Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

Synthesis and Cytotoxic Evaluation of Novel Nα-1, 4-Benzenedicarbonyl Bridged Cyclo-Pentapeptide Candidates

Gaber O. Moustafa and Shaima A. El-Mowafi
Peptide Chemistry Department, Chemical Industries Research institution, National Research Centre, 12622-Dokki, Cairo, Egypt

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

Abstract

Cancer is the second most common cause of death worldwide, with mortality about to surpass that from cardiovascular diseases. Therapeutic peptides present several advantages over conventional cancer treatments such as ease of synthesis, higher target selectivity and lower toxicity. As a continuation of our previous recent reports on anticancer cyclic peptides, novel Nα-1, 4-benzenedicarbonyl bridged cyclo-pentapeptides were newly synthesized and evaluated for cytotoxic activity using MTT growth inhibition assay. Interestingly, compound 9, namely Cyclo-[Nα-1, 4-benzenedicarbonyl-bis-(Gly-L-Phe)-L–Lys]-OMe showed higher inhibitory activity on breast (MCF-7) and liver (HepG-2) cell lines, with IC50 values 4.04 and 2.82 µg/ml respectively, compared to the reference drugs Tamoxifen (8.31 and 10.9 µg/ml) and Doxorubicin (2.97 and 3.73 µg/ml). Cytotoxic activity of compound 9 was further investigated on five other cell lines, namely colon (CaCo-2), prostate (PC-3), cervical (HeLa), larynx (Hep-2) and breast (T47D) cancer cells. Compound 9 was found to be more potent than doxorubicin in three cell lines, (T47D, HeLa and PC-3), and almost as potent in CaCo-2 cell line, consequently representing a very promising anticancer candidate. Assessment of its possible mechanism of action as multi-targeted kinase inhibitor will be investigated.

Keywords: cancer, cyclic pentapeptides, Nα-1, 4-benzenedicarbonyl-bis-peptides, cytotoxicity

1. Introduction

Cancer represents one of the main causes of deaths worldwide. Globally, there were 9.5 million cancer-related deaths and 18.1 million new cases in 2018. It is anticipated that by 2040, there would be 29.5 million new instances of cancer annually, and 16.4 million cancer-related deaths [1]. The detection, treatment, and prevention of various forms of cancer have advanced significantly in recent years. Surgery, radiation, chemotherapy, and hormone therapy are among the cancer treatments currently in use [2-4]. However, the primary issues with these increasingly concerning procedures are their high cost and unfavorable side effects. For example, it has been demonstrated that doxorubicin, a common chemotherapeutic drug used to treat malignancies, can harm the kidney, heart, and brain through oxidative stress [5-7]. When treating breast cancer, other medications like taxanes and anthracyclines are less successful than they formerly were since the tumor has become resistant to them. Nowadays, the possibility of tumor recurrence and metastasis persists as a serious problem, even in cases when early cancer treatments are successful [8–10].

Therapeutic peptides are short sequences of amino acids that can be rationally synthesized with high specificity to bind a desired protein interaction, such as an inhibitor of oncogenic protein interactions [11, 12]. Compared to proteins or antibodies, these peptides have several significant benefits, including extensive biological diversity, low drug-drug interactions, high target specificity and selectivity, and potency of action [13–15]. Peptides do not accumulate in tissues like the liver or kidney, which minimizes harmful side effects and is a major advantage of employing them in cancer treatment [16, 17]. Moreover, they are less immunogenic than recombinant proteins or antibodies and are readily synthesized and modified. Therapeutic peptides do, however, have several significant disadvantages, including short half-lives, limited cell permeability, poor oral bioavailability, and metabolic instability [18–21].

Among chemically synthesized therapeutic peptides, macrocyclic compounds composed of cyclic peptides have been developed to overcome the major limitations of peptides. Indeed, lower entropy levels are accompanied by conformational limitations imposed by molecular cyclization. As a result, cyclopeptides have better pharmacological qualities overall and provide more stability against the activity of proteolytic enzymes, which prolongs their bioavailability. According to earlier studies, it was possible to successfully synthesize macrocyclic peptide derivatives chemically and produce compounds with strong

---

*Corresponding author e-mail: shaimamowafi@gmail.com
Receive Date: 26 December 2023, Revise Date: 07 February 2024, Accept Date: 12 February 2024
DOI: 10.21608/ejchem.2024.258031.9086

antibacterial, anti-inflammatory, and anti-cancer effects [22, 23].

Herein, drawing from past research and our ongoing peptide synthesis endeavors, we have identified Nα-1, 4-benzenedicarbonyl bridging cyclo-pentapeptides with the following structure: Cyclo-[Nα-1, 4-benzenedicarbonyl-bis-(Glycine - Amino Acid)-L-Lys] -Y, **(9–14),** in which "Amino Acid" denotes "L-Phenylalanine" or "Glycine," and "Y" denotes newly synthesized methyl ester, carboxylic, or hydrazide group. These compounds were assessed for their anticancer potential in comparison to commonly used anticancer medications.

