



## Design, Synthesis and Molecular Docking Studies of Novel Amino Acids and Peptide Derivatives based on Phthaloyl Chloride with Expected Anticancer Activity

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*In Loving Memory of Late Professor Doctor "Mohamed Refaat Hussein Mahran"*

### Abstract

This paper discusses the synthesis and characterization of  $N\alpha$ -phthaloyl-bis-[amino acid]-X,  $N\alpha$ -phthaloyl-bis-[dipeptide]-X derivatives were conducted, followed by a cytotoxicity evaluation against human cancer cell lines. Compounds 12 and 16 exhibited notable cytotoxic activity against the human colon (CaCo-2) cancer cell line. Molecular docking of these promising derivatives (12 and 16) revealed favorable binding within the active site of the EGFR enzyme, suggesting potential anticancer activity. These docking results suggest a well-fitted interaction involving diverse hydrogen bond interactions with protein residues, indicating a potential for eliciting anticancer activity through this mechanism. This comprehensive approach integrates synthesis, cytotoxicity evaluation, and molecular docking, providing valuable insights into the anticancer properties of these compounds, especially against colon cancer cells.

Keywords: amino acid, linear peptide, cyclic pentapeptide,  $N\alpha$ -phthaloyl-bis-peptides, cytotoxicity.

### 1. Introduction

Therapeutic peptides have many merits over proteins or antibodies: they are not large in size and have the ability to penetrate the cell membranes. They also have high activity, specificity and affinity; minimal drug-drug interaction; and biological and chemical diversity. Another benefit of using peptides as a drug is that they do not accumulate in specific organs (e.g. kidney or liver), which can help to decrease their toxic side effects [1]. Besides, therapeutic peptides have been easily modified [2] and are less immunogenic than recombinant antibodies or proteins [3]. Peptides are intrinsically able to interact with biological systems and are therefore potent therapeutics [4-6]. Therapeutic peptides are considered as novel and promising approach for the development of anticancer agents [7-8]. Peptides, both natural and synthetic, have seen an increase application as therapeutic agents in recent years [9-12]. In 2012, in addition to about 80 peptides appeared on the market, as well as, 200 peptides reported to be in clinical phases and 400 peptides are in advanced preclinical stages [13]. Designing drugs and peptides with anticancer properties pose ongoing challenges in contemporary research [14-19]. Our

group's exploration of peptide candidates for multiple therapeutic purposes, such as anticancer, anti-inflammatory, analgesic, and antimicrobial activities, aligns with the versatile nature of peptides in medical research. This approach allows for a broad spectrum of potential applications [20-47].

### 2. Experimental Details

#### Chemistry

IR Spectra were obtained using the Perkin Elmer FT-IR Spectrum BX apparatus. Melting points are uncorrected. Specific optical rotations were measured with a A. Krawss, Optronic, P8000 polarimeter. NMR Spectra were scanned in DMSO- $d_6$  on a Bruker NMR spectrophotometer at 400 MHz for  $^1H$  NMR and 100 MHz for  $^{13}C$  NMR. Mass spectra were obtained using a GCMS-QP1000 EX spectrometer (70 e.V.) Elemental analyses were performed at the Microanalytical Center of Cairo University. ( $R_f$ ) was determined using Thin Layer Chromatography (TLC) eluted with silica gel aluminum sheets, 60 F254 (E. Merck), it was eluted with (S<sub>1</sub>; Butanol/water/acetic acid/ pyridine; 120/48/12/40) or ethyl acetate).

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**2.1.1. Synthesis of phthaloyl dichloride, (2)**, was prepared according to the reported method [48]

**2.1.2. General procedure for synthesis of (N<sup>α</sup>-phthaloyl)-bis-[amino acids]-OMe, (3 and 4)**

**A. Acid chloride method:**

A dichloromethane (DCM) solution of compound, (2), (3 gm, 14.78 mmol) was added dropwisely to a cold and stirred DCM solution (-20 °C, 50 ml) of 2 equivalents of free L-Ala-methyl ester or DL-NVa-methyl ester (5.4 gm, 29.56 mmol. The reaction mixture was stirred for additional 3 hours at the same temperature then for 24 hours at room temperature, washed with water, 1N sodium bicarbonate, 1N potassium hydrogen sulphate and water then dried for 24 hours at 0 °C, over anhydrous sodium sulphate. The volatile materials were evaporated till dryness and the obtained residue was solidified by trituration with pet. ether (B.P. 40-60 °C.). The obtained solid was filtered off and recrystallized from MeOH to give the compound (3).

**B. Mixed anhydride method:**

Ethyl chloroformate (ECF) (2.9 ml, 30.1 mmol) was added to a stirred and a cold DCM solution (-20 °C, 50 ml) solution of phthalic acid (1), (5 gm, ~30 mmol) and triethylamine (TEA) (6.6 ml, 60.2 mmol). The reaction mixture was stirred for additional 30 minutes, and then a DCM solution (-20 °C, 50 ml) of free L-Ala-methyl ester (11gm; 60.2 mmol) was added. Stirring was maintained for 3 hours at -20 °C, then for 24 hours at room temperature. The reaction mixture was then washed with water, 1N sodium bicarbonate, 1N potassium hydrogen sulphate and water and finally dried over anhydrous sodium sulphate, the volatile materials were evaporated till dryness and the obtained oily residue was solidified by trituration with pet. ether (B.P. 40-60 °C). The obtained solid was collected by filtration and recrystallized from MeOH to give compounds (3 and 4), as identified by melting point and thin layer chromatography (TLC) in comparison with authentic samples prepared according to method A.

**N<sup>α</sup>-Phthaloyl-bis-[L-Ala- methyl ester]; (3)** was prepared according to the reported method [49]

**N<sup>α</sup>-Phthaloyl-bis-[DL-NVa- methyl ester]; (4)**

**4:** Yield: [A]: (61.2%); [B]: (82.5%); melting point;

m.p. 118-120 °C;  $[\alpha]_D^{25} = -266.7$  (C = 0.06). Rf x100 (the eluent) = 85.4 (S1). IR (cm<sup>-1</sup>): (KBr): ν= 3305(NH stretching), 3066.26 (CH, aromatic), 2874.38 (CH, aliphatic), 1743.33(C=O, ester), 1639.2 and 1546.63 (C=O, amide I and II, respectively). <sup>1</sup>H-NMR (500 MHz, ppm, DMSO-d<sub>6</sub>): δ= 8.64-8.60 (s, 2H, 2NH, D<sub>2</sub>O exchangeable), 7.60-7.54 (m, 4H, aromatic), 4.41-4.35 (t, 2H, 2CH, NHCH<sub>2</sub>CH<sub>2</sub>, DL-NVa, α CH), 3.66, 3.55 (s, 6H, 2COOCH<sub>3</sub>), 1.90-1.80 (q, 4H, 2CH<sub>2</sub>, DL-NVa, β CH<sub>2</sub>), 1.40-1.35 (m, 4H, 2CH<sub>2</sub>, DL-NVa, γ CH<sub>2</sub>), 1.00-0.95 (t, 6H, 2CH<sub>3</sub>, DL-NVa, δ CH<sub>3</sub>). - MS (EI, 70 eV): m/z (%) = **392.5 (M<sup>+</sup>, 0.28%)**, 393.5 (M<sup>+</sup>+1,

0.09%), 394.5(M<sup>+</sup>+2, 0.10), 290(0.13), 278 (0.44), **261.9 (100%)**, 159.9 (79.82 %), 91(5.42 %), 72 (56.40%). - Molecular formula (M.wt.), C<sub>20</sub>H<sub>28</sub>N<sub>2</sub>O<sub>6</sub> (392.45):- calculated analysis; C 61.21, H 7.19, N 7.14; found analysis; C 61.20, H 7.18, N 7.12.

