RESEARCH ARTICLE

Synthesis, Molecular Docking studies, and Anti-proliferative Activity of Peptide Derivatives of 1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic Acid.

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Abstract

Background: In the current research, initially tri/tetrapeptide methyl ester derivatives of different amino acids were synthesized followed by the synthesis of 1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid and subsequently 1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid was coupled with tri/tetrapeptide methyl esters to obtain novel peptide derivatives.

Methods: The synthesis of peptide derivatives of 1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid along with their antibacterial, antifungal, and anthelmintic activity have been reported in our previous research. The structures of peptide derivatives were elucidated by IR, ^{1}H-NMR and mass spectral analysis and compounds were investigated for their anti-proliferative activity against three different human cancer cell lines using 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Results: All the synthesized peptide derivatives of 1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid exhibited anti-proliferative activity. The peptide derivative (AA_6) exhibited (IC_{50} 3.90±0.25 µg/mL) against Hela cancer cell lines. Hence, it was found to be more active than doxorubicin (IC_{50} 4.02±0.22 µg/mL). Moreover, the same peptide derivative (AA_6) indicated highest binding interactions with 3G5K enzyme pocket in the molecular docking studies.

***Conclusion**:* The results confirmed that the peptide derivatives of 1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid can be used as cytotoxic agents and further future studies should be performed to explore their bio-targets and in-vivo activity.

Keywords: Peptide, Amino acid, Anti-proliferative, Docking, Enzyme, Doxorubicin, Bio-targets.

1. INTRODUCTION

The predominance of cancer is escalated globally and currently it ranks the second most common cause of death [1]. Cancer drug resistance is still a major problem in cancer therapy. Therefore, it is important to develop a new, safer and effective anti-cancer agent [2]. Quinoxaline and its derivatives have been reported to exhibit different pharmacological activities like anti-inflammatory, antioxidant, anti-viral, antibacterial, anti-cancer, anti-amoebic, and anti-malarial [3-11]. Moreover, various tri, tetra, and cyclic peptide derivatives of heterocyclic compounds have been reported to exhibit antibacterial, antifungal, anthelmintic, cytotoxic activity [12-20]. Hence, taking into consideration the bio-activity of both quinoxaline and peptide derivatives, tri and tetrapeptide derivatives of 1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid were synthesized and investigated for their anti-proliferative activity.

2. MATERIALS AND METHODS

2.1. Experimental

All the chemicals and solvents of laboratory grade were purchased from Merck (Darmstadt, Germany). The purity of synthesized derivatives was checked by TLC on pre-coated silica gel-G plates (Kieselgel 0.25 mm, 60GF254, Merck, Germany) and R_f values were determined. Open capillary method was used to record the melting point range. IR spectrum of synthesized derivatives was recorded in KBr discs on Shimadzu 8700 FT-IR spectrophotometer (Shimadzu, Japan). ^{1}H-NMR was recorded on Bruker AC NMR spectrometer (300MHz), (Bruker, USA) using $CDCl_3$ as solvent. The Mass spectroscopy of the synthesized derivatives (AA_{1-9}) was done on mass spectrophotometer (Waters, Q-TOF micromass).

2.1.1. Synthesis of 1, 2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid (AA)

A warm solution of pyruvic acid (10 mmol) in 8 mL of absolute ethanol was transferred to the solution of 3,4-diaminobenzoic acid (10 mmol) in 8 mL of same solvent. The resulting mixture was refluxed for 1.5 h at 80 ºC and poured into a beaker having ice-cold water. The precipitate was filtered and recrystallized with aqueous ethyl alcohol.

IR (KBr, ν, cm^{-1}): 3467.38 (O-H str., COOH), 3350.71 (NH str.), 3078.80 (C-H str., aromatic ring), 1506 (C-C Str., aromatic ring), 1036 (C-H bend.), 2864.49 (C-H str., CH3), 1700.52 (C=O str., COOH), 1681.69 (C=O str.). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 2.411 (s, 3H, CH_3), 7.293- 8.165 (m, 3H, aromatic H), 9.281 (s, 1H, aromatic NH), 12.5 (s, 1H, COOH). Mass: m/z 204 (M^+).

2.1.2. General procedure for synthesis of Boc-di/tri/tetrapeptide methyl esters

Boc-dipeptide (1-9), tripeptide (17-21), and tetrapeptide (24-27) were synthesized by coupling of Boc-aminoacids/dipeptides with the corresponding L-amino acid methyl ester hydrochlorides/dipeptide methyl esters employing DCC as coupling agent and NMM as base following Bodanzsky-Bodanzsky procedure [11, 19] with suitable modifications.

Boc-Pro-Phe-OMe (1): IR (KBr, ν, cm^{-1}): 1650 (C=O str., ester); 1475 (C=C str., aromatic ring); 3000 (C-H str., aromatic); 2850 (C-H str., CH_3); 1206 (C-O str., ester); 3600 (N-H str.); ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 8.11 (s, 1H, NH); 6.68-6.88 (m, 1H, Phe); 2.24-4.23 (m, 1H, CH); 1.10-1.78 (d, 2H, CH_2); 1.55 (s, 9H, *t*-butyl); 4.73-4.70 (t, 1H, δ-H of Pro); 4.02-3.97 (t, 2H, δ-H, Pro); 3.85 (s, 3H, OCH_3); 2.40-2.31 (m, 2H, β-H, Pro).

Boc-Trp-Phe-OMe (2): IR (KBr, ν, cm^{-1}): 2826 (CH str., OCH_3); 3125 (NH str., amide); 3065, 3052 (CH str., rings). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 10.1(s, 1H, NH, Trp); 7.42 (d, 1H, Trp); 7.39 (d, 1H, Trp); 3.06-2.81 (m, 2H, CH_2); 1.55 (s, 9H, *t*-butyl); 8.0 (s, 1H, NH); 7.53-7.49 (t, 1H, *p*-H, Phe), 4.09 (s, 3H, OCH_3).

Boc-Phe-Tyr-OMe (3): IR (KBr, ν, cm^{-1}): 3373 (OH str., Tyr); 3125 (NH str., amide); 3065, 3052 (CH str., rings); 1749 (C=O str., ester); 1645, 1638 (C=O str., amide). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 7.51-7.47 (t, 2H, *m*-H, Phe); 6.92-6.88 (m, 3H, *p*-H, Phe and *o*-H, Tyr), 6.66 (s, 1H, NH); 5.95 (s, 1H, OH, Tyr); 4.72-4.69 (q, 1H, α-H, Phe); 3.54 (s, 3H, OCH_3); 3.02-2.76 (m, 4H, β-H, Phe and Tyr); 1.55 (s, 9H, *t*-butyl).

Boc-Gly-Leu-OMe (4): IR (KBr, ν, cm^{-1}): 3125 (N-H str., amide); 2927 (-CH str., asym., CH_2); 1742 (C=O str., ester); 1642, 1639 (C=O str., 2° amide); 1535, 1529 (N-H bend., 2° amide); 1392, 1368 (C-H bend., *t*-butyl). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 6.52 (br. s, 1H, NH); 6.02 (br. s, 1H, NH); 4.25-4.20 (q, 1H, α-H, Leu); 3.62 (s, 3H, OCH_3); 3.50-3.48 (d, 2H, CH_2, Gly); 1.54 (s, 9H, *t*-butyl); 1.51-1.41 (m, 3H, β-H, γ-H, Leu); 0.96-0.94 (d, 6H, δ-H, Leu).

