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Design, Synthesis and Molecular Docking Studies of Novel Cyclic Pentapeptides Based on Phthaloyl Chloride with Expected Anticancer Activity

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A SERIES of Nα-phthaloyl bridged cyclic pentapeptide derivatives were synthesized and characterized on the basis of spectral and elemental analyses. A preliminary cytotoxicity evaluation of all novel compounds was carried out against four human cancer cell lines, human lung (A-549), colon (CaCo-2), prostate (PC-3) and breast (MCF-7) cancer cells at 100 µM concentration using MTT growth inhibition assay. Compound 3 gave the highest cytotoxic activity towards the human colon (CaCo-2) cancer cell line (Growth Inhibition = 72.4 %). Further molecular docking of the promising derivative 3 was developed to study its binding mode within the active site of EGFR enzyme. The docking results suggest good fitting through different hydrogen bond interactions with the protein residues to elicit anticancer activity

Keywords: Amino acid, Linear peptide, Cyclic pentapeptide, N^{α}-phthaloyl-bis-peptides, cytotoxicity.

Introduction

Therapeutic peptides have several important advantages over proteins or antibodies: they are small 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. An added 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 minimize their toxic side effects [1]. As well as, 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]. Drug design and discovery of anticancer peptides is, consequently, an updated research challenge [14-19]. So recently, our research group has focused on peptide candidates, as anticancer activities, antiinflammatory, analgesic agents and antimicrobial [20-39].

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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, in a 1 dm length observation tube, at the indicated conditions. NMR Spectra were scanned in DMSO-d_6 on a Brucker NMR spectrophotometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Chemical shifts are expressed in δ-values (parts per million) relative to an internal standard (TMS at 0.0 ppm) and coupling constants (J) in Hz. 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_1; Butanol/water/acetic acid/ pyridine; 120/48/12/40) or S_2; ethyl acetate).

Synthesis of phthaloyl dichloride, was prepared according to the reported method [40]

These compounds were prepared

General procedure for synthesis of (N$^{\alpha}$ - phthaloyl)-)-bis-[L-Ala]-OMe, (3)

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 ^{0}C, 50 ml) of 2 equivalents of free L-Ala-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 ^{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 ^{0}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 ^{0}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 ^{0}C, 50 ml) of free L-Ala-methyl ester (11gm; 60.2 mmol) was added. Stirring was maintained for 3 hours at -20 ^{0}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 ^{0}C). The obtained solid was collected by filtration and recrystallized from MeOH to give compound **(3)**, as identified by melting point and thin layer chromatography (TLC) in comparison with authentic samples prepared according to method A.

N$^{\alpha}$ - Phthaloyl-bis-[L-Ala- methyl ester]; (3)

3: Yield: [A]: (61.2%); [B]: (63%); melting point; m.p.96-98 ^{0}C; $[\alpha]_D^{25}$ = - 266.7 (C = 0.06).Rf x100 (the eluent) = 68.65(S_2),*IR (cm^{-1}): (KBr):*ν=3288 (NH stretching), 3070 (CH aromatic), 2989(CH aliphatic), 1752 (C=O ester), 1641 and 1551 (C=O amide I and amide II, respectively).*^{1}H-NMR* (500 MHz, ppm, DMSO-d_6): δ= 8.64 (s, 2H, 2NH, D2O exchangeable), 7.92, 7.55 (m, 4H, aromatic), 4.40 (m, 2H, 2CH, NH<u>CH</u>CH_3, L-Ala, α CH), 3.65 (s, 6H, 2COO<u>CH_3</u>), 1.56, 1.37 (d, 6H, 2CH_3, NHCH$_2$<u>CH_3</u>, L-Ala, β CH_3). - *MS (EI, 70 eV): m/z (%)* =336 (M^+, 0.05), 337 (M^++1, 0.27), 338 (M^++2, 0.06), 234 (67.64), 174 (100), 104 (18.44), 76 (23.06), 50 (5.68). - *Molecular formula (M.wt.),* $C_{16}H_{20}N_2O_6$ (336.34):- *calculated analysis;* C 57.14, H 5.99, N 8.33; *found analysis;* C 57.13, H 5.96, N 8.30.

General Procedure for synthesis of N$^{\alpha}$ - Phthaloyl-bis-[L-Ala]; (4)

To a stirred and cold methanolic solution (-5 ^{0}C, 20 ml) of ester **(3)** (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 acid **(4).**

N$^{\alpha}$ - Phthaloyl-bis-[L-Ala]; (4)

4: Yield: 89.5%; m.p. 201-203 ºC. ; $[\alpha]_D^{25}$ = - 600 (C = 0.02). Rf x100 (the eluent) =59.7 (S_2), *IR (cm^{-1}): (KBr):*ν= 3078 (NH stretching), 3007 (CH, aromatic), 2887 (CH, aliphatic), 1686 (C=O, acid), 1585 and 1495

(C=O amide I and II, respectively). *^{1}H-NMR* (500 MHz, ppm, DMSO-d_6): δ= 13.07 (s, 2H, 2OH, D_2O exchangeable), 8.50 (s, 2H, 2NH, D2O exchangeable), 7.80-7.60 (m, 4H, aromatic), 3.55 (m, 2H, 2CH, NHCHCH$_3$, L-Ala, α CH),1.56-1.23 (d, 6H, 2CH$_3$, NHCH$_2$CH$_3$, L-Ala, β CH$_3$). *MS (EI, 70 eV): m/z (%)* =308 (M^+, 0.05), 309 (M^+ +1, 0.05), 166 (1.59), 122 (75.69), 105 (100), 76 (43.30), 65 (32.37), 50 (43.81).*Molecular formula (M.wt.),* $C_{14}H_{16}N_2O_6$(308.29):*calculated analysis;* C 54.54, H 5.23, N 9.09; *found analysis;* C 54.50, H 5.19, N 9.06.