**2.1.3. General Procedure for synthesis of N<sup>α</sup>-Phthaloyl-bis-[amino acids]; (5 and 6)**

To a stirred and cold methanolic solution (-5 °C, 20 ml) of N<sup>α</sup>-Phthaloyl-bis-[amino acids- methyl ester]; (3 and 4) (2 mmol), sodium hydroxide (1N, 25 ml) was added dropwisely. The reaction mixture was stirred for 4 hours at the same temperature then for 24 hours at room temperature. The solvent was distilled off under reduced pressure, and the remaining aqueous solution was cooled and acidified with 1N hydrochloric acid (pH = 3). The obtained solid was filtered off, washed with water, dried and recrystallized from EtOH to give the both acids, N<sup>α</sup>-Phthaloyl-bis-[L-Ala]; (5) and N<sup>α</sup>-Phthaloyl-bis-[DL-NVa]; (6).

**N<sup>α</sup>-Phthaloyl-bis-[L-Ala]; (5)** was prepared according to the reported method [49]

**N<sup>α</sup>-Phthaloyl-bis-[DL-NVa]; (6)**

**6:** Yield: 85.2 % %; m.p. 159-165 °C. ;  $[\alpha]_D^{25} = -600$  (C = 0.02). Rf x100 (the eluent) = 30.3(S2). IR (cm<sup>-1</sup>): (KBr): ν= 3353.6 (NH stretching), 3063.37 (CH, aromatic), 2962.13 (CH, aliphatic), 1739(C=O, acid), 1624 and 1545 (C=O, amide I and II, respectively). <sup>1</sup>H-NMR (500 MHz, ppm, DMSO-d<sub>6</sub>): δ= 12.85 (s, 2H, 2OH, D<sub>2</sub>O exchangeable), 8.47 (s, 2H, 2NH, D<sub>2</sub>O exchangeable), 7.53 (m, 4H, aromatic), 4.33 (t, 2H, 2CH, NHCH<sub>2</sub>CH<sub>2</sub>, DL-NVa, α CH), 1.85-1.75 (q, 4H, 2CH<sub>2</sub>, DL-NVa, β CH<sub>2</sub>), 1.55-1.50 (m, 4H, 2CH<sub>2</sub>, DL-NVa, γ CH<sub>2</sub>), 0.95-1.00 (t, 6H, 2CH<sub>3</sub>, DL-NVa, δ CH<sub>3</sub>). MS (EI, 70 eV): m/z (%) = **364.3 (M<sup>+</sup>, 0.09%)**, 365.3 (M<sup>+</sup>+1, 0.07%), 366.4 (0.13%), 281.85 (0.06%), 247.85 (9.26%), 159.85 (92.71%), **72 (100%)**, 55 (55.59%). Molecular formula (M.wt.), C<sub>18</sub>H<sub>24</sub>N<sub>2</sub>O<sub>6</sub> (364.39) :calculated analysis; C 59.33, H 6.64, N 7.69; found analysis; C 59.32, H 6.61, N 7.68.

**2.1.4. General Procedure for Synthesis of N<sup>α</sup>-phthaloyl)-bis-[amino acids -NHNH<sub>2</sub>]; (7 and 8)**

Hydrazine hydrate (0.35ml, 10 mmol) was added to a stirred methanolic solution (1 mmol, 50ml) of of N<sup>α</sup>-Phthaloyl-bis-[amino acids- methyl ester]; (3 and 4). The reaction mixture was refluxed for 3 hours, after which the volatile materials were evaporated. The obtained residue was triturated with ether, filtered off and recrystallized from MeOH /ether to afford the corresponding hydrazides (7 and 8).

**N<sup>α</sup>-Phthaloyl-bis-[L-Ala- NHNH<sub>2</sub>]; (7)** was prepared according to the reported method [49]

**N<sup>α</sup>-phthaloyl)-bis-[DL-NVa -NHNH<sub>2</sub>]; (8)**

**5:** Yield: (66.6 %); m.p. decomposition at above 300 °C.  $[\alpha]_D^{25} = -450$  (C = 0.02). Rf x100 (the eluent)

=57.2 (S1). IR ( $cm^{-1}$ ): (KBr):  $\nu$ = 3267.79 (NH stretching), 3152 (CH, aromatic), 2923 (CH, aliphatic), 1620, 1471 and 1451 (C=O amide I, II and III, respectively).  $^1H-NMR$  (500 MHz, ppm, DMSO- $d_6$ ):  $\delta$ = 9.15 (s, 2H, CONHNH $_2$ , D $_2$ O exchangeable), 8.80 (s, 2H, 2NH, D $_2$ O exchangeable), 7.82-7.79 (m, 4H, aromatic), 5.16 (t, 2H, 2CH, NHCHCH $_2$ , DL-NVa,  $\alpha$  CH), 4.25 (s, 2H, CONHNH $_2$ ), 2.51, 2.50 (q, 4H, 2CH $_2$ , DL-NVa,  $\beta$  CH $_2$ ), 1.70-1.60 (m, 4H, 2CH $_2$ , DL-NVa,  $\gamma$  CH $_2$ ), 0.95-0.90 (t, 6H, 2CH $_3$ , DL-NVa,  $\delta$  CH $_3$ ). MS (EI, 70 eV):  $m/z$  (%) = **392.45** ( $M^+$ , **0.02%**), 393.4 ( $M^+$  +1, 0.02%), 319.05 (0.10%), 281.95 (0.22%), 204.90 (0.21%), 161.85 (17.92%), **58.95** (**100%**), 55 (68.20%), 53 (7.42%). Molecular formula (M.wt.), C $_{18}$ H $_{28}$ N $_6$ O $_4$  (392.45):calculated analysis; C 55.09, H 7.19, N 21.41; found analysis; C 55.08, H 7.17, N 21.40.

### 2.1.5. General Procedure for Synthesis of N $^{\alpha}$ - Phthaloyl - bis-[dipeptide -OMe]; (9-11)

ECF (2.9 ml, 30.1 mmol) was added to a stirred and a cold DCM solution (-20  $^{\circ}C$ , 50 ml) of N $^{\alpha}$  - Phthaloyl-bis-[L-Ala]; (5) or N $^{\alpha}$  - Phthaloyl-bis-[DL-NVa]; (6), (5 gm, ~30 mmol) and TEA (6.6 ml, 60.2 mmol). The reaction mixture was stirred for additional 30 minutes, and then a DCM solution (-20  $^{\circ}C$ , 50 ml) of free DL-NVa or L-Phe ala-acid (11 gm; 60.2 mmol) was added. Stirring was maintained for 3 hours at -20  $^{\circ}C$ , then for 24 hours at room temperature. The reaction mixture was then washed with water, 1N sodium bicarbonate, 1N potassium hydrogen sulphate and water and finally dried over anhydrous sodium sulphate, the volatile materials were evaporated till dryness and the obtained oily residue was solidified by trituration with pet. ether (B.P. 40-60  $^{\circ}C$ ). The obtained solid was collected by filtration and recrystallized from MeOH to give N $^{\alpha}$  - Phthaloyl-bis-[L-Ala- Phe-OMe]; (9), N $^{\alpha}$  - phthaloyl)-bis-[DL-NVa - DL-NVa -OMe]; (10) and N $^{\alpha}$  - Phthaloyl) - bis-[DL-NVa - L-Phe-OMe]; (11),