Boc-Phe-Gly-OMe (5): IR (KBr, ν, cm^{-1}): 3129 (NH str., amide); 1745 (C=O str., ester); 1639 (C=O str, 2º amide); 1390, 1365 (CH bend, *t*-butyl); 1273 (C-O str., ester). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 7.09 (1H, br. s, NH); 4.96 (1H, td, α-H, Phe); 4.11 (2H, d, α-H, Gly); 3.65 (3H, s, OCH_3); 2.97 (2H, d, β-H, Phe); 1.54 (9H, s, *t*-Butyl).

Boc-Ala-Gly-OMe (6): IR (KBr, ν, cm^{-1}): 3126 (N-H str, amide); 2826 (CH str., OCH_3); 1462 (CH def., CH_2); 1747 (C=O str., ester); 1638 (C=O str., 2º amide); 1390, 1365 (CH bend, *t*-butyl); 1533 (NH bend, 2º amide). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 4.59 (1H, q, α-H, Ala); 4.12 (2H, d, α-H, Gly); 3.64 (3H, s, OCH_3); 1.55 (9H, s, *t*-Butyl); 1.34 (3H, d, β-H, Ala).

Boc-Pro-Gly-OMe (7): IR (KBr, ν, cm^{-1}): 3128 (N-H str., amide); 2997, 2994 (CH str., cyclic CH_2 and CH); 2848 (CH str., sym., CH_2); 2826 (CH str., OCH_3); 1752 (C=O str., ester); 1672, 1635 (C=O str., amide); 1390, 1362 (CH bend, *t*-butyl); 1270 (s, C-O str, ester). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 5.76 (1H, br. s, -NH); 3.98-3.96 (2H, d, CH_2, Gly); 3.86-3.82 (1H, t, α-H, Pro); 3.64 (3H, s, OCH_3); 3.25-3.20 (2H, t, δ-H, Pro); 2.55-2.46 (2H, m, β-H, Pro); 1.94-1.86 (2H, m, γ-H, Pro); 1.48 (9H, s, *t*-butyl).

Boc-Pro-Trp-OMe (8): IR (KBr, ν, cm^{-1}): 3052 (CH str., rings); 1749 (C=O str., ester); 1645, 1638 (C=O str., amide); 1587, 1479, 1476 (skeletal bands, rings); 1365 (CH bend, *t*-butyl); 825, 710, 695 (CH def., rings); 3125 (NH

str., amide). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 4.73-4.70 (t, 1H, δ-H, Pro); 4.02-3.97 (t, 2H, α-H, Pro); 2.48-2.41 (m, 2H, γ-H, Pro); 2.40-2.31 (m, 2H, β-H, Pro); 1.54 (s, 9H, *t*-butyl); 3.66 (s, 3H, OCH_3); 7.39 (s, 1H, NH, Trp).

Boc-Ala-Phe-OMe (9): IR (KBr, ν, cm^{-1}): 3123 (N-H str., amide); 3052 (C-H str., aromatic. ring); 1750 (C=O str., ester); 1643 (C=O str., amide); 710, 690 (C-H bend., phenyl ring). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 7.13-7.09 (1H, t, *p*-H, Phe); 7.00-6.97 (2H, t, *m*-H, Phe); 6.89 (1H, br. s, N-H); 4.60- 4.48 (2H, m, α-H, Phe and Ala); 3.54 (3H, s, OCH_3); 3.00-2.98 (2H, d, β-H, Phe); 1.54 (9H, s, *t*-butyl).

Boc-Pro-Phe-Tyr-OMe (17): IR (KBr, ν, cm^{-1}): 1650 (C=O str.); 3480 (OH str.); 1475 (C=C aromatic ring str.); 3000 (CH aromatic ring str.); 2850 (CH str., CH_3); 3600 (NH str.); 3200 (OH str.). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 4.73-4.70 (t, 1H, δ-H, Pro); 4.02-3.97 (t, 2H, α-H, Pro); 8.11 (s, 1H, NH); 7.13-7.09 (t, 1H, *p*-H, Phe); 7.00-6.97 (2H, t, *m*-H, Phe); 6.86- 1.48 (s, 9H, *t*-butyl); 4.11 (s, 1H, OH-Tyr); 3.66 (s, 3H, OCH_3).

Boc-Trp-Phe-Leu-OMe (18): IR (KBr, ν, cm^{-1}): 1205 (C-O str., ester); 732, 695 (CH bend., out-of-plane, monosub. ring); 3300 (NH str.); 2925 (CH str., asym., CH_2); 2822 (CH str., OCH_3); 1535 (NH bend., amide). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 10.84 (s, 1H, NH, Trp); 7.53 (d, 1H, Trp); 7.34 (d, 1H, Trp); 1.32 (s, 9H, *t*-butyl); 6.80 (s, 1H, NH-Phe); 7.13-7.09 (t, 1H, *p*-H, Phe); 7.00-6.97 (2H, t, m-H, Phe); 4.12 (s, 3H, OCH_3).

Boc-Phe-Tyr-Phe-OMe (19): IR (KBr, ν, cm^{-1}): 3373 (OH str., Tyr); 3125 (NH str., amide); 1749 (C=O str., ester); 1645, 1638 (C=O str., amide). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 7.51-7.47 (t, 2H, *m*-H, Phe); 6.92-6.88 (m, 3H, *p*-H, Phe and *o*-H, Tyr), 6.86-6.84 (d, 2H, *o*-H, Phe); 6.58 (s, 1H, NH); 5.95 (s, 1H, OH, Tyr); 4.72-4.69 (q, 1H, α-H, Phe); 1.55 (s, 9H, *t*-butyl).

Boc-Gly-Leu-Gly-OMe (20): IR (KBr, ν, cm^{-1}): 3125 (NH str., amide); 2927 (CH str., asym., CH_2); 2853 (CH str., sym., CH_2); 1742 (C=O str.); 1642, 1639 (C=O str., 2º amide); 1535, 1529 (NH bend., 2º amide); 1368 (CH bend., *t*-butyl). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 6.52 (br. s, 1H, NH, Gly); 6.02 (br. s, 1H, NH, Leu); 3.50-3.48 (d, 2H, CH_2, Gly); 1.54 (s, 9H, *t*-butyl), 3.67 (s, 3H, OCH_3).

Boc-Phe-Gly-Trp-OMe (21): IR (KBr, ν, cm^{-1}): 1644, 1639 (C=O str., 2º amide), 3050 (C-H Aromatic str.); 1670 (C=C aromatic ring str.); 1640 (C=O str.); 1240 (C-O str.). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 7.09 (1H, br. s, NH); 4.96 (1H, td, α-H, Phe); 4.11 (2H, d, α-H, Gly); 2.97 (2H, d,β-H, Phe); 1.54 (9H, s, *t*-butyl); 3.66 (s, 3H, OCH_3); 7.39 (s, 1H, NH, Trp); 3.25 (m, 2H, CH_2); 7.16-7.15(d, 1H, Trp).