2. Experimental

Melting points were uncorrected and determined in opened glass capillary tubes with an "Electro Thermal" Digital melting point apparatus, (model: IA9100). Elemental micro-analysis for carbon, hydrogen and nitrogen (Micro-analytical Unit, NRC) was found within the acceptable limits of the calculated values. Infrared spectra (KBr) were recorded on a Nexus 670 FTIR Nicolet, Fourier Transform infrared spectrometer.

^{1}H-NMR spectra and ^{13}C-NMR were run in ([D$_6$] DMSO) on JöEL 270 MHz or 500 MHz instruments. Mass spectra were run on a MAT Finnigan SSQ 7000 spectrometer, using the electron impact technique (EI).

Analytical thin layer chromatography (TLC) was performed on silica gel aluminum sheets, 60 F254 (E. Merck). The following solvent systems (by volume) were used as eluents for the development of the plates: S: chloroform/methanol/acetic acid (85/10/5 v/v); S$_1$: S/ petroleum ether (B.p. 40- 60 ^{o}C) (3/2) and S$_2$: butanol/water/acetic acid/pyridine (120/48/12/40 v/v). Specific optical rotations were measured with a A. Krawss, Optronic, P8000 polarimeter, in a 1 dm length observation tube, at the indicated conditions, and according to the equation: $[\alpha] = (100 \times \alpha)/(c \times l)$, where: α : observed rotation angle, D: sodium line (λ= 589 nm), c : concentration (g/100ml), l = path length in dm and T = experimental temperature (^{o}C).

2.1. Chemistry

2.1.1. Synthesis of N$^{\alpha}$-1, 4-benzenedicarbonyl- *bis*-[glycine ethyl ether], 3

A. Acid chloride method

A dichloromethane (DCM) solution of Compound **2** (3 gm, 14.78 mmol) (50 ml) was dropwisely added to a cold (-20 ^{o}C) and stirred DCM solution (50 ml) of free glycine ethyl ester (4.13 gm, 2 equivalents 29.56 mmol, obtained by the addition of two equivalents amount of N-methylmorpholine (3.25 ml; 29.56 mmol) to the amino acid methyl ester hydrochloride to a stirred and cold (-20 C) DCM, 50ml).

The reaction mixture was stirred for additional 3 hours at the same temperature then for 24 hours at room temperature, washed with dist. water, 1N sodium bicarbonate, 1N potassium hydrogen sulphate and dist. water then for dried for (24 hours at 0 ^{o}C) over sodium sulphate anhydrous. The solvent was evaporated to dryness and the obtained residue was solidified by Pt.ether (B.P. 40-60 ^{0}C). The obtained solid was filtered off and crystallized from methanol to give Compound **3.**

B. Mixed anhydride method

Ethyl chloroformate (2.9 ml, 30.1 mmol) was added to a stirred and cold (-20 ^{o}C) a DCM solution (50 ml) of terephthalic acid (5 gm, 30.1 mmol) and N-methylmorpholine (6.6 ml, 60.2 mmol).

The reaction mixture was stirred for additional 30 minutes, then a DCM solution (50 ml) free Gly ethyl ester (8.4 gm; 60.2 mmol, -20 ^{o}C) in dichloromethane (50 ml) was added. Stirring was maintained for 3 hours at -20 ^{o}C then for 24 hours at room temperature. The reaction mixture was then washed with dist. water, 1N sodium bicarbonate, 1N potassium hydrogen sulphate and water then dried over sodium sulphate anhydrous (24 hours at 0 0C).

The solvent was evaporated to dryness and the obtained oily residue was solidified by trituration with pt.ether (B.P. 40-60 ^{0}C), collected by filtration and crystallized from methanol to give compound **3** as identified by melting point and TLC in comparison with authentic sample prepared according to method A.

3: Yield: [A]: 93; [B]: 85; m.p. 113-115 ^{0}C. Rf x100 (solvent system) 65 (S$_1$). IR (cm^{-1}): (KBr): 3861 and 3318 (NH stretching) 3091 (CH aromatic) 2983 (CH aliphatic) 1752 (C=O ester) 1644 and 1551 (C=O amide I and amide II). MS (EI, 70 eV): m/z (%) =336 (M^{+}, 3.84%), 337 (M^{+}+1, 0.8%), 338 ((M^{+}+2, 0.17%), 339 (M^{+}+3, 0.03%), 291(5%), 263(17%), 234(100%), 160(14%), 104(31%), 76(14%), 65(0.39), 50(4.04%). 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; C 57.00, H 6.00, N 8.30.