N $^{\alpha}$  - Phthaloyl - bis-[L-Ala - L-Phe -OMe]; (9)

**9.** Yield: (90.95 %); m.p. (oily compound); Rf x100 (the eluent) = 70.3 ((Ethyl acetate: methanol). IR ( $cm^{-1}$ ): (KBr):  $\nu$ = 3370 (NH stretching), 2924 (CH aromatic), 2856 (CH aliphatic), 1648.84 (C=O ester), 1529, 1458 and 1313 (C=O amide I, II and amide III, respectively).  $^1H-NMR$  (500 MHz, ppm, DMSO- $d_6$ ):  $\delta$ = 8.90 (s, 4H, 4NH, D $_2$ O exchangeable), 7.71-7.27 (m, 14H, aromatic), 4.60 (t, 2H, NHCHCH $_2$ Phe,  $\alpha$  CH), 4.50 (m, H, 2CH, NHCHCH $_3$ , L-Ala,  $\alpha$  CH), 3.77, 3.61 (d, 4H, 2CH $_2$ , CH $_2$ Phe,  $\beta$  CH $_2$ ), 3.26 (s, 6H, 2COOCH $_3$ ), 1.70-1.60 (d, 6H, 2CH $_3$ , L-Ala,  $\beta$  CH $_3$ ). MS (EI, 70 eV):  $m/z$  (%) = **654.71** ( $M^+$ , **0.02%**), 655.7 ( $M^+$  +1, 0.01%), 532.25 (0.02%), 407 (0.04%), 352(0.21%), 249.95(10.45%), 148.05 (54.35%), **88(100%)**, 67 (2.13%). Molecular formula (M.wt.), C $_{36}$ H $_{38}$ N $_4$ O $_8$  (654.71): calculated analysis; C 66.04, H 5.85 N 8.56; found analysis; C 66.02, H 5.82, N 8.53.

N $^{\alpha}$  - Phthaloyl - bis-[DL-NVa - DL-NVa -OMe]; (10)

**10.** Yield: (78.12%); m.p. 161-166  $^{\circ}C$ .  $[\alpha]_D^{25}$  = - 500 (C = 0.02). Rf x100 (the eluent)= 68.4 (ethyl acetate). IR ( $cm^{-1}$ ): (KBr):  $\nu$ = 3292.86 (NH stretching), 3059 (CH, aromatic), 2959 (CH aliphatic), 1746 (C=O, ester), 1646 and 1535 (C=O amide I and II, respectively).  $^1H-NMR$  (500 MHz, ppm, DMSO- $d_6$ ):  $\delta$ = 8.47, 8.45 (s, 4H, 4NH, D $_2$ O exchangeable), 7.53, 7.51 (m, 4H, aromatic), 4.37 (t, 4H, 4CH, NHCHCH $_2$ , DL-NVa,  $\alpha$  CH), 3.37 (s, 6H, 2COOCH $_3$ ), 1.72 (q, 8H, 4CH $_2$ , DL-NVa,  $\beta$  CH $_2$ ), 1.39 (m, 2H, 2CH $_2$ , DL-NVa,  $\gamma$  CH $_2$ ), 0.90, 0.89 (t, 12H, 4CH $_3$ , DL-NVa,  $\delta$  CH $_3$ ). MS (EI, 70 eV):  $m/z$  (%) = **590.71** ( $M^+$ , **0.72%**), 591.71 ( $M^+$ +1, 0.28%), 508.85 (0.12%), 433.75 (0.73%), **360.85** (**100**), 315.8 (0.10%), 159.85 (8.14%), 94.95 (0.76%), 59.95 (1.15%). Molecular formula (M.wt.), C $_{30}$ H $_{46}$ N $_4$ O $_8$  (590.71) :calculated analysis; C 61, H 7.85, N 9.48; found analysis; C 60.99, H 7.84, N 9.85.

N $^{\alpha}$  - Phthaloyl) - bis-[DL-NVa - L-Phe-OMe]; (11)

**11.** Yield: (83.33%); m.p. (oily compound);  $[\alpha]_D^{25}$  = - 400 (C = 0.02). Rf x100 (the eluent) =78.9 (ethyl acetate). IR ( $cm^{-1}$ ): (KBr):  $\nu$ = 3320 (NH, stretching), 3033 (CH, aromatic), 2955 (CH, aliphatic), 1733 (C=O, ester), 1650 and 1533 (C=O, amide I and II, respectively).  $^1H-NMR$  (500 MHz, ppm, DMSO- $d_6$ ):  $\delta$ = 8.87, 8.37 (s, 4H, 4NH, D $_2$ O exchangeable), 7.87-7.22 (m, 14H, aromatic), 4.55-4.45 (t, 2H, NHCHCH $_2$ Phe,  $\alpha$  CH), 4.30 (t, 4H, 4CH, NHCHCH $_2$ , DL-NVa,  $\alpha$  CH), 3.90-3.70 (s, 6H, 2COOCH $_3$ ), 3.16, 3.03 (d, 4H, 2CH $_2$ , CH $_2$ Phe,  $\beta$  CH $_2$ ), 2.00 (q, 4H, 2CH $_2$ , DL-NVa,  $\beta$  CH $_2$ ), 1.22 (m, 2H, 2CH $_2$ , DL-NVa,  $\gamma$  CH $_2$ ), 0.84 (t, 6H, 2CH $_3$ , DL-NVa,  $\delta$  CH $_3$ ). MS (EI, 70 eV):  $m/z$  (%) = **686.79** ( $M^+$ , **0.01%**), 687.7 (0.03%), 536.75 (0.02%), 493.75(0.04%), 366.70 (0.23%), 302.85 (0.17%), 226.85 (0.10%), 185.85(0.88%), **72** (**100%**), 55 (10.27%). Molecular formula (M.wt.), C $_{38}$ H $_{46}$ N $_4$ O $_8$  (686.79):calculated analysis; C 66.45, H 6.75, N 8.16; found analysis; C 66.43, H 7.72, N 8.13.

### 2.1.6. General Procedure for Synthesis of N $^{\alpha}$ - Phthaloyl - bis-[DL-NVa - amino acids]; (8 and 9)

To a stirred and cold methanolic solution (-5  $^{\circ}C$ , 20 ml) of N $^{\alpha}$  - Phthaloyl-bis-[L-Ala- Phe-OMe]; (9) or N $^{\alpha}$  - phthaloyl)-bis-[DL-NVa - DL-NVa -OMe]; (10) or N $^{\alpha}$  - Phthaloyl) - bis-[DL-NVa - L-Phe-OMe]; (11), (2 mmol), sodium hydroxide (1N, 25 ml) was added dropwisely. The reaction mixture was stirred for 4 hours at the same temperature then for 24 hours at room temperature. The work up was continued as followed for compound 4. The obtained solid was filtered off and recrystallized from EtOH to give the corresponding dipeptide acids, N $^{\alpha}$

- **Phthaloyl-bis-[L-Ala- Phe]; (12)**, **N<sup>α</sup> - phthaloyl)-bis-[DL-NVa - DL-NVa]; (13)** and **N<sup>α</sup> - Phthaloyl) - bis-[DL-NVa - L-Phe]; (14)**.