Boc-Trp-Phe-Pro-Trp-OMe (24): IR (KBr, ν, cm^{-1}): 732, 695 (CH bend., out-of-plane, monosub., ring); 3300 (NH str.); 3480 (OH str.); 825, 710, 695 (CH def., rings). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 10.84 (s, 1H, NH trp); 7.53 (d, 1H, Trp); 1.32 (s, 9H, *t*-butyl); 6.80(s, 1H, NH-Phe); 7.13-7.09 (t, 1H, *p*-H, Phe); 4.73-4.70 (t, 1H, δ-H, Pro); 3.66 (s, 3H, OCH_3).

Boc-Phe-Tyr-Pro-Trp-OMe (25): IR (KBr, ν, cm^{-1}): 3373 (OH str., Tyr); 3125 (NH str., amide); 1638 (C=O str., amide); ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 11.0 (s, 1H, OH); 7.51-7.47 (t, 2H, *m*-H, Phe); 6.92-6.88 (m, 3H, *p*-H, Phe and *o*-H, Tyr); 5.95 (s, 1H, OH, Tyr); 4.72-4.69 (q, 1H, α-H, Phe); 1.55 (s, 9H, *t*-butyl); 4.02-3.97 (t, 2H, α-H, Pro); 3.66 (s, 3H, OCH_3); 7.39 (s, 1H, NH, Trp).

Boc-Ala-Gly-Ala-Phe-OMe (26): IR (KBr, ν, cm^{-1}): 2956, 2926 (CH str., asym., CH_3 and CH_2); 3052 (CH str., aromatic ring). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 6.42 (s, 1H, NH); 4.59 (q, 1H, α-H, Ala); 7.13-7.09 (t, 1H, *p*-H, Phe); 3.54 (s, 3H, OCH_3).

Boc-Pro-Gly-Ala-Phe-OMe (27): IR (KBr, ν, cm^{-1}): 2927 (CH str., asym., CH_2); 2997, 2994 (CH str., cyclic CH_2 and CH); 1532 (NH bend., amide); 1269 (C-O str., ester). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 5.76 (s, 1H, NH); 3.98-3.96 (d, 2H, CH_2, Gly); 3.25-3.20 (t, 2H, δ-H, Pro); 2.55-2.46 (m, 2H, β-H, Pro); 1.48 (s, 9H, *t*-butyl); 7.13-7.09 (t, 1H, *p*-H, Phe); 3.54 (s, 3H, OCH_3); 1.59-1.57 (d, 3H, β-H, Ala).

2.1.3. Deprotection of dipeptide at carboxylic end.

The compounds (1-7), (0.01 mol) were dissolved separately in 36 mL of THF/H_2O, (1:1), followed by addition of LiOH (0.015 mol) at 0 °C. The resulting solution was stirred for 1h at room temperature and acidified to pH 3.5 with 0.5 mol/L of sulfuric acid. The aqueous layer was extracted with diethyl ether (3 x 25 mL) and combined organic extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude products were recrystallized with methanol and petroleum ether to obtain compounds (10-16).

Boc-Pro-Phe-OH (10): IR (KBr, ν, cm^{-1}): 1650 (C=O str.); 3480 (O-H str.); 3000 (C-H aromatic ring str.); 3600 (N-H str.). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 11.2 (s, 1H, OH); 4.73-4.70 (t, 1H, δ-H, Pro); 4.02-3.97 (t, 2H, α-H, Pro); 8.11 (s, 1H, NH); 7.13-7.09 (t, 1H, *p*-H, Phe); 1.48 (s, 9H, *t*-butyl).

Boc-Trp-Phe-OH (11): IR (KBr, ν, cm^{-1}): 3076, 3030 (C-H str., ring); 3300 (N-H str.); 3480 (O-H str.). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 10.84 (s, 1H, NH, Trp); 7.53 (d, 1H, Trp); 6.80 (s, 1H, NH-Phe); 11.0 (s, 1H, OH); 7.00-6.97 (2H, t, m- H, Phe); 1.32 (s, 9H, t-butyl).

Boc-Phe-Tyr-OMe (12): IR (KBr, ν, cm^{-1}): 3373 (OH str., Tyr); 3125 (NH str., amide); 1638 (C=O str., amide); 3480 (OH str.). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 11.0 (s, 1H, OH); 6.92-6.88 (m, 3H, *p*-H, Phe and *o*-H, Tyr); 5.95 (s, 1H, OH, Tyr); 1.55 (s, 9H, *t*-butyl).

Boc-Gly-Leu-OH (13): IR (KBr, ν, cm^{-1}): 3125 (NH str., amide); 2927 (CH str., asym., CH_2); 1368 (CH bend., *t*-butyl group); ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 6.52 (s, 1H, NH); 4.25-4.20 (q, 1H, α-H, Leu); 3.50-3.48 (d, 2H, CH_2, Gly); 10.5 (s, 1H, OH); 1.53 (s, 9H, *t*-butyl).

Boc-Phe-Gly-OMe (14): IR (KBr, ν, cm^{-1}): 3129 (NH str., amide), 3480 (OH str.). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 7.09 (s, 1H, NH); 4.96 (d, 1H, H-α, Phe); 4.16 (d, 2H, H-α, Gly); 10.8 (s, 1H, OH).

Boc-Ala-Gly-OH (15): IR (KBr, ν, cm^{-1}): 3126 (NH str., amide); 1641, 1638 (C=O str., 2º amide); 1533 (NH bend, 2º amide); 3480 (OH str.); 1650 (C=O str.). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 7.05 (s, 1H, NH); 6.42 (s, 1H, NH); 4.59 (s, 1H, q, α-H, Ala); 4.12 (d, 2H,α-H, Gly); 1.55 (s, 9H, *t*-butyl); 1.34 (d, 3H, β-H, Ala); 11.03 (s, 1H, OH).

Boc-Pro-Gly-OH (16): IR (KBr, ν, cm^{-1}): 3480 (OH str.); 3128 (NH str., amide); 2997, 2994 (CH str., cyclic CH_2 and CH); 1532 (NH bend., amide). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 5.76 (s, 1H, NH); 11.0 (s, 1H, OH); 1.48 (s, 9H, *t*-butyl); 3.25-3.20 (t, 2H, δ-H, Pro); 3.98-3.96 (d, 2H, CH_2, Gly).

2.1.4. Deprotection of di/tri/tetrapeptide at amino end

0.01 mol of each compound (8-9, 17-21, 24-27) was taken separately and dissolved in chloroform (15 mL), followed by treatment with trifluoroacetic acid (0.02 mol). The mixture was stirred at room temperature for 1h and washed with saturated sodium bicarbonate solution (25 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude products were recrystallized with chloroform and petroleum ether to obtain pure compounds (22, 23, and 28-36) respectively.