General Procedure for Synthesis of N$^\alpha$ - phthaloyl)-bis-[L-Ala-NHNH$_2$]; (5)

hydrazine hydrate (0.35ml, 10 mmol) was added to a stirred methanolic solution (1 mmol, 50ml) of L-Ala methyl ester **(3)**. 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 hydrazide **(5)**.

N$^\alpha$ - phthaloyl)-bis-[L-Ala-NHNH$_2$]; (5)

5: Yield: (55.5 %); m.p. decomposition at 290-292 ^{0}C. $[\alpha]_D^{25}$ = - 450 (C = 0.02). Rf x100 (the eluent) =34.32 (S_2).*IR (cm^{-1}): (KBr):*ν= 3266 (NH stretching), 3151 (CH, aromatic), 2931 (CH, aliphatic), 1622, 1579 and 1469 (C=O amide I, II and III, respectively). *^{1}H-NMR* (500 MHz, ppm, DMSO-d_6): δ= 9.18 (s, 1H, CONHNH$_2$, D_2O exchangeable), 8.08, 8.06 (s, 2H, 2NH, D2O exchangeable), 7.84, 7.83 (m, 4H, aromatic), 4.52, 4.51 (m, 2H, 2CH, NHCHCH$_3$, L-Ala, α CH), 4.26 (s, 2H, CONHNH$_2$), 1.29, 1.11 (d, 6H, 2CH$_3$, NHCH$_2$CH$_3$, L-Ala, β CH$_3$).*MS (EI, 70 eV): m/z (%)* = 336 (M^+, 0.03), 162 (71.22), 132 (22.63), 105 (23.69), 104 (100), 76 (45.15), 57 (5.08), 50 (33.17).*Molecular formula (M.wt.),* $C_{14}H_{20}N_6O_4$(336.35):*calculated analysis;*C49.99, H 5.99, N 24.99; *found analysis;* C 49.97, H 5.950, N 24.98.

General Procedure for Synthesis of N$^\alpha$ - Phthaloyl - bis-[L-Ala – L-Ala -OMe]; (6)

ECF (2.9 ml, 30.1 mmol) was added to a stirred and a cold DCM solution (-20 ^{0}C, 50 ml) of compound **4**, (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 ^{0}C, 50 ml) of free L-Ala-acid (11gm; 60.2 mmol) was added. Stirring was maintained for 3 hours at -20 ^{0}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 ^{0}C). The obtained solid was collected by filtration and recrystallized from MeOH to give compound **(6)**, as identified by melting point and TLC.

N$^\alpha$ - Phthaloyl - bis-[L-Ala – L-Ala -OMe]; (6)

6. Yield: (80.6%); oily compound. $[\alpha]_D^{25}$ = - 500 (C = 0.02). Rf x100 =65 (S_1).*IR (cm^{-1}): (KBr):*ν= 3337 (NH stretching), 2989 (CH aromatic), 2954 (CH aliphatic), 1723 (C=O ester), 1656, 1534 and 1454 (C=O amide I, II and amide III, respectively). *^{1}H-NMR* (500 MHz, ppm, DMSO-d_6): δ= 8.15, 8.00 (s, 4H, 4NH, D2O exchangeable), 7.85, 7.80 (m, 4H, aromatic), 4.75-4.30 (m, 4H, 4CH, NHCHCH$_3$, L-Ala, α CH), 3.70 (s, 6H, 2COOCH$_3$), 1.65-1.30 (d, 12H, 4CH$_3$, NHCH$_2$CH$_3$, L-Ala, β CH$_3$). *MS (EI, 70 eV): m/z (%)* =478 (M^+, 0.06), 479 (M^++1, 0.05), 304 (5.24), 244 (18.30), 174 (100), 130 (32.34), 105 (11.08), 76 (31.17), 58 (2.83), 50 (7.49). *Molecular formula (M.wt.),* $C_{22}H_{30}N_4O_8$(478.50):*calculated analysis;* C55.22, H 6.32, N 11.71;*found analysis;* C 55.21, H 6.30, N 11.70.

General Procedure for Synthesis ofN$^\alpha$ - Phthaloyl - bis-[L-Ala –L-Ala -COOH]; (7)

To a stirred and cold methanolic solution (-5 ^{0}C, 20 ml) of ester, (**6**) (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 acid, (**7**).

N$^\alpha$ - Phthaloyl - bis-[L-Ala –L-Ala -COOH]; (7)

7. Yield: (89.23%); m.p.170 -172 ^{0}C. $[\alpha]_D^{25}$ = - 266.7 (C = 0.03). Rf x100 (the eluent) = 55.4 (S_1).*IR (cm^{-1}): (KBr):*ν= 3078 (NH stretching), 2887 (CH, aromatic), 2644 (CH, aliphatic), 1690 (C=O, acid), 1584, 1492 and 1400 (C=O amide I, II and III, respectively). *^{1}H-NMR* (500 MHz, ppm, DMSO-d_6): δ= 12.58 (s, 2H, 2OH, D_2O exchangeable), 8.08, 8.02 (s, 4H, 4NH, D2O exchangeable), 7.90, 7.89 (m, 4H, aromatic), 4.90-4.14 (m, 4H, 4CH, NHCHCH$_3$, L-Ala, α CH), 1.56-1.25 (d, 12H, 4CH$_3$, NHCH$_2$CH$_3$, L-Ala, β CH$_3$). *MS (EI, 70 eV): m/z (%)* = 450 (M^+, 0.54), 451 (M^++1, 1.24), 452 (M^++2, 0.99), 368 (10.66),

239 (8.06), 148 (8.93), 81 (37.37), 69 (52.32), 57 (100), 50 (6.72). *Molecular formula (M.wt.),* $C_{20}H_{26}N_4O_8$ (450.44): *calculated analysis;* C53.33, H 5.82, N 12.44; *found analysis;* C 53.30, H 5.81, N 12.40.

