbon peak at 52.19 ppm is not present in the ^{13}C-APT NMR spectrum of the Boc–Tyr–Val–OH. These results indicate that the –OCH_3 group is converted to the –OH functional group. The presence of carboxylic acid –OH 1-proton peak at 12.73 ppm is a result of the conversion of the –CO–OCH_3 ester group to the –CO–OH group. Rest of peaks in both spectrums are compatible with the structure.

The peak of the singlet –CO–OH group of one proton at 12.70 ppm of the Boc–Tyr–Phe–OH compound has not appeared in the ^{1}H-NMR of the Boc–Tyr–Phe–Phe–OCH_3 compound. In addition, a three-proton singlet peak of –OCH_3 is observed at 3.58 ppm of the formed tripeptide compound. Likewise, with the binding of the Phe–OCH_3 amino acid, a third –NH proton was observed at



SCHEME 1 General synthetic route for tripeptides. Reaction conditions: (i) CDMT, NMM, MeCN, rt, up to 12 h, (ii) 8% NaOH, MeOH, 0°C to rt, up to 24 h (iii) CDMT, NMM, MeCN, rt, up to 18 h. CDMT, 2-chloro-4,6-dimethoxy-1,3,5-triazine; NMM, *N*-methyl morpholine; rt, room temperature.

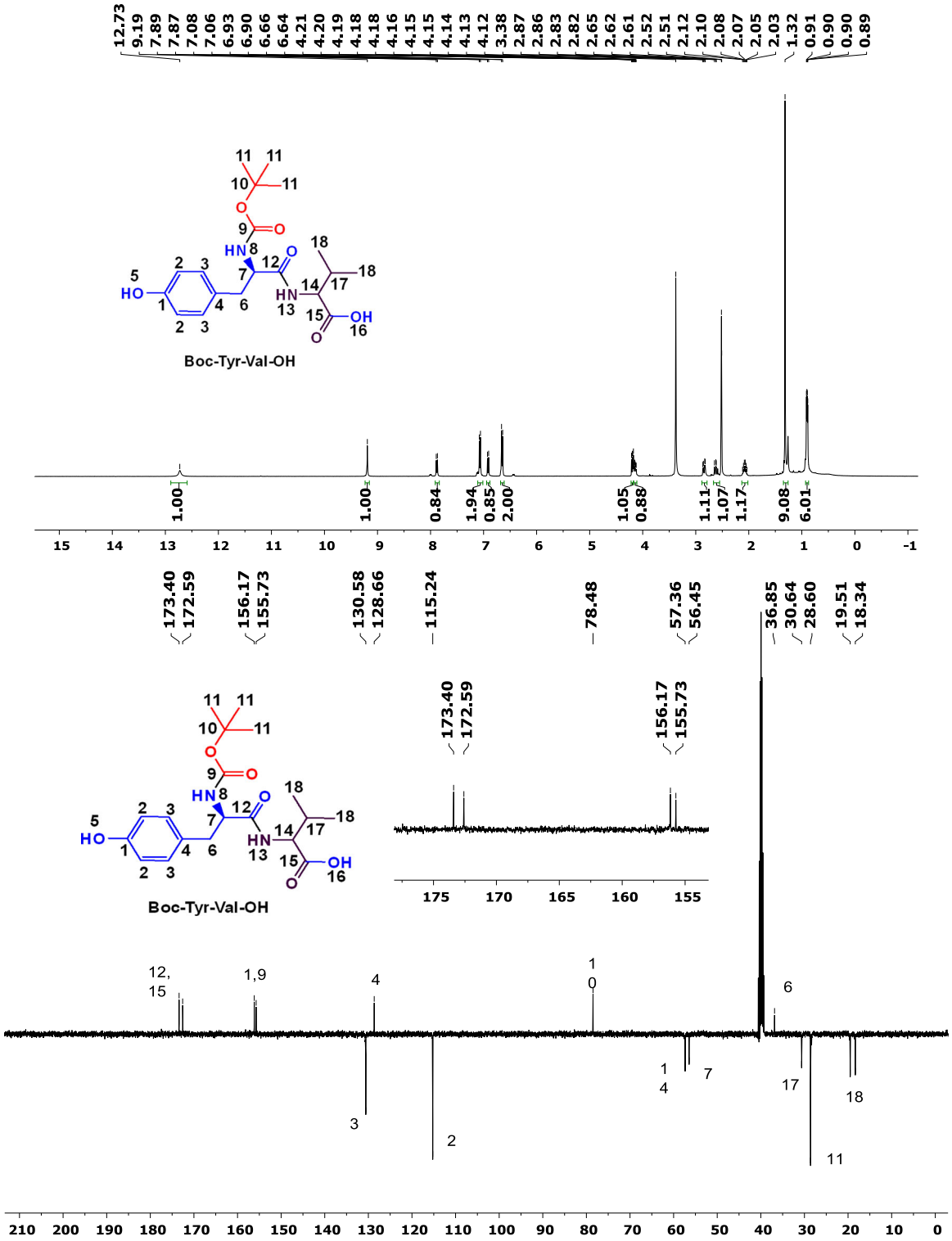


FIGURE 1 ^{1}H and ^{13}C NMR spectra of **Boc–Tyr–Val–OH**. NMR, nuclear magnetic resonance.

6.99–7.01 ppm. Aromatic peaks in phenylalanine at 7.19–7.30 ppm (10H, m, H21–26/28–33) and the proton peaks of the aliphatic $-CH_2$ (H20) group were observed at 2.93–2.98 and 3.00–3.07 ppm (2H, H27). The proton peak of the –CH (H17) group was at 3.97–4.07 ppm (1H, H17) is appeared. The carbon peaks of the same groups at 37.09 (C20), 37.24 (C27), and 53.66 (C17) ppm, respectively, and the $-OCH_3$ ester carbon peak is clearly present at 52.32 ppm. The compatibility of integral heights and carbon peaks with the structure and the observation of expected peaks indicate that the structure was formed (Figures 2 and 3).