Boc-Pro-Trp-OMe (22): IR (KBr, ν, cm^{-1}): 1752 (C=O str., ester); 1648 (C=O str., amide); 1540 (NH bend., amide); 1280 (C-O str., ester); 3130 (m, NH str., amide). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 4.73-4.70 (t, 1H, δ-H, Pro); 4.02-3.97 (t, 2H, α-H, Pro); 2.48-2.41 (m, 2H, γ-H, Pro); 2.40-2.31 (m, 2H, β-H, Pro); 3.66 (s, 3H, OCH_3); 7.39 (s, 1H, NH, Trp), 7.42-7.41(d, 1H, Trp); 3.25 (m, 2H, CH_2).

Boc-Ala-Phe-OMe (23): IR (KBr, ν, cm^{-1}): 1750 (C=O str, ester); 1643 (C=O str., amide); 1584, 1475 (skeletal bands, aromatic ring); 1535 (NH bend., amide). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 7.13-7.09 (t, 1H, *p*-H, Phe); 7.00-6.97 (t, 2H, *m*-H, Phe); 4.60- 4.48 (m, 2H, α-H, Phe and Ala); 3.54 (s, 3H, OCH_3).

Boc-Pro-Phe-Tyr-OMe (28): IR (KBr, ν, cm^{-1}): 1650 (C=O str.); 3480 (OH str.); 1475 (C=C str., aromatic ring). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 4.73-4.70 (t, 1H, δ-H, Pro); 8.11 (s, 1H, NH); 7.13-7.09 (t, 1H, *p*-H, Phe); 4.11 (s, 1H, OH-Tyr); 2.3 (s, 1H, NH Pro).

Boc-Trp-Phe-Leu-OMe (29): IR (KBr, ν, cm^{-1}): 3076, 3030 (CH str., ring); 2926 (CH str., asym., aliph., CH_2); 3300 (NH str.). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 10.84 (s, 1H, NH, Trp); 7.00-6.97 (t, 2H, *m*-H, Phe); 4.03-3.99 (t, 2H, CH_2); 4.12 (s, 3H, OCH_3).

Boc-Phe-Tyr-Phe-OMe (30): IR (KBr, ν, cm^{-1}): 3373 (OH str., Tyr); 3125 (NH str., amide); 3065, 3052 (CH str, rings); 1749 (C=O str., ester). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 7.51-7.47 (t, 2H, *m*-H, Phe); 6.92-6.88 (m, 3H, *p*-H, Phe and *o*-H, Tyr), 5.95 (s, 1H, OH, Tyr); 3.54 (s, 3H, OCH_3); 3.02-2.76 (m, 4H, β-H, Phe and Tyr); 2.0 (s, 2H, NH_2).

Boc-Gly-Leu-Gly-OMe (31): IR (KBr, ν, cm^{-1}): 3125 (m, NH str., amide); 2927 (m, CH str., asym., CH_2); 2853 (m, CH str., sym., CH_2); 1742 (s, C=O str.); 1642, 1639 (C=O str., 2° amide); 1535, 1529 (NH bend., 2° amide). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 6.52 (s, 1H, NH, Gly); 6.02 (s, 1H, NH, Leu); 4.25- 4.20 (q, 1H, H, Leu); 3.50-3.48 (d, 2H, CH_2, Gly); 2.0 (s, 2H, NH_2); 3.67 (s, 3H, OCH_3).

Boc-Phe-Gly-Trp-OMe (32): IR (KBr, ν, cm^{-1}): 3129 (NH str., amide), 1644, 1639 (C=O str., 2º amide), 1532, 1526 (NH bend., 2° amide), 3050 (CH str., aromatic ring); 1670 (C=C str., aromatic ring); 1640 (C=O str.); 1240 (C-O

str.); 2960 (CH str., methyl). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 7.09 (s, 1H, NH); 4.96 (d, 1H, α-H, Phe); 4.11 (d, 2H, α-H, Gly); 2.97 (d, 2H, β-H, Phe); 3.66 (s, 3H, OCH_3); 7.39 (s, 1H, NH, Trp).

Boc-Trp-Phe-Pro-Trp-OMe (33): IR (KBr, ν, cm^{-1}): 2926 (CH str., asym., aliph., CH_2); 2894 (CH str., >CH–); 1742 (C=O str., ester).). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 2.0 (s, 2H, NH_2); 10.84 (s, 1H, NH, Trp); 7.53 (d, 1H, Trp); 7.15-7.11(m, 3H, Trp); 6.80(s, 1H, NH-Phe); 7.00-6.97 (t, 2H, *m*-H, Phe); 4.02-3.97 (t, 2H, α-H, Pro); 2.48-2.41 (m, 2H, γ-H, Pro); 3.66 (s, 3H, OCH_3); 7.39 (s, 1H, NH, Trp).

Boc-Phe-Tyr-Pro-Trp-OMe (34): IR (KBr, ν, cm^{-1}): 3373 (OH str., Tyr); 3125 (NH str., amide); 3065, 3052 (CH str., rings); 2925, 2846 (CH str., asym. and sym., CH_2); 1638 (C=O str., amide). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 11.0 (s, 1H, OH); 7.51-7.47 (t, 2H, m-H, Phe); 6.92-6.88 (m, 3H, *p*-H, Phe and *o*-H, Tyr); 4.02–3.97 (t, 2H, α-H, Pro); 3.66 (s, 3H, OCH3); 7.39 (s, 1H, NH, Trp).

Boc-Ala-Gly-Ala-Phe-OMe (35): IR (KBr, ν, cm^{-1}): 1533 (NH bend, 2º amide); 1650 (C=O str.); 1535 (NH bend., amide); 1269 (C-O str., ester). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 7.05 (s, 1H, NH); 4.59 (q, 1H, α-H, Ala); 4.12 (d, 2H, α-H, Gly); 7.13-7.09 (t, 1H, *p*-H, Phe); 3.54 (s, 3H, OCH3); 2.0 (s, 2H, NH_2).

Boc-Pro-Gly-Ala-Phe-OMe (36): IR (KBr, ν, cm^{-1}): 2927 (CH str., asym., CH_2); 2848 (CH str., sym, CH_2); 1672, 1635 (C=O str., amide); 1532 (NH bend., amide). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 3.86-3.82 (t, 1H, α-H, Pro); 2.55-2.46 (m, 2H, β-H, Pro); 3.98-3.96 (d, 2H, CH_2, Gly); 4.62 (q, 1H, α-H, Ala); 7.00-6.97 (t, 2H, *m*-H, Phe); 3.54 (s, 3H, OCH_3).

2.1.5. General procedure for the synthesis of tri/tetrapeptide derivatives of 1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid (AA_{1-9})

0.01 mol of each tri/tetrapeptide methyl ester (28-36) was dissolved separately in 75 mL of THF followed by addition of 2.3 mL, NMM at 0 °C. The mixture was stirred for 15 min. The compound 1, 2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid (AA), (0.01 mol in 75 mL of THF) and 2.1 g of DCC were added to the previous mixture with stirring. After 36h, the mixture was filtered and residue was washed with 25 mL of THF and filtrate was washed with 15 mL of mixture of 5% sodium bicarbonate and saturated sodium chloride solution. The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated under vacuum. The crude product was recrystallized from a mixture of chloroform and *n*-hexane (3:1).