**N<sup>α</sup> - Phthaloyl-bis-[L-Ala- Phe]; (12)**

**12.** Yield: (85 %); m.p. (119-125 °C); Rf x100 (the eluent) = 43.6 (S<sub>1</sub>). *IR* (cm<sup>-1</sup>): (KBr): ν= 3376 (NH stretching), 2915 (CH, aliphatic), 1703 (C=O, acid), 1651 and 1593 (C=O amide I and II, respectively). <sup>1</sup>H-NMR (500 MHz, ppm, DMSO-d<sub>6</sub>): δ= 12.60 (s, 2H, 2OH, D<sub>2</sub>O exchangeable), 8.69, 8.67 (s, 4H, 4NH, D<sub>2</sub>O exchangeable), 7.83-7.12 (m, 14H, aromatic), 5.14-5.11 (t, 2H, NHCHCH<sub>2</sub>Phe, α CH), 4.62, 4.60 (m, 2H, 2CH, NHCHCH<sub>3</sub>, L-Ala, α CH), 3.51 (d, 4H, 2CH<sub>2</sub>, CH<sub>2</sub>Phe, β CH<sub>2</sub>), 1.98-1.91 (d, 6H, 2CH<sub>3</sub>, L-Ala, β CH<sub>3</sub>). *MS* (EI, 70 eV): *m/z* (%) = **658.65 (M<sup>+</sup>, 0.09%)**, 659.65 (M<sup>+</sup>+1, 0.11 %), 593.05(0.89%), 500.05 (0.11%), 353(1.71%), 269 (1.07%), 211 (1.19%), 170.95 (2.87 %), **57 (100%)**. *Molecular formula* (M.wt.), C<sub>34</sub>H<sub>34</sub>N<sub>4</sub>O<sub>10</sub> (658.65): *calculated analysis*; C 62, H 5.20 N 8.51; *found analysis*; C 61.98, H 5.18, N 8.50.

**N<sup>α</sup> - Phthaloyl - bis-[DL-NVa - DL-NVa]; (13)**

**13.** Yield: (87.7 %); m.p. 190-195 °C. Rf x100 (the eluent) = 47.89(ethyl acetate).

*IR* (cm<sup>-1</sup>): (KBr): ν= 3301 (NH stretching), 3069 (CH aromatic), 2962 (CH aliphatic), 1690 (C=O acid), 1725, 1644 and 1536 (C=O amide I, II and III, respectively). <sup>1</sup>H-NMR (500 MHz, ppm, DMSO-d<sub>6</sub>): δ= 12.55 (s, 2H, 2OH, D<sub>2</sub>O exchangeable), 8.50-8.38 (s, 4H, 4NH, D<sub>2</sub>O exchangeable), 8.12-7.41 (m, 4H, aromatic), 4.31-3.36 (t, 4H, 4CH, NHCHCH<sub>2</sub>, DL-NVa, α CH), 1.68, 1.66 (q, 8H, 4CH<sub>2</sub>, DL-NVa, β CH<sub>2</sub>), 1.34 (m, 2H, 2CH<sub>2</sub>, DL-NVa, γ CH<sub>2</sub>), 0.88, 0.86 (t, 12H, 4CH<sub>3</sub>, DL-NVa, δ CH<sub>3</sub>). *MS* (EI, 70 eV): *m/z* (%) = **562.6 (M<sup>+</sup>, 0.02%)**, 563.6 (0.13%), 454.65 (0.03%), 408.75 (0.94%), 301.85 (1.28%), 201.85 (29.89%), 159.90(46.58%), **72 (100%)**, 55 (26.09%). *Molecular formula* (M.wt.), C<sub>28</sub>H<sub>42</sub>N<sub>4</sub>O<sub>8</sub> (562.66): *calculated analysis*; C 59.77, H 7.52, N 9.96; *found analysis*; C 59.75, H 7.50, N 9.94.

**N<sup>α</sup> - Phthaloyl) - bis-[DL-NVa - L-Phe]; (14)**

**14.** Yield: (81.5% %); m.p. (oily compound); [α]<sub>D</sub><sup>25</sup> = - 650 (C = 0.02). Rf x100 (the eluent) = 60 (S<sub>1</sub>). *IR* (cm<sup>-1</sup>): (KBr): ν= 3314 (NH stretching), 3066 (CH, aromatic), 2958 (CH, aliphatic), 1721 (C=O, acid), 1644 and 1533(C=O amide I and II, respectively). <sup>1</sup>H-NMR (500 MHz, ppm, DMSO-d<sub>6</sub>): δ= 12.40 (s, 2H, 2OH, D<sub>2</sub>O exchangeable), 8.72-8.02 (s, 4H, 4NH, D<sub>2</sub>O exchangeable), 7.48-7.21 (m, 14H, aromatic), 4.50, 4.31 (t, 2H, NHCHCH<sub>2</sub>Phe, α CH), 3.54 (t, 2H, 2CH, NHCHCH<sub>2</sub>, DL-NVa, α CH), 3.08, 3.06 (d, 4H, 2CH<sub>2</sub>, CH<sub>2</sub>Phe, β CH<sub>2</sub>), 1.99, 1.64 (q, 4H, 2CH<sub>2</sub>, DL-NVa, β CH<sub>2</sub>), 1.21, 1.07 (m, 2H, 2CH<sub>2</sub>, DL-NVa, γ CH<sub>2</sub>), 0.82, 0.67 (t, 6H, 2CH<sub>3</sub>, DL-NVa, δ CH<sub>3</sub>). *MS* (EI, 70 eV): *m/z* (%) = **658.7 (M<sup>+</sup>, 0.01%)**, 659.7 (0.03%), 519 (0.01%), 423.85 (0.07%), 395.75 (0.14%), 278.95 (0.19%), 203

(7.98%), 147 (46.29%), **91 (100%)**, 59 (19.78%). *Molecular formula* (M.wt.), C<sub>36</sub>H<sub>42</sub>N<sub>4</sub>O<sub>8</sub> (685.74):*calculated analysis*; C 65.64, H 6.43, N 8.51; *found analysis*; C 65.63, H 6.41, N 8.50.

**2.1.7. General Procedure for Synthesis of N<sup>α</sup> - Phthaloyl - bis-[DL-NVa - amino acids - NHNH<sub>2</sub>]; (10 and 11).**

Hydrazine hydrate (0.35ml, 10 mmol) was added to a stirred methanolic solution (1 mmol, 50ml) of **N<sup>α</sup> - Phthaloyl-bis-[L-Ala- Phe-OMe]; (9)** or **N<sup>α</sup> - phthaloyl)-bis-[DL-NVa - DL-NVa -OMe]; (10)** or **N<sup>α</sup> - Phthaloyl) - bis-[DL-NVa - L-Phe-OMe]; (11)**. The reaction mixture was refluxed for 3 hours, after which the volatile materials were evaporated. The obtained residue was triturated with ether, filtered off and recrystallized from MeOH /ether to afford the **N<sup>α</sup> - Phthaloyl-bis-[L-Ala- Phe- NHNH<sub>2</sub>]; (15)** or **N<sup>α</sup> - phthaloyl)-bis-[DL-NVa - DL-NVa - NHNH<sub>2</sub>]; (16)** or **N<sup>α</sup> - Phthaloyl) - bis-[DL-NVa - L-Phe- NHNH<sub>2</sub>]; (17)**.