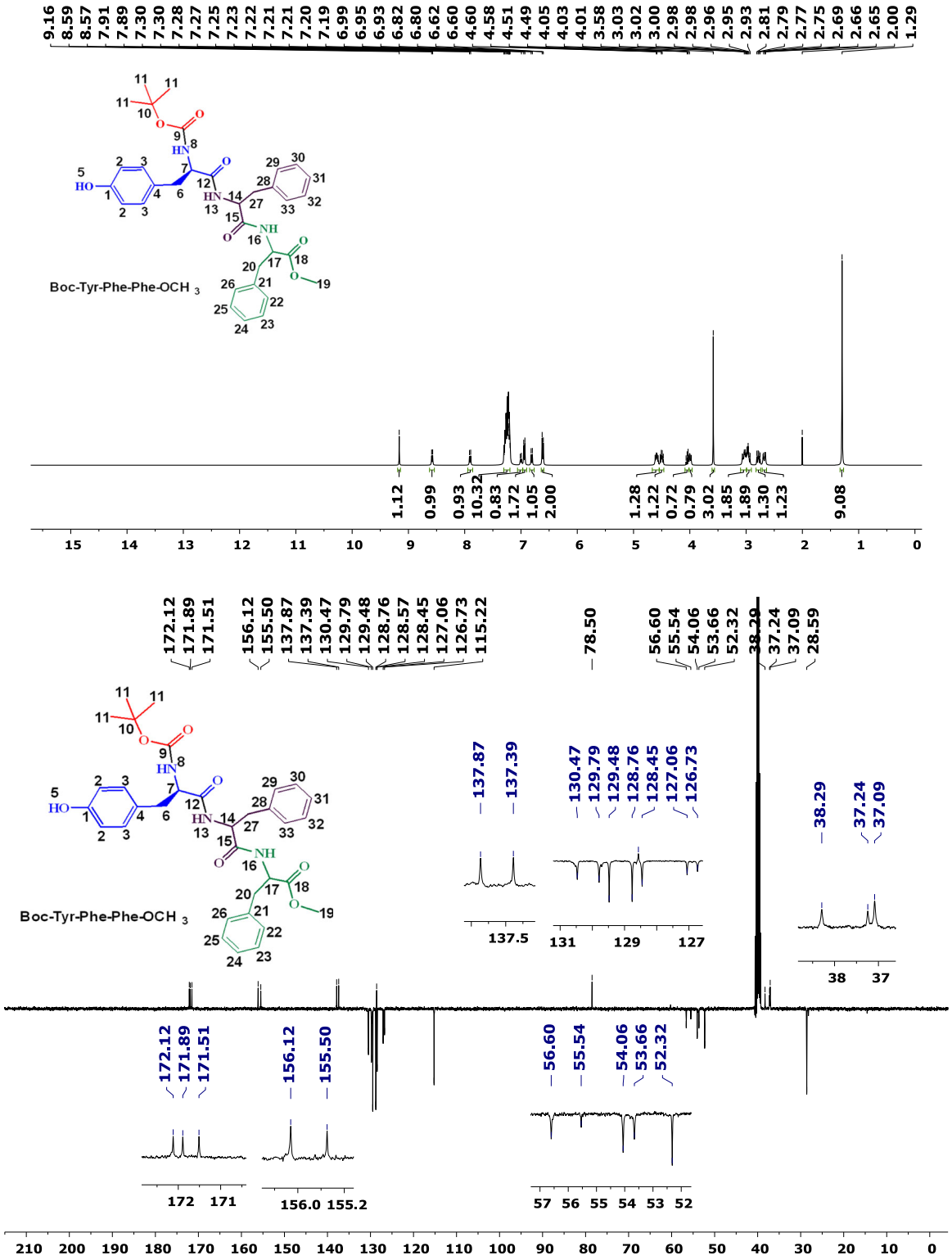


FIGURE 2 ^{1}H and ^{13}C NMR spectra of Boc–Tyr–Phe–Phe–OMe. NMR, nuclear magnetic resonance.

3.2 | Cytotoxicity and genotoxicity studies

TGG, TVV, TVA, TVP, TVM, TAV, and TPG coded compounds were dose-dependent, while other tripeptides caused significant reductions against MCF-7 cell lines, especially at 25, 50, and 100 μM doses ($p < 0.05$). Cytotoxic activity results of tripeptides on the A2780 cell line show that TGG, TVA compounds together with TAG, TAV, TAP, TPP, TPG, TPV and TPA, TPL (alanine and phenylalanine derivatives) cause significant decreases in cell viability in a dose-dependent manner ($p < 0.05$). In addition, considering the concentration values