2.1.6. Spectral analysis of Compound AA_1

IR (KBr, ν, cm^{-1}): 1465 cm^{-1} (-C=N str., quinoxaline ring); 3350.71 (NH str.); 3078.80 (C-H str., aromatic ring); 1506 (C-C str., aromatic ring); 1681 (C=O str.); 3600 (NH str.); 1639 (C=O str., 2º amide). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 2.411 (s, 3H, CH_3); 7.293-8.165 (m, 3H, aromatic H); 9.281 (s, 1H, aromatic NH); 4.73-4.70 (t, 1H, δ-H, Pro); 4.02-3.97 (t, 2H, α-H, Pro); 8.11 (s, 1H, NH); 7.13-7.09 (t, 1H, *p*-H, Phe); 7.00-6.97 (t, 2H, *m*-H, Phe); 6.86-6.84 (d, 2H, *o*-H, Phe); 4.11 (s, 1H, OH-Tyr**)**; 3.66 (s, 3H, OCH_3); 7.31-7.32 (d, 1H, *m*-H, Tyr); 7.53–7.49 (t, 1H, *o*-H, Tyr), 4.92 (s, 1H, CH); 2.92-3.02 (m, 2H, CH_2); 2.3(s, 1H, NH, Pro). Mass: m/z 625 (M^+).

2.1.7. Spectral analysis of Compound AA_2

IR (KBr, ν, cm^{-1}): 1742 (C=O str., ester); 3300 (NH str.); 2925 (CH str., asym., CH_2); 2822 (CH str., OCH_3); 1535 (NH bend., amide). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 10.84 (s, 1H, NH, Trp); 7.53 (d, 1H, Trp); 7.15–7.11(m, 3H, Trp); 6.80(s, 1H, NH, Phe); 7.16-7.12 (t, 1H, *p*-H, Phe); 7.00-6.97 (t, 2H, *m*-H, Phe); 4.12 (s, 3H, OCH_3); 4.03-3.99 (t, 2H, CH_2); 2.4(s, 1H, NH, Trp); 2.411 (s, 3H, CH_3); 7.29-8.16 (m, 3H, aromatic H); 9.28 (s, 1H, aromatic NH); 4.03-3.99 (t, 2H, β-H, Leu); 3.55-3.52 (m, 1H, α-H, Leu). Mass: m/z 664 (M^+).

2.1.8. Spectral analysis of Compound AA_3

IR (KBr, ν, cm^{-1}): 3350.71 (NH str.); 1506 (C-C str., aromatic ring); 2864.49 (C-H str., CH_3); 1681 (C=O str.); 1642 (C=O str., 2º amide); 1535 (NH bend., 2° amide). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 2.32 (s, 3H, CH_3), 7.3-8.2 (m, 3H, aromatic H), 9.1 (s, 1H, aromatic NH); 7.51-7.47 (t, 2H, *m*-H, Phe); 6.92-6.88 (m, 3H, *p*-H, Phe and *o*-H, Tyr), 6.80-6.78 (d, 2H, *m*-H, Tyr); 6.58 (s, 1H, NH); 5.95 (s, 1H, OH, Tyr); 3.68 (s, 3H, OCH_3). Mass: m/z 445 (M^+).

2.1.9. Spectral analysis of Compound AA_4

IR (KBr, ν, cm^{-1}): 1475 cm^{-1} (-C=N str., quinoxaline ring); 3072 (C-H str., aromatic ring); 3129 (NH str., amide), 1670 (C=C str., aromatic ring); 1640 (C=O str.). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 3.62 (s, 3H, OCH_3); 2.411 (s, 3H, CH_3), 7.00-8.12 (m, 3H, aromatic H), 8.8 (s, 1H, aromatic NH); 4.57-4.53 (q, 1H, α-H, leu); 3.74-3.72 (d, 2H, CH_2, Gly); 3.65 (s, 3H, OCH_3); 1.86-1.82 (t, 2H, β-H, Leu); 1.50-1.42 (m, 1H, γ-H, Leu). Mass: m/z 594 (M^+).

2.1.10. Spectral analysis of Compound AA_5

IR (KBr, ν, cm^{-1}): 3340 (NH str.); 3070 (C-H str., aromatic ring); 1506 (C-C Str., Aromatic ring); 1036 (C-H bend.); 2864.49 (C-H str., CH_3); 3373 (OH str., Tyr); 3125 (NH str., amide); 1749 (C=O str., ester); 1645, 1638 (C=O str., amide). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 2.2 (s, 3H, CH_3), 6.92- 7.35 (m, 3H, aromatic H), 9.1 (s, 1H, aromatic NH); 7.51-7.47 (t, 2H, *m*-H, Phe); 6.92-6.88 (m, 3H, *p*-H, Phe and *o*-H, Tyr), 6.80-6.78 (d, 2H, *m*-H, Tyr); 6.60 (s, 1H, NH); 5.95 (s, 1H, OH, Tyr); 4.72-4.69 (q, 1H, α-H, Phe); 4.60- 4.56 (m, 1H, α-H, Tyr); 3.54 (s, 3H, OCH_3); 3.02-2.76 (m, 4H, β-H, Phe and Tyr); 1.98 (s, 2H, NH_2). Mass: m/z 675 (M^+).

2.1.11. Spectral analysis of Compound AA_6

IR (KBr, ν, cm^{-1}): 1205 (C-O str., ester); 732, 695 (CH bending, out-of-plane, monosub., ring); 3300 (NH str.); 825, 710, 695 (CH def., rings). 3350.71 (NH str.); 3078.80 (C-H str., aromatic ring); 1506 (C-C str., aromatic ring); ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 2.2 (s, 2H, NH_2); 10.84 (s, 1H, NH, Trp); 7.53 (d, 1H, Trp); 7.15–7.11(m, 3H, Trp); 6.80 (s, 1H, NH-Phe); 7.13-7.09 (t, 1H, *p*-H, Phe); 7.00-6.97 (t, 2H, *m*-H, Phe); 4.73–4.70 (t, 1H, δ-H of Pro); 4.02-3.97 (t, 2H, α-H, Pro); 2.48-2.41 (m, 2H, γ-H, Pro); 2.40-2.31 (m, 2H, β-H, Pro); 3.66 (s, 3H, OCH_3); 7.39 (s, 1H, NH, Trp), 7.42-7.41(d, 1H, Trp); 3.25 (m, 2H, CH_2); 7.16-7.15 (d, 1H, Trp); 2.411 (s, 3H, CH_3), 7.0-8.1 (m, 3H, aromatic H), 8.75 (s, 1H, aromatic NH). Mass: m/z 834 (M^+).