**N<sup>α</sup> - Phthaloyl - bis-[L-Ala-L-Phe-NHNH<sub>2</sub>]; (15)**

**15.** Yield: (85 %); m.p. (Decompose at 280 °C); [α]<sub>D</sub><sup>25</sup> = - 350 (C = 0.02). Rf x100 (the eluent) = 75.4 (S<sub>1</sub>). *IR* (cm<sup>-1</sup>): (KBr): ν= 3273 (NH stretching), 3157 (CH, aromatic), 2992 (CH, aliphatic), 1625, 1575 and 1470 (C=O amide I, II and III, respectively). <sup>1</sup>H-NMR (500 MHz, ppm, DMSO-d<sub>6</sub>): δ= 8.90 (s, 1H, CONH<sub>2</sub>, D<sub>2</sub>O exchangeable), 8.09-8.07 (s, 4H, 4NH, D<sub>2</sub>O exchangeable), 7.87-7.18 (m, 14H, aromatic), 4.48 (t, 2H, NHCHCH<sub>2</sub>Phe, α CH), 4.50 (m, H, 2CH, NHCHCH<sub>3</sub>, L-Ala, α CH), 4.25 (s, 2H, CONH<sub>2</sub>), 3.39-3.32 (d, 4H, 2CH<sub>2</sub>, CH<sub>2</sub>Phe, β CH<sub>2</sub>), 1.30-1.25 (d, 6H, 2CH<sub>3</sub>, L-Ala, β CH<sub>3</sub>). *MS* (EI, 70 eV): *m/z* (%) = **630.69 (M<sup>+</sup>, 0.01%)**, 631.6 (M<sup>+</sup>+1, 0.02 %), 507.8 (0.03%), 368 (0.13%), 300 (0.03%), 249.85 (5.59%), 161.90 (67.23%), **104 (100%)**, 77 (51.76%), 55 (18.18 %). *Molecular formula* (M.wt.), C<sub>32</sub>H<sub>38</sub>N<sub>8</sub>O<sub>6</sub> (630.96): *calculated analysis*; C 60.94, H 6.07 N 17.77; *found analysis*; C 60.92, H 6.05, N 17.75.

**N<sup>α</sup> - Phthaloyl - bis-[DL-NVa - DL-NVa - NHNH<sub>2</sub>], (16)**

**16.** Yield: (62.5 %); m.p. decomposition at 241-260 °C. [α]<sub>D</sub><sup>25</sup> = - 250 (C = 0.04). Rf x100 (the eluent) = 83.01(S<sub>1</sub>). *IR* (cm<sup>-1</sup>): (KBr): ν= 3201 (NH stretching), 3050 (CH, aromatic), 2960 (CH, aliphatic), 1670, 1460 and 1331 (C=O amide I, II and III, respectively). <sup>1</sup>H-NMR (500 MHz, ppm, DMSO-d<sub>6</sub>): δ= 9.20 (s, 1H, CONH<sub>2</sub>, D<sub>2</sub>O exchangeable), 8.11-8.07 (s, 4H, 4NH, D<sub>2</sub>O exchangeable), 7.90-7.87 (m, 4H, aromatic), 4.50 (t, 4H, 4CH, NHCHCH<sub>2</sub>, DL-NVa, α CH), 4.15 (s, 2H, CONH<sub>2</sub>), 1.70-1.60 (q, 8H, 4CH<sub>2</sub>, DL-NVa, β CH<sub>2</sub>), 1.35-1.33 (m, 2H, 2CH<sub>2</sub>, DL-NVa, γ CH<sub>2</sub>), 0.89-0.85 (t, 12H, 4CH<sub>3</sub>, DL-NVa, δ CH<sub>3</sub>). *MS* (EI,

70 eV):  $m/z$  (%) = 590.7 ( $M^+$ , 0.04%), 591.7 ( $M^+$ +1, 0.05%), 507.95 (0.10%), 436.90 (0.15%), 368 (1.43%), 287 (0.25%), 206.95 (1.98%), 136.05 (2.02%), **72** (100%), 59 (42.87), 53 (9.56 %). *Molecular formula* (*M.wt.*),  $C_{28}H_{46}N_8O_6$  (590.71): *calculated analysis*; C 56.93, H 7.85, N 18.97; *found analysis*; C 56.90, H 7.82, N 18.95.

### $N^\alpha$ - Phthaloyl) - bis-[DL-NVa – L-Phe-NHNH<sub>2</sub>], (17)

**17.** Yield: (74 %); m.p. decomposition at 276-278 °C;  $[\alpha]_D^{25} = -300$  (C = 0.02). Rf x100 (the eluent) = 73.5 (S<sub>1</sub>). *IR* ( $cm^{-1}$ ): (KBr):  $\nu = 3276$  (NH stretching), 3159 (CH, aromatic), 2954 (CH, aliphatic), 1638, 1451 and 1375 (C=O amide I, II and III, respectively). *<sup>1</sup>H-NMR* (500 MHz, ppm, DMSO-*d*<sub>6</sub>):  $\delta = 9.00$  (s, 1H, CONHNH<sub>2</sub>, D<sub>2</sub>O exchangeable), 8.09-8.06 (s, 4H, 4NH, D<sub>2</sub>O exchangeable), 7.86-7.20 (m, 14H, aromatic), 3.96 (t, 2H, NHCH<sub>2</sub>CH<sub>2</sub>Phe,  $\alpha$  CH), 3.50 (t, 2H, 2CH, NHCH<sub>2</sub>CH<sub>2</sub>, DL-NVa,  $\alpha$  CH), 4.00 (s, 2H, CONHNH<sub>2</sub>), 3.12 (d, 4H, 2CH<sub>2</sub>, CH<sub>2</sub>Phe,  $\beta$  CH<sub>2</sub>), 2.00 (q, 4H, 2CH<sub>2</sub>, DL-NVa,  $\beta$  CH<sub>2</sub>), 1.15, 1.07 (m, 2H, 2CH<sub>2</sub>, DL-NVa,  $\gamma$  CH<sub>2</sub>), 0.69, 0.58 (t, 6H, 2CH<sub>3</sub>, DL-NVa,  $\delta$  CH<sub>3</sub>). *MS* (*EI*, 70 eV):  $m/z$  (%) = 686.80 ( $M^+$ , 0.01%), 620 (0.07%), 490 (0.02%), 408.85 (0.06%), 338.95 (0.16%), 278.90 (0.33%), 201 (3.38%), 176.90 (0.49%), 105 (10.44%), **72** (100 %), 55 (17.10%). *Molecular formula* (*M.wt.*),  $C_{36}H_{46}N_8O_6$  (686.80): *calculated analysis*; C 62.96, H 6.75 N 16.32; *found analysis*; C 62.90, H 6.73, N 16.30.