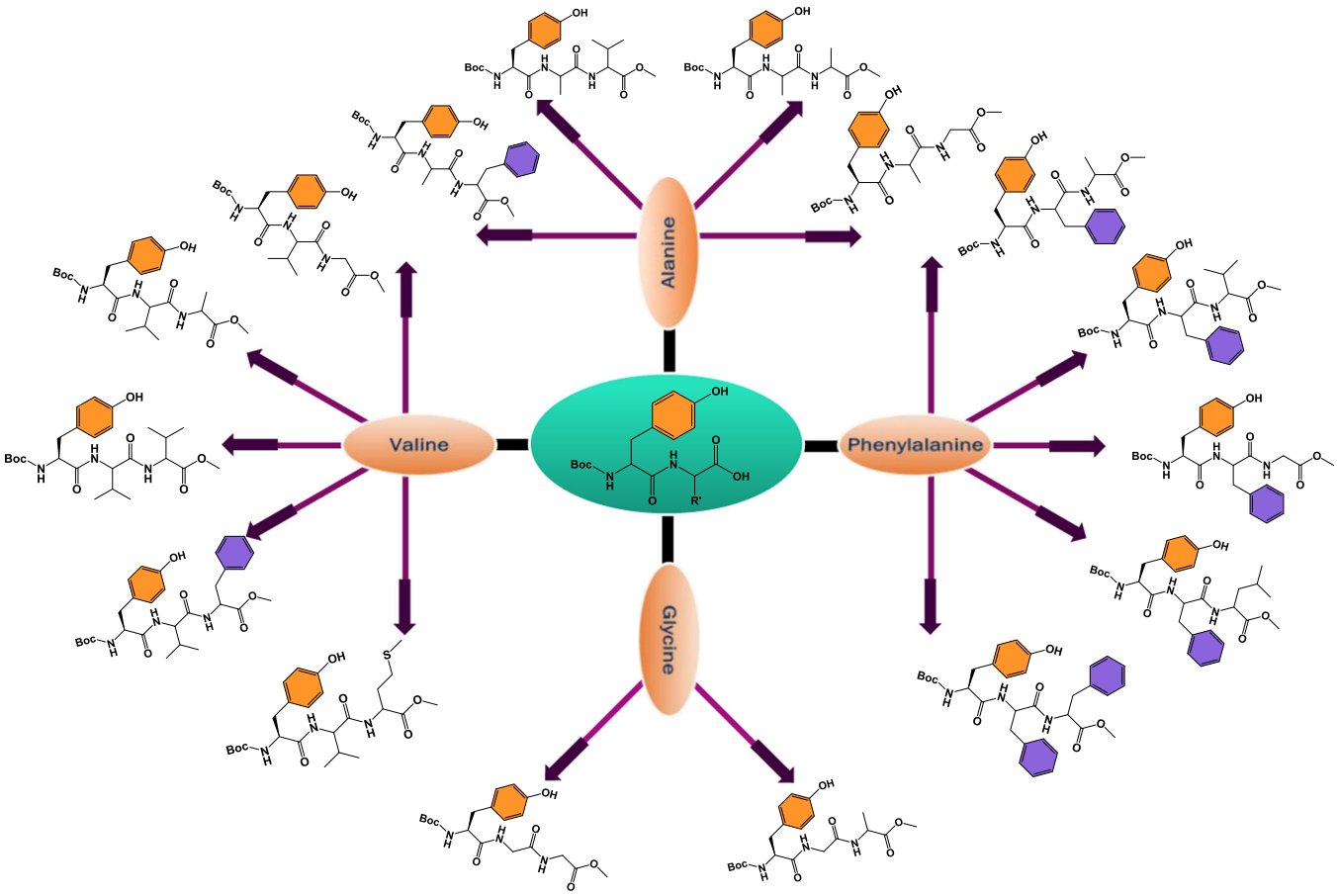


FIGURE 3 General representation of tripeptides.

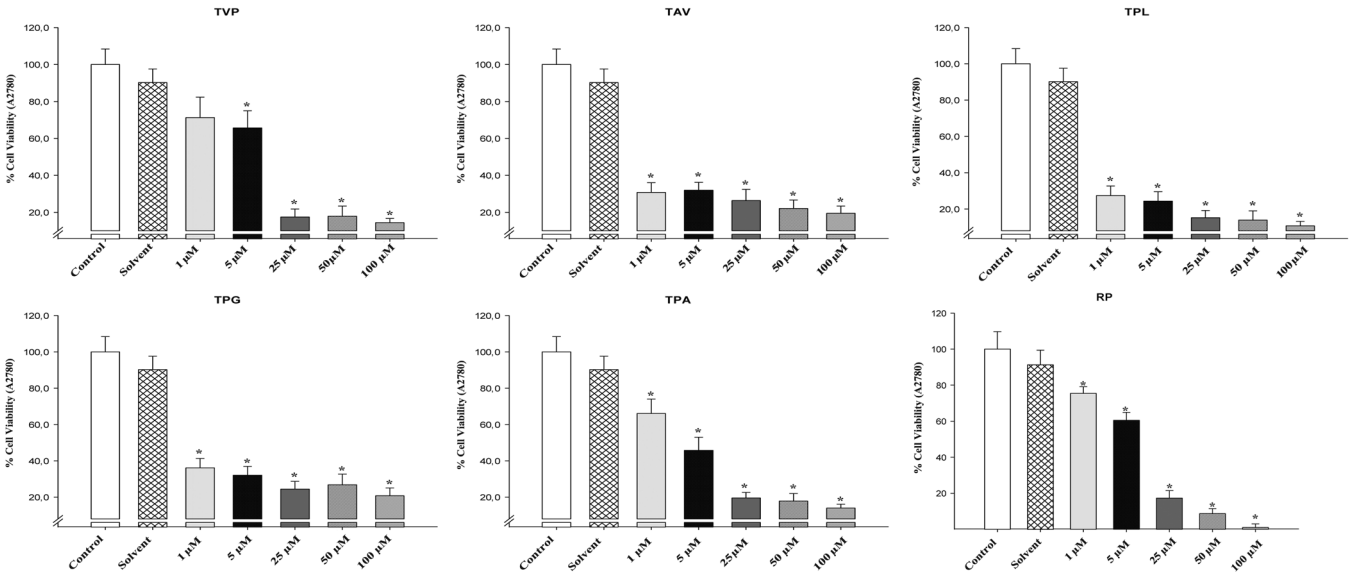


FIGURE 4 Cell viability results of tripeptides against A2780 cell lines.

that cause 50% Inhibition, it is quite remarkable that the concentration of 0.6 μM in the TPL compound is lower than that of the reference drugs, and the reduction in cell viability of these compounds. It has been observed that decreases of 50%–60% in the lowest doses and 80%–90% in the high doses. Considering all the results, it shows that the tripeptide compounds have a very significant effect against the A2780 human ovarian cancer cell line in general ($p < 0.05$). In the activity studies of tripeptides against the