2.1.12. Spectral analysis of Compound AA_7

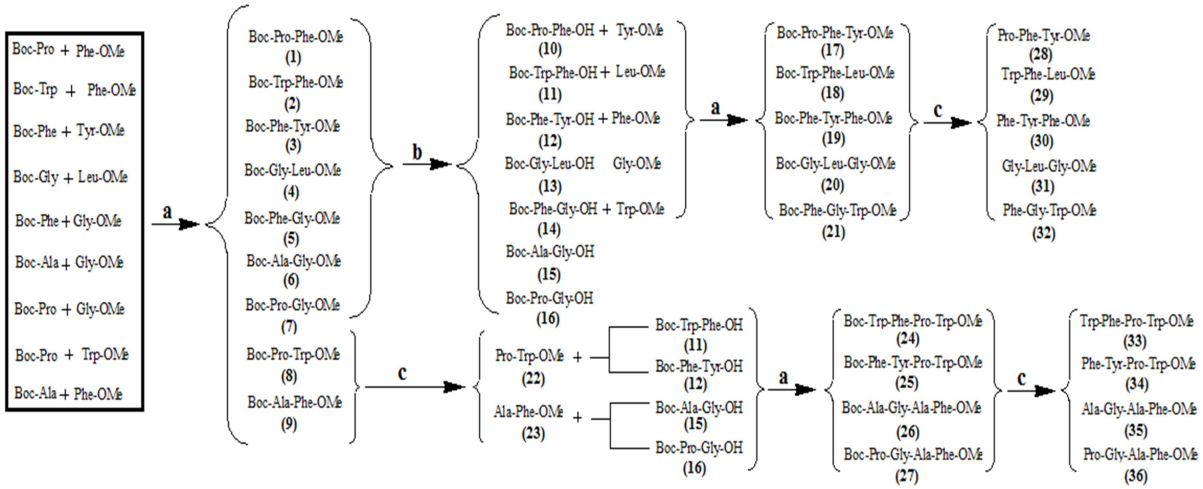
IR (KBr, ν, cm^{-1}): 1475 cm^{-1} (-C=N str., quinoxaline ring); 3345 (NH str.); 3138 (NH str., amide); 1032 (C-H bend.). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 5.55 (s, 1H, NH); 4.00-3.98 (d, 2H, CH_2, Gly); 3.82-3.78 (t, 1H, α-H, Pro); 3.30-3.25 (t, 2H, δ-H, Pro); 2.58-2.49 (m, 2H, β-H, Pro); 1.92-1.87 (m, 2H, γ-H, Pro); 6.83 (s, 1H, NH); 6.86-6.84 (d, 2H, *o*-H, Phe); 2.38 (s, 3H, CH_3), 7.19-8.02 (m, 3H, aromatic H), 8.92 (s, 1H, aromatic NH); 6.48 (s, 1H, NH); 3.39 (s, 3H, OCH_3); 1.62-1.60 (d, 3H, β-H, Ala); 2.2 (s, 1H, NH, Pro). Mass: m/z 590 (M^+).

2.1.13. Spectral analysis of Compound AA_8

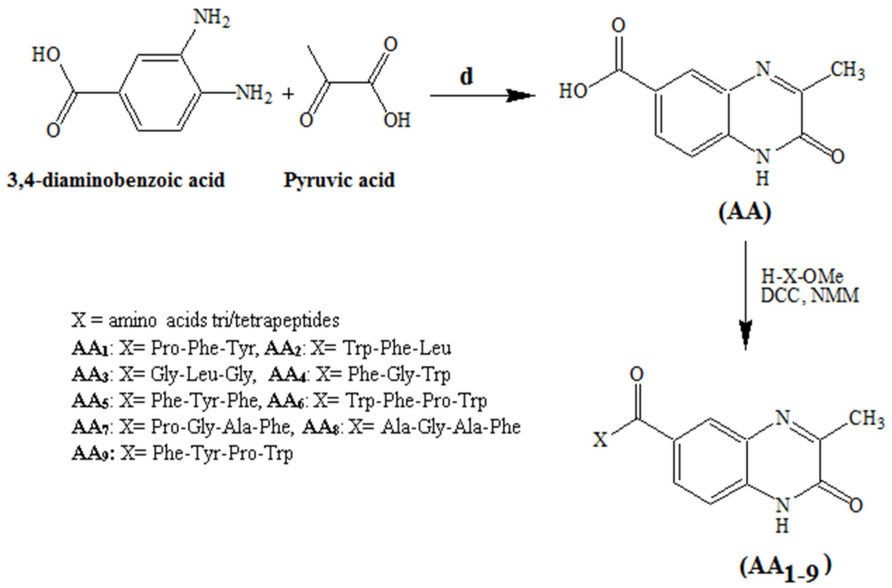
IR (KBr, ν, cm^{-1}): 3120 (NH str., amide); 1635 (C=O str, 2° amide); 1274 (C-O str., ester); 3042 (CH str., aromatic ring); 1468 (C-C Str., aromatic ring). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 6.99 (s, 1H, NH); 4.12-4.10 (d, 2H, α-H, Gly); 1.30- 1.28 (d, 3H, β-H, Ala); 7.13-7.09 (t, 1H, *p*-H, Phe); 7.00-6.97 (t, 2H, *m*-H, Phe); 6.80 (s, 1H, NH); 6.86-6.84 (d, 2H, *o*-H, Phe); 6.50 (s, 1H, NH); 3.58 (s, 3H, OCH_3); 2.0 (s, 2H, NH_2); 2.39 (s, 3H, CH_3), 6.19-7.29 (m, 3H, aromatic H), 9.10 (s, 1H, aromatic NH). Mass: m/z 564 (M^+).

2.1.14. Spectral analysis of Compound AA_9

IR (KBr, ν, cm^{-1}): 1530 cm^{-1} (-C=N str., quinoxaline ring); 1028 (C-H bend.); 3370 (OH str., Tyr); 3120 (NH str., amide); 1630 (C=O str., amide); 1587, 1479, 1476 (skeletal bands, rings). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 2.40 (s, 3H, CH_3), 7.39-8.26 (m, 3H, aromatic H), 9.12 (s, 1H, aromatic NH); 7.51-7.47 (t, 2H, *m*-H, Phe); 6.92-6.88 (m, 3H, *p*-H, Phe and *o*-H, Tyr); 6.80-6.78 (d, 2H, *o*-H, Phe); 6.60 (s, 1H, NH); 4.02–3.97 (t, 2H, α-H, Pro); 2.48-2.41 (m, 2H, γ-H, Pro); 2.40-2.31 (m, 2H, β-H, Pro); 3.66 (s, 3H, OCH_3). Mass: m/z 811 (M^+).



Scheme 1. Reaction scheme for the synthesis of different tri and tetrapeptides (1-36). Reagents and conditions: (a) DCC, NMM, $CHCl_3$, r.t., 36 h; (b) LiOH, THF/H_2O (1:1), r.t., 1 h; (c) TFA, $CHCl_3$, r.t., 1h.



Scheme 2. Reaction scheme for synthesis of tri/tetrapeptide derivatives of 1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid (AA_{1-9}). Reagents and conditions: (d) rectified spirit, reflux 1.5 h.

Table 1. The physical characterization data of synthesized compounds.