1.1.2. Synthesis of N$^{\alpha}$-1, 4-benzenedicarbonyl-*bis*-[glycine], 4

To a stirred and cold ethanolic solution (-5 ^{o}C, 20 ml) of the corresponding ester **3** (2 gm, 5.94 mmol), sodium hydroxide (1N, 35 ml) was dropwisely added. The reaction mixture was stirred for 3 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 1 N hydrochloric acid to pH≈3. The obtained solid was filtered off, washed with water, dried and crystallized from ethanol/water to give the corresponding amino acid **4**.

4: Yield: 78; m.p. 224-226 ^{0}C. Rf x100 (solvent system) 50 (S$_1$). IR (cm^{-1}): (KBr): 3310 (NH stretching) 3059 (CH aromatic) 2937 (CH aliphatic) 1708 (C=O acid) 1642 and 1552 (C=O amide I and amide II). MS (EI, 70 eV): m/z (%) =280 (M^+, 0.6%), 277 (0.62%), 236(1.93%), 206 (6.81%), 179(27.88%), 160(3.15%), 149(100%), 121(27.65%), 104(31%), 103(9%), 76(17.40%), 65(47.82), 50(18.37%). Molecular formula (M.wt.), $C_{12}H_{12}N_2O_6$ (280.07). Calculated analysis; C: 46.16, H: 3.87, N: 8.97, Found; C 46.01, H 3.75, N 9.00.

1.1.3. Synthesis of N^{α}-1, 4-benzenedicarbonyl-*bis*-[dipeptide ester]; (5 and 6)

Ethyl chloroformate (2.9 ml, 30.1 mmol) was added to a stirred and cold (-20 °C) solution of 1, 4-benzenedicarbonyl-*bis*-[Glycine], **4**, with the same sequence (15.05 mmol) and N-methylmorpholine (3.3 ml, 30.1 mmol) in dichloromethane (50 ml). The reaction mixture was stirred for additional 30 minutes, then the two equivalents amount of free amino acid esters (L-Phe, Gly) (30.1mmol) (-20 °C) in dichloromethane (50 ml) 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 dist. water, 1N sodium bicarbonate, 1N potassium hydrogen sulphate and water then dried over sodium sulphate anhydrous (24 hours at 0 ^{0}C). The obtained solid was filtered off and crystallized from ethanol or methanol to give the esters (5 and 6).

5: Yield: 75; m.p. 108-111 0C. Rf x100 (solvent system) 78 (S1); [α] : - 12.04 (C, 0.02, MeOH). IR (cm^{-1}): (KBr): 3355 (NH stretching) 3029 (CH aromatic) 2949 (CH aliphatic) 1739 (C=O ester) 1720 and 1638 (C=O amide I and amide II). MS (EI, 70 eV): m/z (%) = 422 (M^+. 0.07%), 423 (M^+ +1, 0.16%), 424 (M^+ +2, 1.01%), 425 (M^+ +3, 0.38%), 426 (M^+ +4, 0.10%), 409(1.01%), 166 (80.71%), 161(2.30%), 149(100%), 121(39.08%), 104(4.97%), 103(4.46%), 76(17.32%), 65(53.37), 51(25.72%),50(37.37%). Molecular formula (M.wt.), $C_{32}H_{34}N_4O_8$ (602.6). Calculated analysis; C, 63.78; H, 5.69; N, 9.30; O, 21.24, Found; C 63.70, H 5.71, N 9.35.

6: Yield: 70; m.p. 108-110 ^{0}C. Rf x100 (solvent system) 70 (S_1). IR (cm^{-1}): (KBr): 3375 (NH stretching) 2940 (CH aromatic) 2830 (CH aliphatic) 1710 (C=O ester), 1640 and 1460 (C=O amide I and amide II). MS (EI, 70 eV): m/z (%) = 450 (M^+, 13%), 260 (19.73%), 200 (11.55), 170 (15.43%), 130 (22.19%), 109 (18.60%), 78(18.70%), 67(100%), 50(16.60%). Molecular formula (M.wt.), $C_{20}H_{26}N_4O_8$ (450.4). Calculated analysis; C, 53.33; H, 5.82; N, 12.44, Found; C 53.19, H 5.75, N 12.38.

1.1.4. Synthesis of N^{α}-1, 4-benzenedicarbonyl-bis-[dipeptide amino acid]; (7 and 8).

To a stirred and cold methanolic solution (-5 °C, 20 ml) of the corresponding esters, (**5** and **6**) (2 mmol), sodium hydroxide (1N, 25 ml) was dropwisely added. 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 1 N hydrochloric acid to pH≈3. The obtained solid was filtered off and crystallized from ethanol to give the corresponding amino acid (**7** and **8**).