General Procedure for Synthesis of N$^{\alpha}$ - Phthaloyl - bis-[L-Ala – L-Ala –NHNH$_2$]; 8

hydrazine hydrate (0.35ml, 10 mmol) was added to a stirred methanolic solution (1 mmol, 50ml) of phthaloyl - bis-[L-Ala – L-Ala –methyl ester], (**6**). 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 hydrazide, **(8).**

N$^{\alpha}$ - Phthaloyl - bis-[L-Ala – L-Ala –NHNH$_2$]; (8)

8. Yield: (50.0 %); m.p. decomposition at 260-263 ^{0}C. $[\alpha]_D^{25}$ = - 250 (C = 0.04). Rf x100 (the eluent) = 79.2 (S_1).*IR (cm^{-1}): (KBr):*ν= 3274 (NH stretching), 3137 (CH, aromatic), 2959 (CH, aliphatic), 1654, 1575 and 1480 (C=O amide I, II and III, respectively).*^{1}H-NMR* (500 MHz, ppm, DMSO-d$_6$): δ= 9.03 (s, 1H, CONHNH$_2$, D2O exchangeable), 8.20, 8.11 (s, 4H, 4NH, D2O exchangeable), 8.00, 7.92 (m, 4H, aromatic), 5.05-4.42 (m, 4H, 4CH, NHCHCH$_3$, L-Ala, α CH), 4.40 (s, 2H, CONHNH$_2$), 1.75-1.33 (d, 12H, 4CH$_3$, NHCH$_2$CH$_3$, L-Ala, β CH$_3$). *MS (EI, 70 eV): m/z (%)* = 478 (M^+, 0.04), 479 (M^++1, 0.08), 480 (M^++2, 0.03), 368 (0.15), 235 (0.44), 181 (1.44), 161 (67.38), 104 (100), 132 (20.47), 76 (42.62), 75 (55.73), 72 (48.75), 50 (49.23).*Molecular formula (M.wt.),* $C_{20}H_{30}N_8O_6$ (478.50):*calculated analysis;* C50.20, H 6.32, N 23.42;*found analysis;* C 50.17, H 6.30, N 23.41.

General Procedure for Synthesis of Cyclo - (N$^{\alpha}$ – Phthaloyl) - bis-[L-Ala –L-Ala] – L – Lys –OMe; (9)

ECF (2.9 ml, 30.1 mmol) was added to a stirred and a cold DCM solution (-20 ^{0}C, 50 ml) of phthaloyl-[L-Ala – L-Ala -COOH], (**7**), (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 ^{0}C, 50 ml) of free L-Lys-OMe, (11gm; 60.2 mmol) was added. Stirring was maintained for 3 hours at -20 ^{0}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 ^{0}C). The obtained solid was collected by filtration and recrystallized from MeOH to give compound, (**9**), as identified by melting point and TLC.

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

9. Yield: (92.8 %); m.p. 78 -80^{0}C. [α] = - 400 (C = 0.02). Rf x100 (the eluent) =76.66 (S_1). *IR (cm^{-1}): (KBr):*ν= 3335 (NH stretching), 3071 (CH aromatic), 2942 (CH aliphatic), 1716 (C=O ester), 1655, 1536 and 1452 (C=O amide I, II and amide III, respectively).*^{1}H-NMR* (500 MHz, ppm, DMSO-d$_6$): δ= 8.16-8.14 (s, 6H, 6NH, D2O exchangeable), 7.88-7.14 (m, 4H, aromatic), 4.89 (m, 4H, 4CH, NHCHCH$_3$, L-Ala, α CH),, 4.22 (t, 1H, CH$_2$CHNH, α CH, Lys), 3.96, 3.61 (s, 6H, 2COOCH$_3$), 3.52, 3.32 (m, 2H, NHCH$_2$CH$_2$, εCH$_2$, Lys), 2.18 - 1.96 (m , 6H, 3CH$_2$, NHCH$_2$CH$_2$CH$_2$CH$_2$CHNH, γ, δ, β CH$_2$, Lys), 1.55-1.22 (d, 12H, 4CH$_3$, NHCH$_2$CH$_3$, L-Ala, β CH$_3$). *MS (EI, 70 eV): m/z (%)* = 574 (M^+, 0.04), 575 (M^++1, 0.07), 576 (M^++2, 0.10), 445 (2.08), 330 (10.86), 287 (18.97), 231 (9.37), 143 (26.00), 115 (100), 84 (62.13), 55 (7.79), 50 (2.48).*Molecular formula (M.wt.),* $C_{27}H_{38}N_6O_8$(574.63):*calculated analysis;*C56.43, H 6.67, N 14.36;*found analysis;* C 56.40, H 6.65, N 14.35.