Compound	Mol. Formula	Physical State	M.P. (°C)	R_f value $CHCl_3$:CH_3OH(9:1)	% Yield
Boc- Pro-Phe-OMe	$C_{20}H_{28}N_2O_5$	Semi solid	-	0.64	78
Boc-Trp-Phe-OMe	$C_{27}H_{31}N_3O_5$	Semi solid	-	0.68	76
Boc-Phe-Tyr-OMe	$C_{24}H_{30}N_2O_6$	White crystals	117-118	0.73	78
Boc-Gly-Leu-OMe	$C_{14}H_{26}N_2O_5$	Semi solid	-	0.73	74
Boc-Phe-Gly-OMe	$C_{16}H_{21}N_2O_5$	Semi solid	-	0.75	77
Boc-Ala-Gly-OMe	$C_{11}H_{20}N_2O_5$	Semi solid	-	0.69	75
Boc-Pro-Gly-OMe	$C_{13}H_{22}N_2O_5$	Brown crystals	-	0.72	77
Boc-Pro-Trp-OMe	$C_{22}H_{29}N_3O_5$	Semi solid	-	0.75	79
Boc-Ala-Phe-OMe	$C_{18}H_{26}N_2O_5$	Semi solid	-	0.71	76
Boc- Pro-Phe-OH	$C_{19}H_{26}N_2O_5$	White crystals	140-141	0.68	71
Boc-Trp-Phe-OH	$C_{26}H_{29}N_3O_5$	Brown crystals	150-152	0.71	72
Boc-Gly-Leu-OH	$C_{13}H_{24}N_2O_5$	White crystals	128-130	0.71	72
Boc-Phe-Gly-OH	$C_{15}H_{19}N_2O_5$	White crystals	144-145	0.74	71
Boc-Ala-Gly-OH	$C_{10}H_{18}N_2O_5$	White solid	118-119	0.72	71
Boc-Phe-Tyr-OH	$C_{23}H_{28}N_2O_6$	White crystals	121-123	0.76	71
Boc-Pro-Gly-OH	$C_{12}H_{20}N_2O_5$	White crystals	132-133	0.74	74
Boc-Pro-Phe-Tyr-OMe	$C_{29}H_{37}N_3O_7$	Semi solid	-	0.7	72

Boc-Trp-Phe-Leu-OMe	$C_{32}H_{42}N_4O_6$	Semi solid	-	0.68	71
Boc-Phe-Tyr-Phe-OMe	$C_{33}H_{39}N_3O_7$	Semi solid	-	0.74	71
Boc-Gly-Leu-Gly-OMe	$C_{16}H_{29}N_3O_6$	Semi solid	-	0.72	68
Boc-Phe-Gly-Trp-OMe	$C_{28}H_{34}N_4O_6$	Semi solid	-	0.75	71
Pro-Trp-OMe	$C_{17}H_{21}N_3O_3$	Semi solid	-	0.7	71
Ala-Phe-OMe	$C_{13}H_{18}N_2O_3$	Semi solid	-	0.68	72
Boc-Trp-Phe-Pro-Trp-OMe	$C_{42}H_{48}N_6O_7$	Semi solid	-	0.70	70
Boc-Phe-Tyr-Pro-Trp-OMe	$C_{40}H_{47}N_5O_8$	Semi solid	-	0.73	72
Boc-Ala-Gly-Ala-Phe-OMe	$C_{23}H_{34}N_4O_7$	Semi solid	-	0.71	71
Boc-Pro-Gly-Ala-Phe-OMe	$C_{25}H_{36}N_4O_7$	Semi solid	-	0.75	68
Pro-Phe-Tyr-OMe	$C_{24}H_{29}N_3O_5$	Semi solid	-	0.75	72
Trp-Phe-Leu-OMe	$C_{27}H_{34}N_4O_4$	Semi solid	-	0.73	71
Phe-Tyr-Phe-OMe	$C_{28}H_{31}N_3O_5$	Semi solid	-	0.72	69
Gly-Leu-Gly-OMe	$C_{11}H_{21}N_3O_4$	Semi solid	-	0.68	68
Phe-Gly-Trp-OMe	$C_{23}H_{26}N_4O_4$	Semi solid	-	0.70	71
Trp-Phe-Pro-Trp-OMe	$C_{37}H_{40}N_6O_5$	Semi solid	-	0.76	68
Phe-Tyr-Pro-Trp-OMe	$C_{35}H_{39}N_5O_6$	Semi solid	-	0.71	72
Ala-Gly-Ala-Phe-OMe	$C_{18}H_{26}N_4O_5$	Semi solid	-	0.69	70
Pro-Gly-Ala-Phe-OMe	$C_{20}H_{28}N_4O_5$	Semi solid	-	0.74	71
AA	$C_{10}H_8N_2O_3$	White solid	295-296	0.67	72
AA_1	$C_{34}H_{35}N_5O_7$	Brown solid	307-308	0.71	70
AA_2	$C_{37}H_{40}N_6O_6$	Brown solid	310-311	0.74	68
AA_3	$C_{21}H_{27}N_5O_6$	White solid	300-301	0.70	65
AA_4	$C_{32}H_{30}N_6O_6$	Brown solid	305-307	0.75	69
AA_5	$C_{38}H_{37}N_5O_7$	White crystals	315-316	0.72	71
AA_6	$C_{47}H_{46}N_8O_7$	White solid	322-323	0.76	70
AA_7	$C_{30}H_{34}N_6O_7$	Brown solid	302-303	0.77	69
AA_8	$C_{28}H_{32}N_6O_7$	White crystals	299-300	0.69	69
AA_9	$C_{45}H_{45}N_7O_8$	White crystals	316-317	0.73	73

2.2. Pharmacological Investigation

2.2.1. Cell culture

Three cancer cell lines, human lung adenocarcinoma epithelial cell line (A549), human cervical carcinoma cell line (HeLa) and breast cancer cell line (MCF7) were used to investigate anti-proliferative activity of compounds (AA_{1-9}). The cell lines were cultured in RPMI1640 supplemented with 10% fetal bovine serum and incubated in humidified atmosphere of 5% CO_2 at 37 °C.

2.2.2. Anti-proliferative activity

The anti-proliferative activity of the test compounds was investigated using 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [21]. Cells were plated on to 96 well plates at a cell density of 8 x 10^4 cells per well in 100 µL of RPMI 1640 and allowed to grow in a 5% CO_2 incubator for 24 h at 37 °C. After 24 h, the solution of synthesized compounds (AA_{1-9}) prepared in DMSO at concentration of 0.1, 1, 10 and 50 µg/mL were added to each well and incubated for 48 h. The control groups received the same amount of DMSO and all samples were tested for triplicates. Doxorubicin (0.01, 0.1, 0.5 and 1 µg/mL) was used as positive control. The cells were incubated for 72 h (37 °C, 5 % CO_2). Then, 100 µL of MTT solution (0.5 mg/mL in Dulbecco's modified eagle's medium) was added to each well and incubated for 3 h. The growth of tumoral cells was quantified by ability of living cells to reduce the yellow dye MTT to a blue formazan product. The formazan product of MTT reduction was dissolved in DMSO. The medium was removed and 100 µL DMSO was added to each well to dissolve the MTT metabolic product. Afterward, each plate was shaken at 150 rpm for 5 min and absorbance was measured at 570 nm. The assay was performed in triplicate to calculate IC_{50} values (concentration of test compound that kills 50% of cancer cells after 72 h of incubation as compared to positive control) [21-23].