### 1.1. In-vitro cytotoxic activity against some selected human cancer cell lines

Human lung (A-549), colon (CaCo-2), prostate (PC-3) and breast (MCF) cancer cell were obtained from Karolinska Center, Department of Oncology and Pathology, Karolinska Institute and Hospital, Stockholm, Sweden. IC<sub>50</sub> values were performed using SPSS computer program (SPSS for windows, statistical analysis software package /version 9/ 1989 SPSS Inc., Chicago, USA). The procedure was done in laminar air flow cabinet bio safety class II level. Culturing and sub culturing were carried out according to Thabrew [50]. Doxorubicin was used as a positive control. DMSO used as negative control. Cell Viability Assay was done according to (Selim) [51] as described by Mosmann [52]. The cells were seeded at concentration of 10x10<sup>3</sup> cells per well in case of MCF-7, 20x10<sup>3</sup> cells/well in case of HCT-116 cell lines using 96-well plates at 37 °C. After 48 hours' incubation, the medium was aspirated and 40  $\mu$ l MTT salt (2.5 mg/ml) were added and further incubated for 4 hours. 200  $\mu$ l 10% sodium dodecyl sulphate (SDS) was added. The absorbance was measured at 595nm.

### 1.2. Molecular docking studies

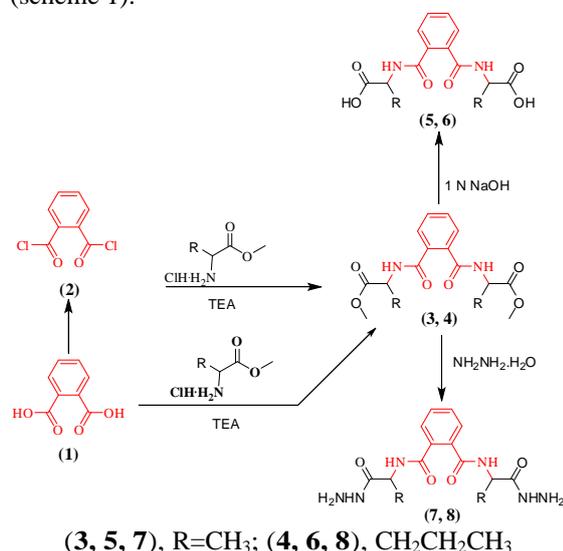
All the molecular modeling calculations and docking simulation studies were performed using Molecular Operating Environment (MOE<sup>®</sup>) [10] 2008.10 [53]. The target compounds **12**, **16** were constructed into a 3D model using the builder interface of the MOE program. Then, they were subjected to a conformational search and energy minimization. All the minimizations were performed with MOE until a RMSD gradient of 0.01 Kcal/mole and RMS distance of 0.1 Å with MMFF94X force-field and the partial charges were automatically calculated. The obtained data base was then saved as MDB file to be used in the docking calculations. The X-ray crystallographic structure of EGFR receptor complexed with [6, 7-Bis (2-methoxy-ethoxy) quinoline-4-yl]-(3-ethynylphenyl) amine, erlotinib (PDB ID: 1M17) [54] was obtained from the Protein Data Bank through the internet. Re-docking of the co-crystalline ligand within the receptor active sites to ensure the docking method was efficient and the active pocket was saved as moe file to be used for docking simulation of the selected compounds. Docking of the conformation database of the target compounds was done using MOE-Dock software.

## 2. Results and Discussion

### 2.1. Chemistry

Peptide synthesis commenced with  $N^\alpha$ -phthaloyl dicarbonyl dichloride (**1**); which was coupled with amino acid methyl esters at low temperature, utilizing triethylamine (TEA) as an organic base. IR spectra confirmed the presence of an aromatic ring, aliphatic groups, amide linkage, and an ester group. Compounds (**3** and **4**) exhibited a benzene ring, represented by a band at 3066.26  $cm^{-1}$  (CH aromatic type). The amide linkage was evidenced by three characteristic bands in the (1641, 1551, and 1453  $cm^{-1}$ ) region (amide I, II, and III, respectively) for both compounds (**3** and **4**). Additionally, the ester group presence was supported by a band in the 1743  $cm^{-1}$  region ( $\nu$  C=O ester) for compounds (**3** and **4**). The <sup>1</sup>H-NMR spectra for compounds (**3** and **4**) revealed a singlet (6H) at  $\delta$  3.60-3.63 ppm for the (2CH<sub>3</sub> ester). Hydrolysis of dipeptide methyl esters (**3** and **4**) with 1 N methanolic sodium hydroxide afforded the corresponding acids, (**5** and **6**). The IR spectra indicated the absence of the  $\nu$  (C=O, ester) signal, while bands at 1739  $cm^{-1}$  were observed, corresponding to the  $\nu$  (C=O, acid) in compounds (**5** and **6**). The <sup>1</sup>H-NMR spectra revealed the appearance of a singlet (1H) at  $\delta$  (12.85-13.07) ppm for carboxylic (OH) protons, which are exchangeable with D<sub>2</sub>O. The hydrazinolysis of (**3** and **4**) using hydrazine hydrate in methanol resulted in the formation of the corresponding hydrazides, namely (**7** and **8**). The IR spectra indicated the amide linkage was well identified by its three characteristic bands in (1620, 1471 and 1451)  $cm^{-1}$  region (amide I, II and III respectively) for compound (**7** and **8**). In

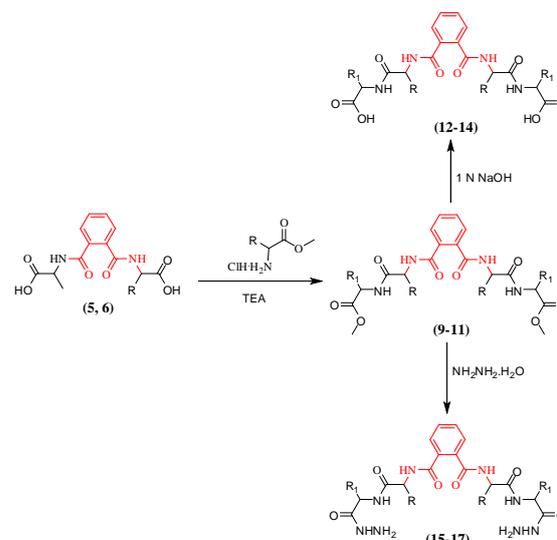
addition, the NH stretching vibrations of the amide and hydrazide groups appeared as a broad band centered at  $3267\text{cm}^{-1}$ . The  $^1\text{H-NMR}$  spectra revealed the appearance of a broad singlet (2H) at  $\delta$  (4.25, 4.26) ppm for the amino protons ( $\text{NH}_2$ ). The amide (NH) protons appeared as singlets (2H) at  $\delta$  (8.60-9.18) ppm which are exchangeable with  $\text{D}_2\text{O}$  (scheme 1).



**Scheme 1.** Synthetic routes for compounds (3-8).

The synthesis of the titled tetrapeptide esters (**9-11**), *via* a mixed anhydride procedure. The mixed anhydride peptide coupling method is a potent peptide bond formation method, associated with easily removable side products, namely, an alcohol and  $\text{CO}_2$  gas. The synthetic procedure is thus based on the formation of a mixed anhydride with a carbonic acid ester (ethylchloroformate) in the presence of a tertiary base triethyl amine (TEA). The *in situ* formed mixed anhydride was subsequently coupled with the required amino acid esters, in the presence of the base, to afford the titled esters (**9-11**). The IR spectra validated the existence of an aromatic ring, aliphatic groups, and an amide linkage, along with the presence of the ester group. The  $^1\text{H-NMR}$  spectra for compounds revealed the presence of a singlet (6H) for the presence of (2CH<sub>3</sub> ester). Hydrolysis of tetrapeptide methyl esters (**9-11**) with 1 N methanolic sodium hydroxide afforded the corresponding acids (**12-14**). The IR spectra verified the existence of  $\nu(\text{C}=\text{O}, \text{acid})$ . The  $^1\text{H-NMR}$  spectra for compounds (**12-14**) revealed the appearance of a singlet (2H) for carboxylic (OH) protons which are exchangeable with  $\text{D}_2\text{O}$ . Hydrazinolysis of  $\text{N}^\alpha$ -phthaloyl-bis-[di-peptide] methyl esters: (**9-11**) with hydrazine hydrate in methanol afforded the corresponding hydrazides (**15-17**). The IR spectra indicated the NH stretching vibrations of the amide and hydrazide groups appeared as a broad band. The  $^1\text{H-NMR}$  spectra revealed the appearance of a broad singlet (2H) for the amino protons ( $\text{NH}_2$ ). The amide (NH) protons appeared as singlets (7H) which are

exchangeable with  $\text{D}_2\text{O}$ . (Scheme 2) for compounds (**3-17**).