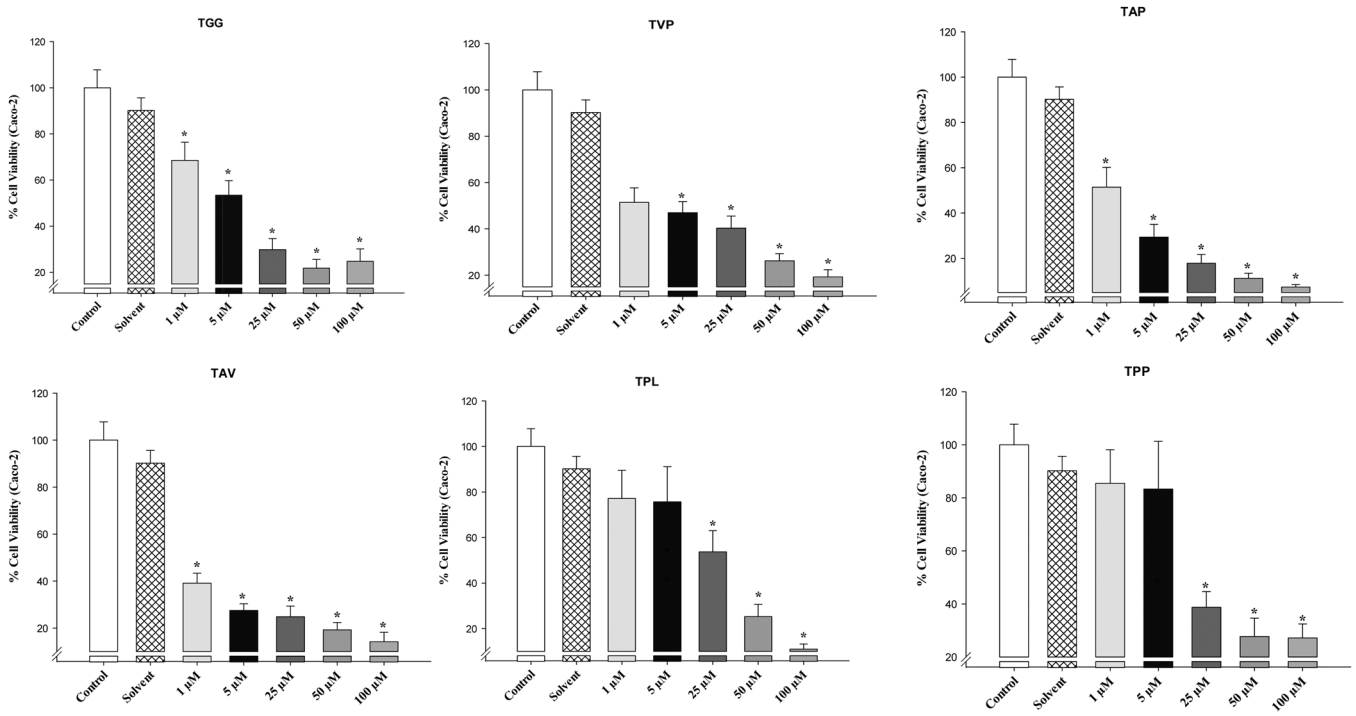


FIGURE 5 Cell viability results of tripeptides against Caco-2 cell lines.

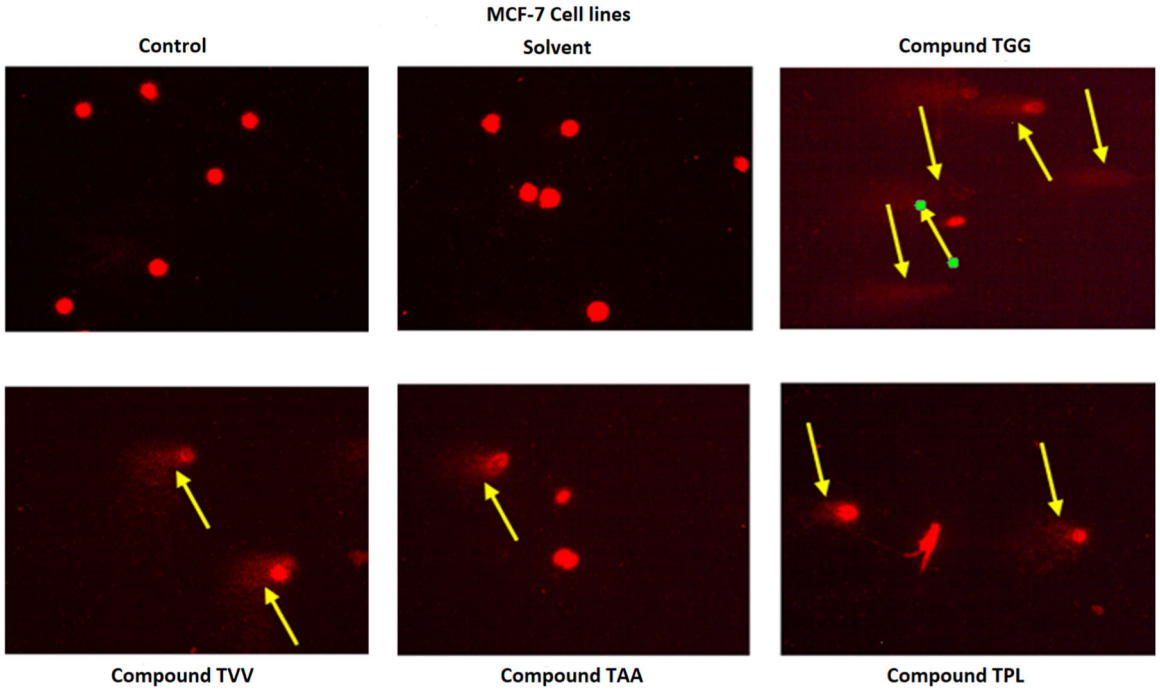


FIGURE 6 Images obtained from MCF-7 cancer cells in which tripeptides were effective within the scope of comet assay trials (yellow arrows indicate comet images showing DNA damage; ×10).