General Procedure for Synthesis of Cyclo - (N$^{\alpha}$ – Phthaloyl) - bis-[L-Ala – L-Ala -OMe] – L – Lys –COOH; (10).

To a stirred and cold methanolic solution (-5 ^{0}C, 20 ml) of ester, (**9**), (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 acid **(10).**

Cyclo - (N$^{\alpha}$ – Phthaloyl) - bis-[L-Ala – L-Ala -OMe] – L – Lys –COOH; (10)

10. Yield: (90.1 %); m.p. 99 -101 ^{0}C. [α] = - 650 (C = 0.02).Rf x100 (the eluent) = 55.7 (S_1).*IR (cm^{-1}): (KBr):*ν= 3369 (NH stretching), 2921 (CH, aromatic), 2855 (CH, aliphatic), 1645 (C=O, acid), 1528and 1455 (C=O amide I and II, respectively).*^{1}H-NMR* (500 MHz, ppm, DMSO-d$_6$): δ= 12.48 (s, 1 H, OH, D$_2$O exchangeable),8.16 (s, 6H, 6NH, D2O

exchangeable), 7.94-7.51 (m, 4H, aromatic), 4.65, 4.43 (m, 4H, 4CH, NHCHCH$_3$, L-Ala, α CH), 4.14 (t, 1H, CH$_2$CHNH, α CH, Lys), 3.50, 3.25 (m, 2H, NHCH$_2$CH$_2$, εCH$_2$, Lys), 2.35-1.80 (m , 6H, 3CH$_2$, NHCH$_2$CH$_2$CH$_2$CH$_2$CHNH, γ, δ, β CH$_2$, Lys), 1.30-1.15 (d, 12H, 4CH$_3$, NHCH$_2$CH$_3$, L-Ala, β CH$_3$).*MS (EI, 70 eV): m/z (%)* = 588 (M^+, 0.27), 589 (M^++1, 0.24), 529 (1.12), 458 (3.26), 272 (20.62), 244 (12.32), 173 (85.87), 127 (21.11), 71 (67.65), 57 (100), 55 (43.02), 50 (4.68).*Molecular formula (M.wt.),* $C_{27}H_{36}N_6O_9$ (588.61):*calculated analysis;* C55.09, H 6.16, N 14.28; *found analysis;* C 55.07, H 6.14, N 14.25.

General Procedure for Synthesis of Cyclo - (N$^{\alpha}$ – Phthaloyl) - bis-[L-Ala – L-Ala -OMe] – L – Lys –NHNH$_2$; (11)

hydrazine hydrate (0.35ml, 10 mmol) was added to a stirred methanolic solution (1 mmol, 50ml) of Cyclo - (N$^{\alpha}$ – Phthaloyl) - bis-[L-Ala – L-Ala -OMe]– L – Lys –OMe (**10**). 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 hydrazide **(11)**.

Cyclo - (N $^{\alpha}$ – Phthaloyl) - bis-[L-Ala – L-Ala -OMe] – L – Lys –NHNH$_2$; (11)

11. Yield: (71.4%); oily compound. $[\alpha]_D^{25}$ = - 300 (C = 0.02). Rf x100 (the eluent) = 67.9 (S_1). *IR (cm^{-1}): (KBr):*ν= 3288 (NH stretching), 3070 (CH, aromatic), 2987 (CH, aliphatic), 1656, 1553 and 1389 (C=O amide I, II and III, respectively). *^{1}H-NMR* (500 MHz, ppm, DMSO-d$_6$): δ= 9.00 (s, 1H, CONHNH$_2$), 8.18-8.08 (s, 6H, 6NH, D2O exchangeable), 7.87-7.50 (m, 4H, aromatic), 4.98(m, 4H, 4CH, NHCHCH$_3$, L-Ala, α CH), 4.55 (t, 1H, CH$_2$CHNH, α CH, Lys), 4.05 (s, 2H, CONHNH$_2$), 3.40-3.20 (m, 2H, NHCH$_2$CH$_2$, εCH$_2$, Lys), 2.45-2.25 (m , 6H, 3CH$_2$, NHCH$_2$CH$_2$CH$_2$CH$_2$CHNH, γ, δ, β CH$_2$, Lys),1.21-1.12 (d, 12H, 4CH$_3$, NHCH$_2$CH$_3$, L-Ala, β CH$_3$).*MS (EI, 70 eV): m/z (%)* = 574 (M^+, 1.65), 550 (10.45), 427 (3.85), 338 (5.12), 161 (44.70), 104 (58.40), 127 (21.11), 116 (100), 84 (99.27), 57 (54.49), 55 (50.11), 50 (26.70).*Molecular formula (M.wt.),* $C_{26}H_{38}N_8O_7$(574.63):*calculated analysis;* C54.34, H 6.67, N 19.50 ;*found analysis;* C 54.33, H 6.65, N 19.46.

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

Human lung (A-549), colon (CaCo-2), prostate (PC-3) and breast (MCF-7) cancer cell were obtained from Karolinska Center, Department of Oncology and Pathology, Karolinska Institute and Hospital, Stockholm, Sweden. IC_{50} 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 *et al*. [41]. Doxorubicin was used as a positive control. DMSO used as negative control. Cell Viability Assay was done according to (Selim *et al*. [42] as described by Mosmann *et al*. [43]. The cells were seeded at concentration of 10x103 cells per well in case of MCF-7, 20x103 cells/well in case of HCT-116 cell lines using 96-well plates at 37 ^{o}C. After 48 hours' incubation, the medium was aspirated and 40 µl MTT salt (2.5 mg/ml) were added and further incubated for 4 hours. 200µl 10% sodium dodecyl sulphate (SDS) was added. The absorbance was measured at 595nm.