(9, 12, 15), R=CH<sub>3</sub>, R<sub>1</sub>=CH<sub>2</sub>Ph; (10, 13, 16), R=CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>, R<sub>1</sub>=CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>; (11, 14, 17), R=CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>, R<sub>1</sub>=CH<sub>2</sub>Ph

**Scheme 2.** Synthetic routes for compounds (9-17).

## 2.2. *In-vitro* anti-cancer activity assessment of compounds 3-17 against carefully chosen human cancer cell lines.

Each of the newly synthesized compounds underwent assessment for their anticancer potential against four cancer cell lines—human lung (A 549), colon (CaCo-2), prostate (PC-3), and breast (MCF-7) cancer cells—at a concentration of 100  $\mu\text{M}$ , employing the MTT growth inhibition assay [52]. Positive and negative controls were established using Doxorubicin and DMSO, respectively. The outcomes are detailed in **Table 1**. As exhibited in **Table 1**, compounds **10, 15** displayed high moderate cytotoxic activity against human lung (A 549), (growth inhibition 67.65 and 63.5% respectively), while other compounds showed low cytotoxic activity. Compound **11** revealed high anti-cancer activity towards prostate (PC-3) cell line and breast (MCF-7) cell line, whereas other compounds displayed from low to moderate cytotoxic activity against them. Regarding to colon (CaCo-2) cell line, compounds **12** and **16** showed the best cytotoxic activity against it (Growth Inhibition 73.1%, 76.67% respectively). Compounds **12** and **16**, displaying inhibition percentages exceeding 70%, underwent further evaluation to determine their median growth inhibitory concentration ( $\text{IC}_{50}$ ). Doxorubicin served as the reference drug, and the results are presented in **table 2**.

## 2.3. Molecular docking studies

Depending on the promising cytotoxic activities of the newly synthesized compounds **12** and **16**, the docking study was applied using Molecular

Operating Environment (MOE®) 2008.10 [53] to illustrate their mechanism of action. Upon continuation of our research on isophthalamide based derivatives that revealed anticancer activities targeting the epidermal growth factor receptor (EGFR) [55], the docking study of compounds **12** and **16** were achieved against EGFR to evaluate the binding affinity and the possible interactions.

The X-ray crystallographic structure of EGFR bound with its ligand erlotinib was downloaded from protein data bank (pdb Id: 1M17) [55]. Redocking of the original ligand was established to evaluate RMSD value (1.20 Å). The original inhibitor (erlotinib) shared the binding with a hydrogen bond acceptor between its quinazoline nitrogen atom and the backbone of **Met769** (distance: 2.70 Å) as previously mentioned [54] (figure 1).

By investigation of figure 2 which showed the docking data of compound **12**, it was noticed that the

sidechain of **Lys721** illustrated two H-bond donors with the two carboxylic OH groups (distance: 2.60 and 2.82 Å, respectively) and arene-cation interaction with the centroid of benzyl moiety. Furthermore, the sidechain of **Asp831** formed hydrogen bond acceptor with the proton of OH group (distance: 1.92 Å).

Referring to figure 3 in which docking of compound **16** was inserted; it was observed that the two terminal amino groups displayed H-bond donors with the backbone of **Met769** and **Gly833** (distance: 2.00 and 2.29 Å, respectively). Additionally, NH of the hydrazide part showed H-bond donor with sidechain of **Gly738** (distance: 1.99 Å). The benzamide moiety improved the fitting via arene-arene interaction with **Phe699** and H-bond acceptor between oxygen and the sidechain of **Lys721** (distance: 2.77 Å).

Table1: Anticancer activity of the promising phthaloyl peptide derivatives against different human carcinoma cell lines at a concentration of 100 µM.

Compound NO	Compound Name	Growth Inhibition (%)			
		A-549	CaCo2	PC-3	MCF -7
3.	<i>N</i> <sup>α</sup> - Phthaloyl - bis-[L-Ala - OMe]	28.95	72.4	38.2	21.55
4.	<i>N</i> <sup>α</sup> - Phthaloyl - bis-[DL-NVa - OMe]	1.24	41.25	38.7	27.9
5.	<i>N</i> <sup>α</sup> - Phthaloyl - bis-[L-Ala -COOH]	47.26	42.9	27	0
6.	<i>N</i> <sup>α</sup> - Phthaloyl - bis-[DL-NVa -COOH]	34.1	60.79	31.85	0
7.	<i>N</i> <sup>α</sup> - Phthaloyl - bis-[L-Ala -NHNH <sub>2</sub> ]	48.25	41.95	37.55	0
8.	<i>N</i> <sup>α</sup> - Phthaloyl - bis-[DL-NVa -NHNH <sub>2</sub> ]	37.1	33.65	21.95	33.15
9.	<i>N</i> <sup>α</sup> - Phthaloyl - bis-[L-Ala - L-Phe -OMe]	14.9	42.85	31	0
10.	<i>N</i> <sup>α</sup> - Phthaloyl - bis-[DL-NVa - DL-NVa -OMe]	67.65	34	34	59.1
11.	<i>N</i> <sup>α</sup> - Phthaloyl - bis-[DL-NVa -L-Phe -OMe]	12.68	64.3	79.23	81.65
12.	<i>N</i> <sup>α</sup> - Phthaloyl - bis-[L-Ala - L-Phe -COOH]	0	76.63	59.05	4.683
13.	<i>N</i> <sup>α</sup> - Phthaloyl - bis-[DL-NVa - DL-NVa -COOH]	33.42	32.75	26.95	50.15
14.	<i>N</i> <sup>α</sup> - Phthaloyl - bis-[DL-NVa -L-Phe -COOH]	0	15.31	12.925	0
15.	<i>N</i> <sup>α</sup> - Phthaloyl - bis-[L-Ala - L-Phe -NHNH <sub>2</sub> ]	63.5	39.8	27.26	58.05
16.	<i>N</i> <sup>α</sup> - Phthaloyl - bis-[DL-NVa - DL-NVa -NHNH <sub>2</sub> ]	37.4	73.1	60.95	50.1
17.	<i>N</i> <sup>α</sup> - Phthaloyl - bis-[DL-NVa -L-Phe -NHNH <sub>2</sub> ]	2.4	25.4	22.45	65.3
	<b>DMSO</b>	0	0	0	0
	<b>Doxorubicin</b>	100	100	100	100

Table 2; The IC<sub>50</sub> values, representing the concentration required to reduce cell viability by 50%, were determined for active compounds exhibiting ≥70% anti-cancer on the human colon carcinoma (CaCo-2) cell line.