PC-3 cell line, it was determined that tripeptide compounds were not very effective at low doses, but were quite effective, especially at high doses of 100 µM ($p < 0.05$). TVP, TAP, TPV, and TPA compounds caused 90% and more decreases in cell viability at 100 µM dose ($p < 0.05$). These compounds showed better efficacy than the reference drugs at this dose. TPL compound consisting of tyrosine–phenylalanine–leucine in Figure 4 was found to be more effective against cancer cell line A2780 at all doses compared to the reference drug. TPA, TPG, and TVP compounds were also effective at all doses against the same cell line. On the other hand, TVP, TAP,

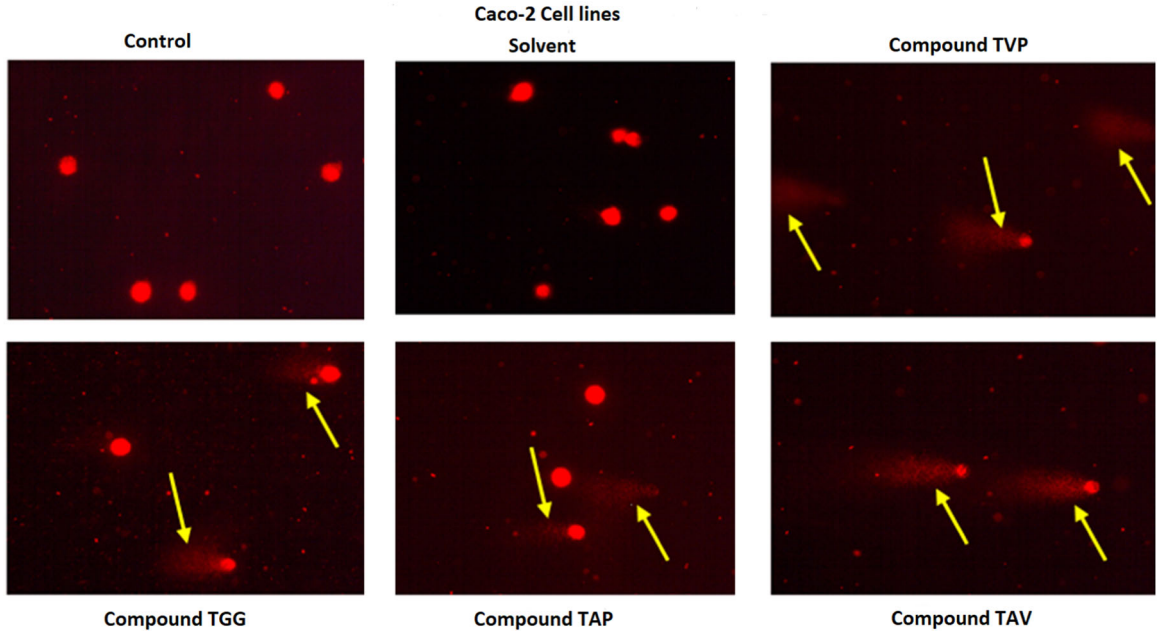


FIGURE 7 Images obtained from Caco-2 cancer cells in which tripeptides were effective within the scope of comet assay trials (yellow arrows indicate comet images showing DNA damage; ×10).

TABLE 1 Docking score and glide energy (kcal/mol) values between dipeptides and tripeptides and proteins.

	3VHE		3C0R		2ZCL		2HQ6	
Dipeptides								
TGO	−5.00	−43.14	−9.48	−71.58	−2.31	−35.27	−6.04	−54.66
TVO	−7.33	−49.32	−8.21	−70.73	−2.48	−35.94	−6.87	−58.79
TAO	−7.21	−49.11	−9.44	−71.55	−2.11	−35.51	−7.15	−61.55
TPO	−4.87	−43.41	−7.66	−70.05	−2.09	−35.15	−6.22	−55.23
Tripeptides								
TGG	−7.45	−49.34	−8.11	−70.61	−2.86	−37.99	−7.51	−61.35
TGA	−2.15	−32.22	−6.14	−65.00	−2.54	−37.14	−5.05	−54.82
TVV	−5.83	−46.58	−7.03	−68.78	−2.44	−37.05	−4.84	−52.74
TVG	−2.89	−32.25	−6.09	−62.14	−1.58	−28.86	−5.15	−55.67
TVA	−8.24	−50.52	−9.55	−76.24	−2.69	−37.24	−6.93	−59.18
TVP	−6.75	−47.99	−9.03	−70.89	−4.14	−41.95	−7.55	−61.44
TVM	−6.59	−47.89	−8.01	−70.65	−2.55	−35.55	−5.14	−55.55
TAA	−5.37	−44.67	−5.99	−61.56	−2.47	−35.49	−5.25	−56.46
TAG	−3.11	−39.37	−7.89	−69.88	−1.54	−24.66	−7.53	−61.42
TAV	−7.24	−49.22	−10.26	−78.08	−2.55	−35.76	−7.85	−61.77
TAP	−5.75	−44.89	−8.23	−70.15	−3.01	−38.41	−7.67	−61.48
TPP	−5.63	−44.91	−9.13	−71.54	−3.48	−38.88	−7.41	−60.74
TPG	−5.21	−44.66	−9.46	−71.57	−2.98	−37.86	−6.23	−57.36
TPV	−4.78	−42.78	−8.88	−71.98	−3.00	−41.00	−7.48	−60.99
TPA	−6.11	−46.76	−9.01	−75.45	−3.44	−41.89	−7.59	−61.45
TPL	−6.16	−46.87	−10.42	−78.66	−3.21	−41.87	−6.98	−59.78
RT	−4.99	−43.15	–	–	–	–	–	–
RP	–	–	−7.74	−65.25	–	–	–	–
RD	–	–	–	–	−3.35	−41.25	–	–

TGG, TAV, and TPL compounds were effective against Caco-2 cancer cell line, especially at 25 and 50 μM concentrations.