2.2.3. Statistical analysis

One Way Analysis of Variance (ANOVA), followed by Student-Newman-Keul's test, was used for doing the statistical comparison between the test and standard groups. The value $p<0.001$ was considered statistically significant using, Graph Pad Prism 5.01 Software.

2.2.4. Molecular docking

Human mitochondrial peptide deformylase (HsPDF) is a potential anticancer target [24]. HsPDF play a crucial role in mammalian cells to remove formyl groups from N-terminal of newly synthesized mitochondrial proteins. Target specificity inhibition of HsPDF in tumor cell leads to arrest the tumor cell growth. Crystal structure for human mitochondrial peptide deformylase (PDB ID: 3G5K) enzyme was retrieved from the protein data bank and resolution 1.7 Å was used to study the binding pattern of synthesized peptide derivatives of 1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid (AA_{1-9}). The 3G5K-BB2 pocket of co-crystalline structure was considered to generate grid/pointing the binding site, which is an essential step in GLIDE docking. Protein preparation wizard (Pep Wizard) of Schrodinger suit was used for protein refinement, energy minimization, and assigning bonds followed by elimination of all water molecules generally present in PDB structures.

3. RESULTS AND DISCUSSION

The current study represented the synthesis of peptide derivatives of 1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid (AA_{1-9}). IR, ^{1}H-NMR and mass spectral analysis, confirmed the chemical structure of all the final compounds (AA_{1-9}). The presence of bands at 1500-1450 cm^{-1} (-C=N str., quinoxaline ring), 1200-1210 cm^{-1} (C-O str., ester), 1536-1520 cm^{-1} (N-H def., amide) and presence of aromatic rings in structures of compounds was confirmed by strong out-of-plane deformation bands (C-H bending) at 886-820 cm^{-1}. Additionally, the structures were also confirmed by appearance of broad singlet at 9.98-6.25 ppm (for imino proton of CO-NH moiety) in ^{1}H-NMR. The anti-proliferative activity of synthesized compounds (AA_{1-9}) was investigated against human lung

adenocarcinoma epithelial cell line (A549), human cervical carcinoma cell line (HeLa) and breast cancer cell line (MCF7) by MTT assay. The results of anti-proliferative activity revealed that all the peptide derivatives (AA_{1-9}) exhibited anticancer potential against cell lines. However, compound (AA_6) represented (IC_{50}: 3.90±0.25 µg/mL) against Hela cancer cell lines. Therefore, it was found to be more active than the standard drug. The result of IC_{50} (µg/mL) values are presented in (Table 2).

Table 2. The IC_{50} values of compounds (AA_{1-9}) against cancer cell lines.

Compound	A549	Hela	MCF7
	IC_{50} values (µg/mL)		
AA_1	12.50±0.39[c]	22.5±0.22[c]	14.10±0.21[c]
AA_2	14.04±0.81[c]	8.50±0.8[c]	10.70±0.11[c]
AA_3	15.20±0.21[c]	20.04±0.11[c]	18.13±0.24[c]
AA_4	11.04±0.82[b]	6.50±0.41[c]	12.70±0.14[c]
AA_5	13.50±0.8[c]	9.20±0.9[c]	11.30±0.12[c]
AA_6	12.50±0.21[c]	3.90±0.25[c]	7.52±0.13[c]
AA_7	38.03±0.31[c]	30.12±0.30[c]	36.02±0.14[c]
AA_8	20.10±0.92[c]	15.20±0.23[c]	13.70±0.15[c]
AA_9	14.50±0.11[c]	4.50±0.2[c]	9.50±0.24[c]
Control	-	-	-
Doxorubicin	8.64±0.21	4.02±0.22	2.51±0.98

Data are expressed in terms of Mean ± SEM of three independent experiments. P values: [a]<0.05, [b]<0.01, [c]<0.001 compared with doxorubicin.

The crystal structure for human mitochondrial peptide deformylase (PDB ID: 3G5K) enzyme was considered to generate grid/pointing of binding site, which is an essential step in GLIDE docking and the compounds (AA_{1-9}) were investigated for their interactions on the binding site. The results revealed that compound (AA_6) represented highest binding score of (-6.6) by binding with Gly residue of enzyme pocket. The docking score of compounds (AA_{1-9}) are presented in (Table 3) and interactions of compound (AA_6) with the enzyme pocket are illustrated in figure 1.

Table 3. Docking score of compounds (AA_{1-9}) against 3G5K enzyme.

Compound	Docking score
	(kcal/mol)
AA_1	-4.199
AA_2	-4.20
AA_3	-2.86
AA_4	-3.47
AA_5	-3.35
AA_6	-6.6

AA_7	-4.35
AA_8	-4.10
AA_9	-5.12

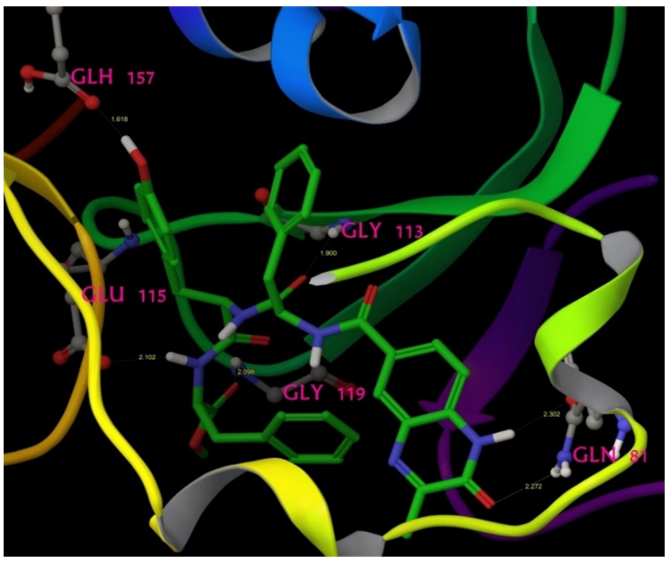


Fig. (1). The interactions of compound (AA_6) with enzyme pocket.

4. CONCLUSION

The present research study reported the synthesis of peptide derivatives of 1,2-dihydro-3-methyl-2-oxoquinoxaline-6-carboxylic acid. The results of anti-proliferative activity revealed good level of activity against cancer cell lines. The synthesized derivative having (Trp-Phe-Pro-Trp) chain in the structure represented maximum activity against Hela cell lines and the same derivative represented highest binding score by binding with (Gly) residue of enzyme pocket in docking studies. The findings suggested that these derivatives have outstanding scope of further future development as anti-cancer agents.

LIST OF ABBREVIATIONS

Pro: Proline, Phe: Phenylalanine, Trp: Tryptophan, Tyr: Tyrosine, Leu: Leucine, Ala: Alanine, Gly: Glycine, DCC: Dicyclohexyl carbodimide, NMM: N-methyl morpholine, Str: Stretching, Bend: Bending.

CONFLCIT OF INTEREST

The authors declare no conflict of interest.

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