Molecular docking studies

The molecular modeling of the compound **3** was carried out using Molecular Operating Environment (MOE, 10.2008) software [44]. The X-ray crystallographic structure of EGFR co-crystallized with erlotinib as inhibitor (PDB ID: 1M17) [45] was downloaded from the protein data bank. The receptor was prepared for docking study using Protonate 3D protocol in MOE with default options followed by water molecules removal. The co-crystalized ligand was used to define the active site for docking. Docking setup was first validated by re-docking of the co-crystallized ligand in the vicinity of the active site of the receptor. The validated setup was then used in predicting the ligand-receptor interactions at the active site for compound **3**.

Results and Discussion

Chemistry

The synthesis of *N*$^{\alpha}$-phthaloyl-*bis*-(L-Ala) methyl ester **(3)** was based on N$^{\alpha}$-phthaloyl dicarbonyl dichloride, which was obtained by conversion of N$^{\alpha}$-phthalic dicarboxylic acid via the reaction with thionyl chloride. This acid chloride was then coupled, at low temperature, with L-Ala-methyl ester in the presence of triethylamine as organic base. Bis-ester **(3)** was also prepared from N$^{\alpha}$-phthalic dicarboxylic acid andL-Ala methyl esterin the presence ethyl chloroformate. Hydrolysis of ester **(3)** with in methanolic NaOH (1N) afforded the corresponding *N*$^{\alpha}$-phthaloyl-

bis-(L-Ala), (**4**). Also, hydrazinolysis of **(3),** with hydrazine hydrate in methanol afforded the corresponding hydrazide **(5)** (Scheme 1).

The IR spectra of **(3)** confirmed the presence of an aromatic ring, aliphatic hydrogens and an amide linkage in addition to the ester group. The amide linkage was confirmed by its two characteristic IR bands in the regions ν = 1641 and 1551 cm^{-1} (amide I and II, respectively). The presence of the ester group is supported by a band in the regions 1752 cm^{-1} ν (C=O), ester). In addition, an absorption band was observed at 3288cm^{-1}, attributed to hydrogen bonded amide ν (NH). Also, the IR spectra of **(4)** showed the absence of ν (C=O, ester), and instead the presence of a band at 1686 $cm^{-1}$and 1469 $cm^{-1}$for ν (C=O, acid and hydrazide, respectively). ^{1}H-NMR of the ester (**3**) revealed the presence of a signal at 3.65 of $2CH_3$, ester, in addition to the D_2O exchangeable signal of imidic protons. Hydrolysis and hydrazinolysis of compound (**3**) led to the corresponding acid (**4**) and hydrazide (**5**), respectively. The mass spectrum of Compounds (**3-5**) showed a peak equal to its molecular weight at m/z = 336, 308 and 336, respectively, (Scheme 1).

Synthetically, hydrolysis of the starting linear tetra peptide bis-ester, (**6**), afforded the corresponding free acid and hydrazide (**7** and **8**), respectively. (Scheme 2). The IR and ^{1}H NMR spectra of **(6)**, supported the presence of the ester group by the observation of a band in the region 1723 cm^{-1} ν (C=O) and the presence of a singlet (6H) at δ = 3.70 ppm for (2 ester-CH_3).

Cyclization of the tetrapeptide (7) with L-lysine methyl ester by mixed anhydride method afforded the corresponding cyclic pentapeptide ester**, (9)**, (Scheme 3). Finally, the methyl groups of the L-Lys-OMe esters of the cyclic pentapeptide**, (9)**, were converted to carboxylic acid groups or hydrazides. Hydrolysis of pentapeptide methyl ester derivative**, (9)**, with 1N sodium hydroxide in methanol afforded the corresponding acid derivative, (**10**). Also, hydrazinolysis of **(9),** with hydrazine hydrate in methanol afforded the corresponding cyclic pentapeptiedie hydrazide derivative**, (11)**, (Scheme 3).

In-vitro cytotoxic activity of compounds 3-11 against some selected human cancer cell lines

All the synthesized compounds **3-11** were evaluated for their antiproliferative activity against four cancerous cell lines, human lung (A-549), colon (CaCo-2), prostate (PC-3) and breast (MCF-7) cancer cells at 100 μM concentration using MTT growth inhibition assay [43]. Doxorubicin and DMSO were used as positive and negative controls, respectively. The results are shown in Table 1.

As exhibited in Table 1, compounds **4-7, 10, 11** displayed weak activity on human breast carcinoma (MCF-7), while compound **8** revealed moderate cytotoxic activity (Growth Inhibition = 44.3 %). Regarding to the other cancer cell lines, all the screened compounds **3-11** represented cytotoxic activity ranging from weak to moderate (Growth Inhibition ~ 0 - 67.7 %). On the other hand, compound **3** afforded the best anticancer activity against human colon carcinoma (CaCo2) (Growth Inhibition = 72.4 %).