In addition, TVV, TVG, TAA, and TAG compounds did not show a statistically significant effect at any dose ($p < 0.05$). All compounds showed activity against Caco-2 cell lines ($p < 0.05$). TGG, TVP, TAG, TAV, TAP, TPV, TPA compounds caused significant decreases in cell viability at all doses in a dose-dependent manner ($p < 0.05$). The decrease in the cell viability of these compounds is quite remarkable and caused a decrease of 80%–90%. Compound TVA at 5, 25, 50, and 100 μM doses, TPP, TPG, TPL at 25, 50, and 100 μM doses, TVM at 50 and 100 μM doses, and TGA, TVV, TVG compounds at 100 μM. showed a significant effect on cell viability ($p < 0.05$). Although tripeptide compounds were generally effective against all cancer cells, their activity against A2780 and Caco-2 cell lines was observed to be more effective than MCF-7 and PC-3 cell lines. It was determined by the results of the comet assay that the cytotoxic compounds caused cell death on MCF-7, A2780, PC-3, and Caco-2 cell lines through DNA damage (Figure 5).

Peptides have features such as small structures, easy synthesis, and the ability to affect tumors, which have made these compounds an important position in potential cancer drug research.[10,39–46] In cancer treatment, peptides are preferred as tumor-targeting agents (hormones, vaccines, and radionuclides) as well as their direct use as cytotoxic drugs.[3,7,47] The ability of peptides to bind to different receptors and be a part of various biochemical pathways allows them to function as potential diagnostic tools and biomarkers.[48–55]

Although the studies carried out in recent years have provided significant advances in the diagnosis and treatment of cancer, intensive research continues for the search for new drug candidates with increased efficacy and least side effects.[56,57] In this context, peptides and peptide conjugates in particular are of great interest because of their potential as anticancer agents.[58–61] One of the possible hypotheses put forward is that most of the anticancer drugs used in clinical practice exert their effects either through the inhibition of DNA-bound proteins/enzymes or by interacting directly with DNA.[62–67] As a result of OTM parameters on A2780, MCF-7, Caco-2, and PC-3 cell lines, it was observed that most of the tripeptides caused genotoxic effects ($p < 0.05$). When the genotoxicity results were examined, it was revealed that these substances were effective on DNA (Figure 6).