Compound NO	IC <sub>50</sub> (Mean ± SD) (µM) a
	CaCo -2 cell line
12	75.5±0.9
16	71.6±1.8
<b>Doxorubicin</b>	0.065 ± 1.00

IC<sub>50</sub> values, along with their standard errors (±), were computed using the SPSS statistical program.

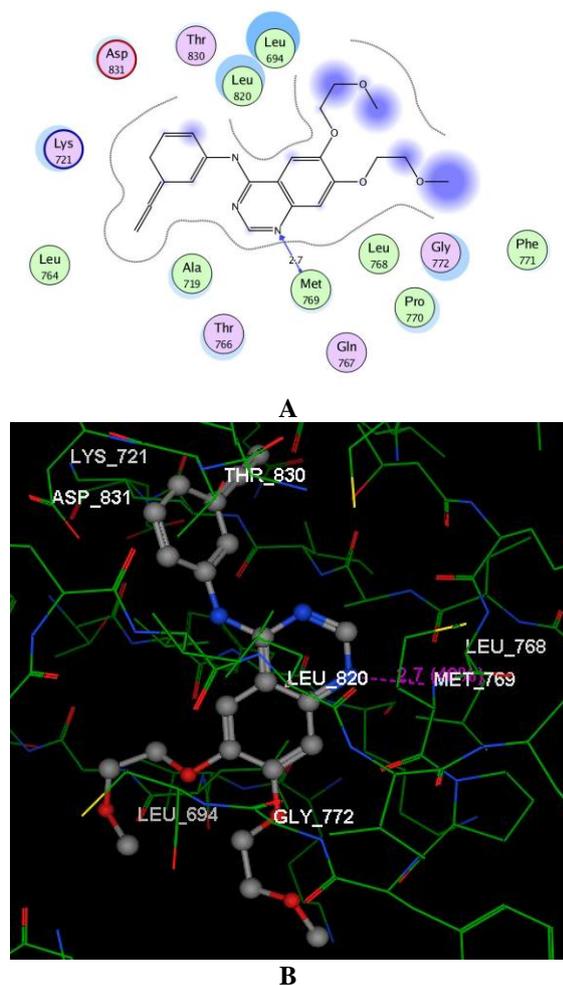
## Conclusion

The objective of this study was to synthesize novel peptides using phthaloyl dichloride (1, 2-

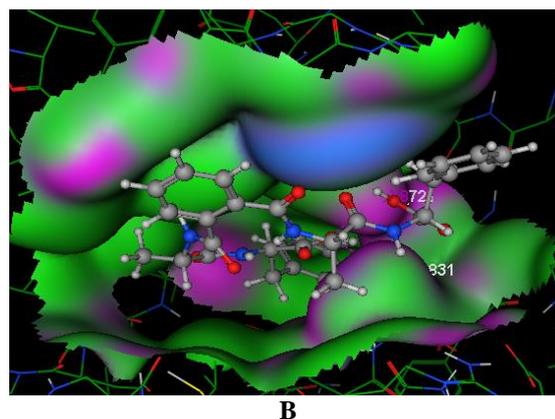
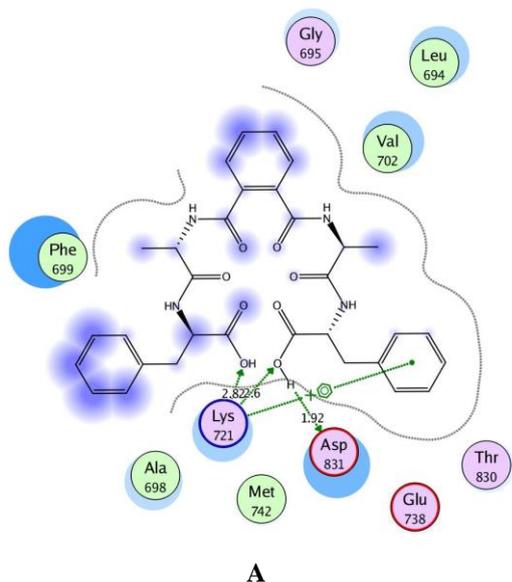
benzenedicarbonyl chloride). These promising synthesized compounds were subsequently characterized through various spectral data analyses.

These novel peptide candidates are of the general structures, N<sup>α</sup>-phthaloyl)-bis-[amino acid]-X, N<sup>α</sup> - phthaloyl) -bis - [dipeptide]-X. Their cytotoxic activities were investigated against four human carcinoma cell lines (CaCo -2, A-549, MCF-7 and PC-3) using MTT assay at 100 µM concentration. Some of these compounds exhibited pronounced cytotoxic activity especially against CaCo -2 cell lines. The molecular docking studies were performed for the highly promising derivatives **12** and **16** within the EGFR binding site to demonstrate their mechanism of action. Exploring comprehensive biological studies, especially traditional anticancer investigations

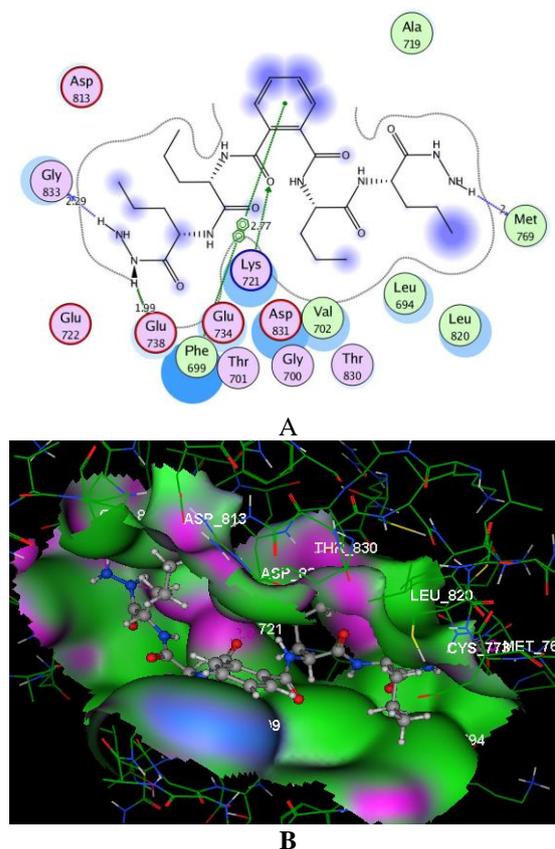
involving experimental animal models, appears to be a valuable pursuit.



**Figure 1.** 2D and 3D visualizations (A & B) display erlotinib's docking into the EGFR binding site (PDB code: 1M17). Dotted lines with arrows represent hydrogen bonds, while C atoms are shaded in gray, N in blue, and O in red.



**Figure 2:** 2D and 3D visualizations (A & B) showcase the docking of compound 12 into the EGFR binding site (PDB code: 1M17). Hydrophobic areas are denoted by green, high polar areas by pink, and mild polar areas by blue. Hydrogen bonds are depicted as dotted lines with arrows, while C atoms are in gray, N in blue, and O in red.



**Figure 3:** 2D and 3D visualizations (A & B), compound 16 is depicted docking into the EGFR binding site (PDB code: 1M17). Hydrophobic areas are highlighted in green, high polar areas in pink, and mild polar areas in blue. Hydrogen bonds are represented as dotted lines with arrows, while C atoms are colored gray, N in blue, and O in red.



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