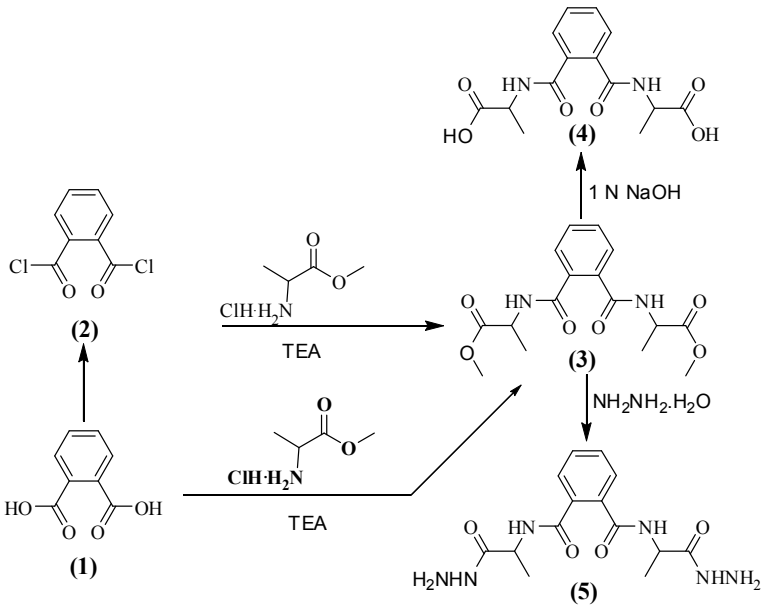
Molecular docking studies

Based on the promising cytotoxic result of compound **3** against human colon (CaCo2) carcinoma and as continuation of our previous work in isophthalamide based derivatives as anticancer compounds targeting the epidermal growth factor receptor (EGFR) [25] , the molecular docking study of compound **3** was performed in a trial to get better understanding of the potential binding, possible interactions, affinity and binding pattern with EGFR. Docking simulations were done using Molecular Operating Environment (MOE, 10.2008) software [43] . Initially our docking protocol was validated via redocking of the co-crystallized ligand, erlotinib within the binding site of EGFR with root mean squared deviation (RMSD) of 1.02 A^o.

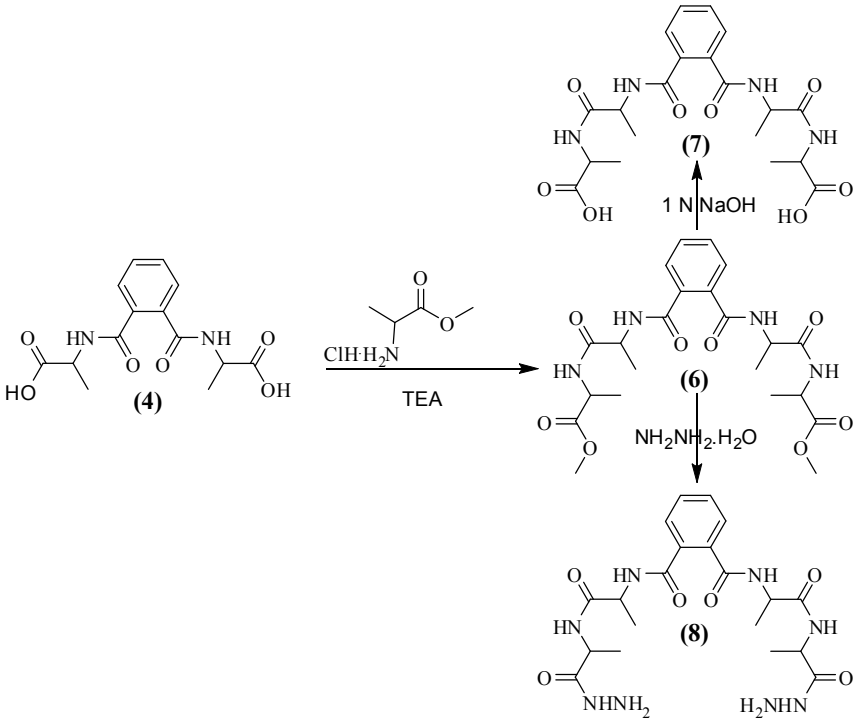
The 4-anilinoquinazoline inhibitor (erlotinib) revealed a hydrogen bond interaction acceptor through its quinazoline nitrogen atom with the backbone of **Met769** (distance: 2.70 Å). (Figure 1).

The docking result of compound **3** was inserted in Figure 2 and displayed that the two carbonyl oxygens neighboring the terminal methoxy groups shared two hydrogen bond acceptors with the sidechain and the backbone of **Thr766** and **Met769** (distance: 2.78 and 2.81 Å), respectively. Furthermore, the amide oxygen established hydrogen bond acceptor with the sidechain of **Thr830** (distance: 2.73 Å).