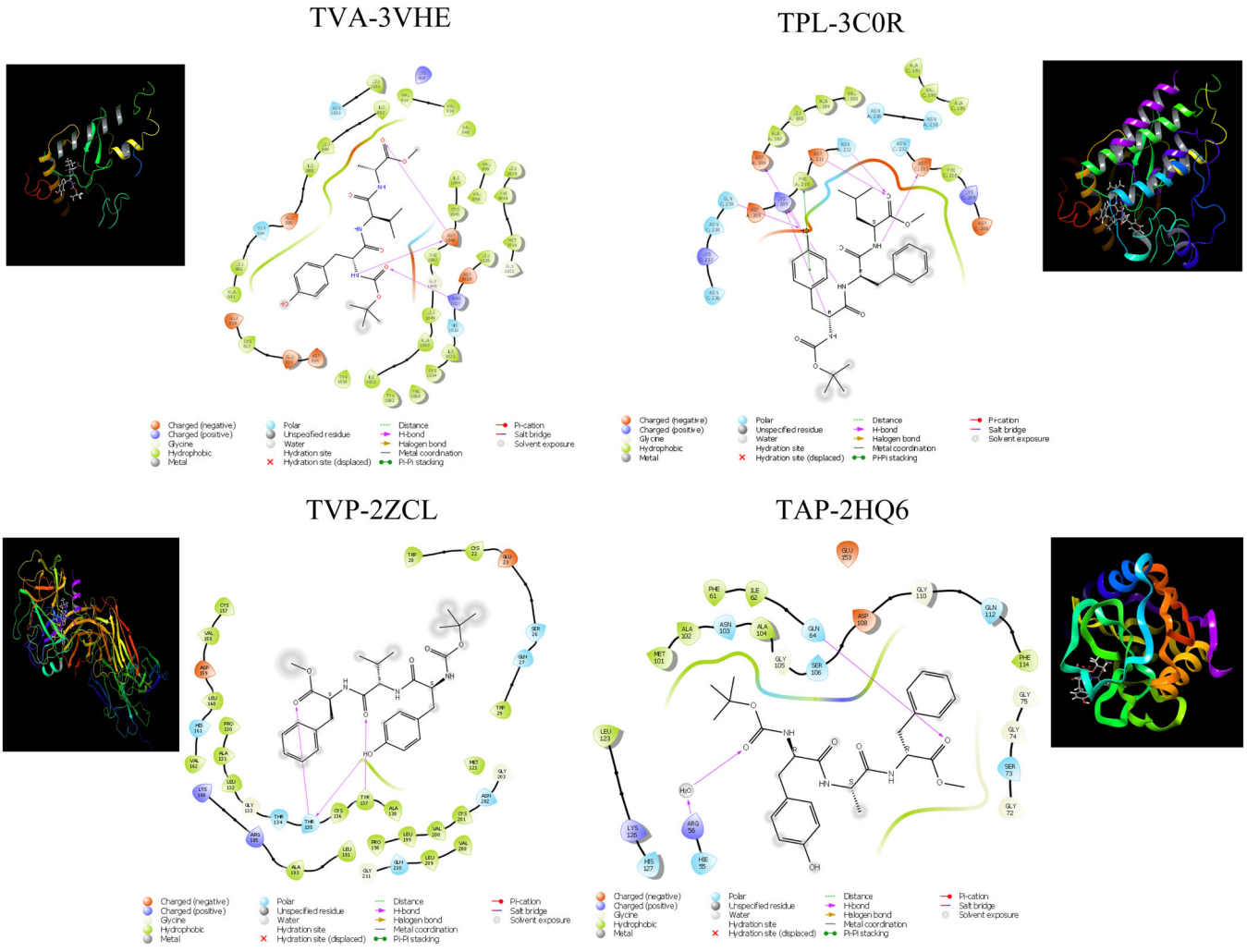


FIGURE 8 The interaction schema of the most active molecule against proteins.

TABLE 2 ADME properties of molecules for A2780.

	TGO	TVO	TAO	TPO	TGG	TGA	TVV	TVG	TVA	TVP	TVM	TAA	TAG	TAV	TAP	TPP	TPG	TPV	TPA	TPL	Ref. range
mol_MW	275	199	201	284	189	305	266	297	144	203	271	276	285	189	263	268	271	283	281	273	130–725
Dipole (D)	5.6	2.4	5.5	4.7	2.1	5.7	5.8	5.1	2.6	4.4	5.4	5.5	5.8	2.1	4.9	5.1	5.1	5.0	4.7	4.9	1.0–12.5
SASA	568	540	543	570	635	715	649	704	689	645	656	666	702	632	648	650	661	701	711	661	300–1000
FOSA	15	2	18	21	0	45	20	23	0	15	25	28	20	10	5	5	18	35	30	25	0–750
FISA	120	89	100	120	75	145	110	120	70	105	120	130	120	95	105	110	125	115	105	115	7–330
PISA	195	185	135	130	115	125	198	122	107	198	204	205	222	120	235	242	245	251	250	226	0–450
WPSA	14	10	10	15	5	25	8	5	5	10	8	4	5	0	0	6	2	9	9	8	0–175
Volume (A^3)	889	901	900	905	904	889	904	918	928	931	945	902	906	911	936	924	909	901	907	934	500–2000
donorHB	2	2	3	2	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	0–6
accptHB	8.0	8.0	7.0	7.0	10.4	13.4	9.8	9.7	11.5	10.5	10.0	10.5	11.0	9.5	10.5	10.5	11.5	10.0	10.0	11.0	2.0–20.0
glob (sphere = 1)	0.8	0.8	0.75	0.8	0.9	0.9	0.9	0.9	0.85	0.9	0.9	0.85	0.8	0.85	0.9	0.8	0.8	0.8	0.8	0.9	0.75–0.95
QPpolrz (A^3)	30.5	31.4	32.6	33.8	20.1	25.3	20.8	21.9	18.4	19.6	20.8	20.9	21.3	19.8	20.4	20.8	19.6	21.8	21.5	209	13.0–70.0
QPlogPC16	11.4	11.4	11.5	11.4	12.2	12.3	12.4	12.1	12.1	12.4	12.1	10.9	10.8	11.2	11.5	11.9	11.5	11.3	12.0	11.9	4.0–18.0
QPlogPoct	15.2	15.1	15.3	15.0	13.5	13.2	13.3	13.4	9.9	10.1	11.1	12.4	13.0	10.7	11.8	11.8	12.0	12.4	12.3	12.0	8.0–35.0
QPlogPw	18.1	17.9	18.0	17.8	15.8	11.4	14.4	12.8	16.0	17.1	15.9	15.2	16.6	15.5	14.1	14.2	14.6	15.3	15.0	14.9	4.0–45.0
QPlogPo/w	0.6	0.7	0.5	0.6	−0.3	−0.9	−0.5	−0.6	−0.1	−0.3	−0.5	−0.6	−0.7	−0.3	−0.4	−0.4	−0.5	−0.6	−0.6	−0.5	−2.0–6.5
QPlogS	−4.1	−4.0	−4.3	−4.1	−3.6	−5.2	−3.9	−4.0	−3.1	−3.4	−3.5	−3.6	−3.9	−3.5	−3.7	−3.8	−3.9	−4.0	−4.0	−3.9	−6.5–0.5
CIQPlogS	−3.8	3.4	−3.5	−3.6	−2.1	−4.5	−3.3	−3.6	−2.0	−2.2	−3.3	−3.4	−3.7	−2.8	−3.2	−3.4	−3.3	−3.5	−3.4	−3.4	−6.5–0.5
QPlogHERG	−3.1	−2.9	−3.5	−3.0	−2.1	−4.8	−3.2	−3.7	−1.8	−2.5	−2.8	−2.9	−3.1	−2.2	−3.2	−3.4	−3.2	−3.3	−3.3	−3.1	(Concern below −5)
QPPCaco (nm/s)	36	10	24	42	19	34	22	25	15	17	26	27	28	16	21	22	24	25	24	23	25 < X < 500
QPlogBB	−2.9	−2.4	−2.6	−2.8	−2.7	−0.4	−2.2	−2.5	−2.9	−2.1	−1.9	−2.8	−2.7	−1.8	−2.3	−2.6	−2.7	−2.8	−2.6	−2.5	−3.0–1.2
QPPMDCK (nm/s)	44	32	31	42	12	47	17	24	10	21	33	32	34	15	28	28	27	26	32	29	25 < X < 500
QPlogKp	−1.6	−2.5	−2.4	−1.7	−3.0	−1.7	−2.5	−2.2	−3.4	−3.1	−2.4	−2.0	−1.8	−3.1	−2.1	−2.0	−1.9	−1.8	−1.8	−1.6	Kp in cm/h
IP (ev)	8.4	8.2	8.1	8.5	9.4	10.1	9.5	9.8	9.9	8.6	8.9	9.1	9.2	8.8	8.9	8.9	9.0	9.1	9.1	9.0	7.9–10.5
EA (eV)	1.5	1.3	1.3	1.6	0.7	1.9	1.2	1.4	0.5	0.9	1.4	1.5	1.6	0.8	1.4	1.4	1.5	1.6	1.6	1.4	−0.9–1.7
#metab	1	2	2	1	3	2	2	2	4	3	4	4	4	3	4	4	3	4	4	3	1–8
QPlogKhsa	−1.0	−0.9	−0.9	−1.0	−0.4	−1.1	−1.3	−1.0	−0.9	−1.2	−1.3	−1.3	−1.1	−0.5	−0.8	−0.9	−1.0	−1.1	−1.0	−1.2	−1.5–1

TABLE 2 (Continued)

	TGO	TVO	TAO	TPO	TGG	TGA	TVV	TVG	TVA	TVP	TVM	TAA	TAG	TAV	TAP	TPP	TPG	TPV	TPA	TPL	Ref. range
% Human oral absorption	12	13	13	12	14	12	13	13	14	12	13	13	13	12	13	13	13	12	12	12	<25% is poor >80% is high
PSA	76	81	81	70	10	66	45	32	8	15	36	38	41	11	28	29	36	42	41	39	7–200
RuleOfFive	1	0	0	1	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	Maximum is 4
RuleOfThree	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Maximum is 3

(Figure 7) Comet analysis results from DNA damage studies of tripeptides in human colon carcinoma (Caco-2), human prostate cancer cell lines (LNCaP, PC-3), and human ovarian cancer (A2780) cell lines at the 100 μM dose TL, tail intensity (TI), and olive tail intensity (OTI) parameters showed that changes were observed and these changes were statistically significant. The results indicate that cell deaths caused by tripeptides occurred over the DNA damage mechanism.

3.3 | Docking studies

Biological receptors and tyrosine-based dipeptide and tripeptide molecules were docked for binding assays. Compounds were inserted into the active conformation of proteins. Experimental results were compared with the obtained docking parameters. The docking score and glide energy were found to be compatible and are given in Table 1.

The docking results yield many parameters, but two parameters are listed that are in agreement with the experimental biological activities of the molecules. These parameters are docking score and glide energy, respectively. Parameter values show a similar trend to the experimental value. The numerical value of the docking score parameter becomes more negative as the interaction between the molecules and the relevant receptor protein increases.[68,69] Thanks to chemical interactions, an effective type of energy can be prescribed between molecules and biological receptors. Chemical interactions are given in Figure 8 to explain the activities of molecules. In Figure 8, the interaction of the most active chemical species against each protein type is illustrated.

As a result of ADME/T analysis, many parameters that allow medical predictions are obtained. Each obtained parameter is used to elucidate the drug properties of 4 tyrosine-based dipeptides and 16 of these dipeptides. ADME/T analysis can explain the effect of 4 tyrosine-based dipeptides and 16 of these dipeptides on organs or tissues. Under the premise of these parameters, the designs of molecules can be improved.[70] ADME/T analysis of compounds more active against the A2780 cell line is given in Table 2. ADME/T analyses between compounds and target proteins representative of other cells are given in Supporting Information: Tables S5–S7. In the table above, molecular weight is a measure of fitness for human metabolism. SASA means saturated carbon and bound hydrogen. QPlogHERG are estimated IC50 values. QPPCaco is the predicted Caco-2 cell permeability in nm/s. This value is important for the gut-blood barrier. QPPMDCK also represents MDCK cell permeability at the blood–brain barrier. This value is not in the desired range for compounds with high inhibitory activity.[71] It can be interpreted, then, that its cells are not a good mimic for the blood–brain barrier. RuleOfFive and RuleOfThree are other important parameters. It is predicted that the molecule will not be a drug candidate when both parameters do not match the reference range. The numerical results obtained in Table 2 show that the examined molecules are the potential drug candidate category and it can be predicted that the

examined molecules will not reveal a potential problem according to ADME analysis.[72]

4 | CONCLUSION

In this study, 4 tyrosine-based dipeptides and 16 tripeptides from these dipeptides were synthesized using the triazine method. Cytotoxicity studies of this peptide library were performed against four different cancer cells, and some tripeptides were found to be more effective than reference drugs in different cell lines. The comet assay method was used to analyze whether cell death was caused by DNA damage, and the results indicate that cell death caused by the majority of the compounds proceeded through the DNA damage mechanism. Theoretical studies of compounds were compared against selected proteins for human cancer cell lines (MCF-7), ovarian (A2780), prostate (PC-3), and colon cancer cell lines (Caco-2). According to the obtained docking parameters, different molecules were found to be more active for each protein. However, the calculations are in good agreement with the experimental results. ADME calculations were made for the drug properties of the studied molecules. The parameters obtained were obtained within the reference range.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the Supporting Information Material of this article.

ORCID

Eray Çalışkan http://orcid.org/0000-0003-2399-4100
Alpaslan Kaplan http://orcid.org/0009-0003-7015-956X
Güldeniz Şekerci http://orcid.org/0000-0002-0811-4454
İrfan Çapan http://orcid.org/0000-0002-9555-1555
Suat Tekin http://orcid.org/0000-0002-2757-1802
Sultan Erkan http://orcid.org/0000-0001-6744-929X
Kenan Koran http://orcid.org/0000-0002-2218-7211
Süleyman Sandal http://orcid.org/0000-0002-8916-3329
Ahmet O. Görgülü http://orcid.org/0000-0003-0632-4834

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