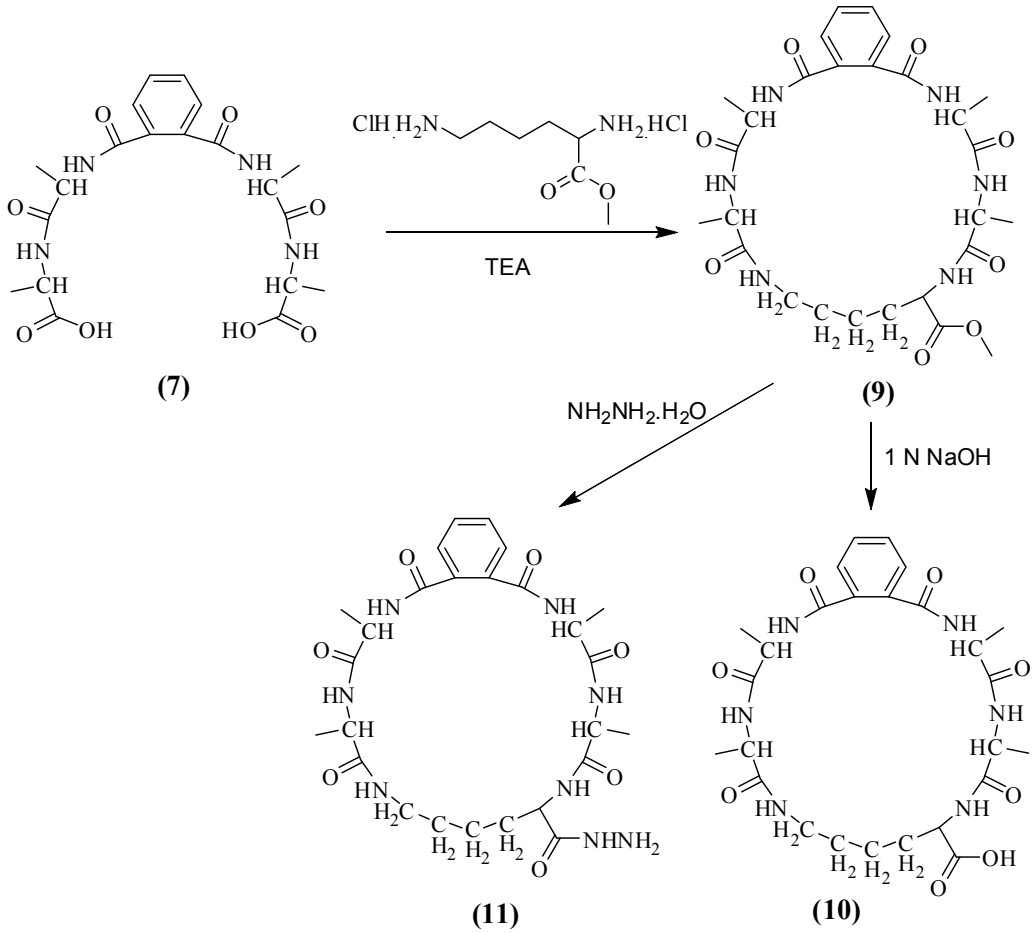
Finally, the previous interactions proved that the compound **3** showed good docking results and nicely fitted within the active site of EGFR.



Scheme 1. Synthetic routes for compounds (3-5).



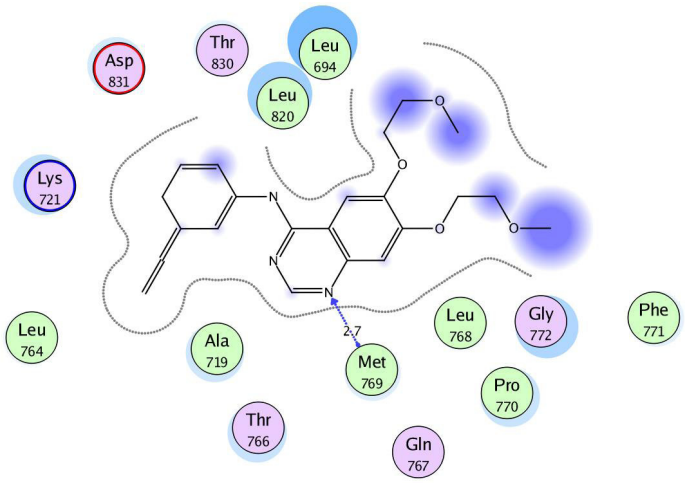
Scheme 2. Synthetic routes for compounds (6-8).



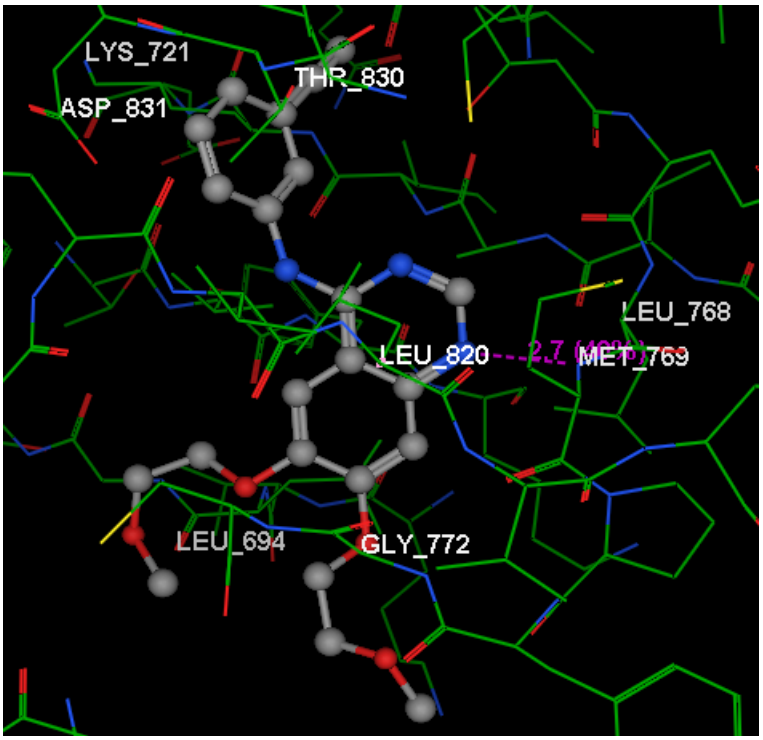
Scheme 3. Synthetic routes for compounds (9-11).

TABLE 1. Cytotoxic activity of nine compounds against four human carcinoma cell lines at 100 µM.

Compound No.	Growth Inhibition (%)			
	A-549	**CaCo-2**	**PC-3**	**MCF-7**
3	28.95	72.4	38.2	21.55
4	47.26	42.9	27	0
5	48.25	41.95	37.55	0
6	16.51	50.58	22.15	0
7	17.84	29.05	27.95	0
8	43.75	52.29	39.6	44.3
9	15.76	67.7	34.95	38.8
10	50.15	21.85	19	0
11	0	49.2	38.65	0
DMSO	0	0	0	0
Doxorubicin	100	100	100	100

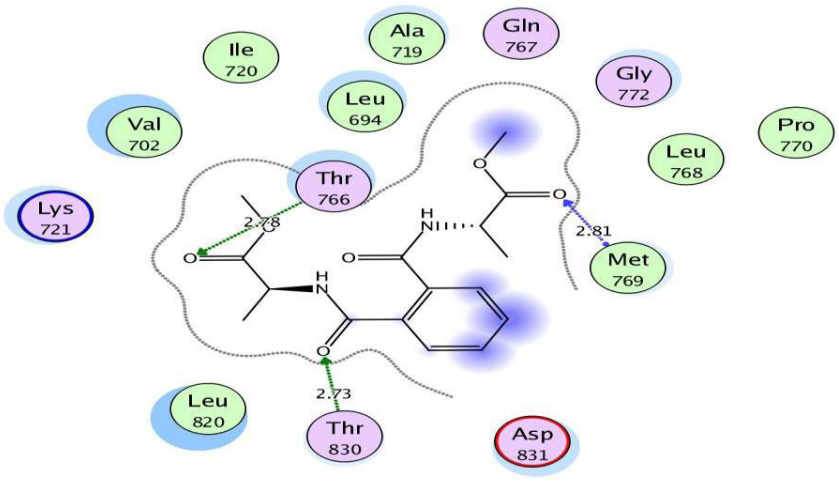


A

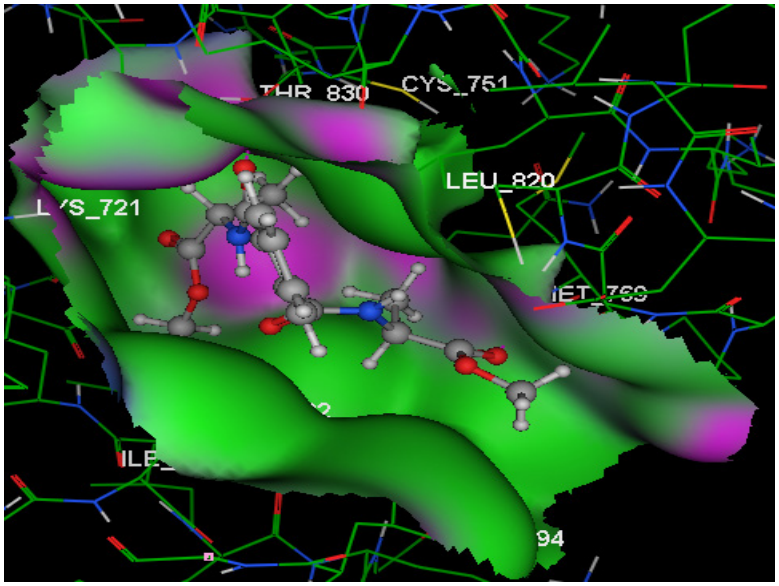


B

Figure 1. A & B represent 2D and 3D views of the original ligand, erlotinib docked into EGFR binding site (PDB code: 1M17). Hydrogen bonds are illustrated as dotted lines and arrows; C atoms are colored gray, N blue and O red.



A



B

Figure 2. A & B represent 2D and 3D views of compound 3 docked into EGFR binding site (PDB code: 1M17). Green color indicates hydrophobic area, pink color indicates high polar area and blue color indicates mild polar area. Hydrogen bonds are illustrated as dotted lines and arrows; C atoms are colored gray, N blue and O red.

Conclusion

The present work aimed to synthesize some novel cyclic pentapeptides based on phthaloyl dichloride (1, 2-benzenedicarbonyl chloride). Synthesized compounds were characterized by different spectral data. Cyclic peptides, which have the general structure: Cyclo-[N^{α}-phthaloyl-bis-(L-ala-L-ala)-L-Lys] ester, acid or hydrazide, appeared promising activity, as cytotoxic, namely, anticancer candidates. Further profound biological, particularly, conventional anticancer investigations, on experimental animal models, seem worthy to be realized.

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تصميم وتشييد ببتيدات حلقية جديدة المستندة إلى كلوريد الفثايل مع دراسة نشاطها المتوقع كمضادات للسرطانات

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يهدف هذا البحث إلى تصميم وتشييد وتعريف الشكل الجزيئى لبعض المشتقات الببتيدية الجديدة خماسية الحلقة و التي يمثلها التركيب العام التالي "فثالويل [ل. آلانين – ل. آلانين] ل.ليزين، والمتوقع لها سمية وتأثير مضاد لخلايا السرطانات البشرية وباستخدام الطرق المتنوعة لتشييد الببتيدات , فقد تم التشييد الكيميائي لتسعة مركبات ببتيدية جديدة . وقد تنوعت تلك الببتيدات مابين ستة مركبات ببتيدية خطية تم تشييدها كببتيدات بادئة للحصول علي الببتيدات الحلقية المقابلة وعددها ثلاثة مركبات. وقد تم التعريف والتحليل الكيميائي والطيفي للمركبات المشيدة بعد تنقيتها وبعد ذلك تم دراسة خواص جميع المركبات المحضرة حديثا كمضادات للسرطانات البشرية المختلفة.