7: Yield: 70; white oily, Rf x100 (solvent system) 50 (S_1); [α] : -23.412 (C, 0.02, MeOH). IR (cm^{-1}): (KBr): 3377 (NH stretching), 2974 (CH aromatic), 2863 (CH aliphatic), 1718 (C=O acid), 1546 and 1447 (C=O amide I and amide II). MS (EI, 70 eV): m/z (%) = 575 (M^+. 2.29%), 576 (M^+ +1, 1.28%), 577 (M^+ +2, 3.30%), 578 (M^+ +3, 1.20%), 579 (M^+ +4, 0.71%), 580 (M^+ +5, 1.15%), 551 (3.4%), 509 (3.82%), 461 (2.04%), 424 (1.69%), 366 (2.50%), 339 (9.33%), 262 (15.78%), 236 (12.91%), 178 (4.19), 149 (11.96%), 123 (23.97%), 109 (36.27%), 95 (61.57), 81 (70.51%), 69 (66.75%), 57 (89.25%), 53 (6.43), 51 (8.06%). Molecular formula (M.wt.), $C_{30}H_{30}N_4O_8$ (574.6). Calculated analysis; C: 62.71, H: 5.26, N: 9.75, Found; C 62.67, H 5.24, N 9.72.

8: Yield: 87; m.p. 115-117 ^{0}C. Rf x100 (solvent system) 60 (S_1). IR (cm^{-1}): (KBr): 3066(NH stretching), 2962 (CH aromatic), 2669 (CH aliphatic), 1690(C=O acid), 1573, 1538 and 1512 (C=O amide I, amide II and amide III). MS (EI, 70 eV): m/z (%) = 423 (M^+ +1, 43.28%), 241 (0.84%), 227 (42.86%), 222 (46.64%), 203 (49.58%), 196 (47.90%), 176 (47.48%), 147 (100%), 125 (14.71%), 108 (52.10%), 80 (92.44%), 70 (22.69%), 64 (40.76%), 59 (21.43%). Molecular formula (M.wt.), $C_{18}H_{22}N_4O_8$ (422.4). Calculated analysis; C: 51.18, H: 5.25, N: 13.26, Found; C 51.17, H 5.16, N 13.18.

1.1.5. Synthesis of Cyclo-(N^{α}-1, 4-benzenedicarbonyl)-*bis*-[dipeptide]-L-Lys-OMe (1, 4-benzenedicarbonyl cyclic pentapeptide methyl esters); (9 and 10)

A. Mixed anhydride method

Ethyl chloroformate (2.9 ml, 30.1 mmol) was added to a stirred and cold (-20 °C) solution of 1, 4-benzenedicarbonyl-bis-[dipeptide amino acid] (**7** and **8)** (15.05 mmol) and N-methylmorpholine (3.3 ml, 30.1mmol) in Dichloromethane (50 ml). The reaction mixture was stirred for additional 30 minutes, then the one equivalent of free L-Lysine methyl ester (3.5 gm, 15.05mmol) (-20 ^{0}C) in Dichloromethane (50 ml) 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 dist. water, 1N sodium bicarbonate, 1N potassium hydrogen sulphate and water then dried over sodium sulphate anhydrous (24 hours at 0 ^{0}C). The obtained solid was filtered off and crystallized from ethanol to give the corresponding cyclic pentapeptide methyl esters (**9** and **10**).

B. N, N\ - Dicyclohexylcarbodiimide method

A cold tetrahydrofuran solution (-5 °C, 20ml) of free L-lysine methyl ester (1 mmol) was added to a stirred dry tetrahydrofuran solution (-5° C, 20 ml) of the corresponding 1, 4-benzenedicarbonyl-*bis*-[dipeptide amino acids] (**7** and **8)** (1mmol). Dicyclohexylcarbodiimide (0.42 gm, 2 mmol) was then added, in portions, over 20 minutes at the same temperature to the reaction mixture. Stirring was maintained for 20 hours at room temperature. The reaction mixture was then diluted with acetonitrile (20 ml) and the formed dicyclohexylurea was filtered off and washed with acetonitrile (2x10 ml). The filtrate was kept in refrigerator overnight and the newly formed dicyclohexylurea was then filtered off.

Tetrahydrofuran was evaporated to dryness and the obtained residue was dissolved in dichloromethane, washed with 1N sodium bicarbonate, 1N potassium hydrogen sulphate and water then dried over anhydrous sodium sulphate. The solvent was evaporated to dryness and the obtained oily residue was solidified by trituration with dry ether/*n*-hexane mixture. The obtained solid was collected by filtration and crystallized from ethanol/n-hexane. The cyclic pentapeptide methyl esters (**9** and **10**) were identified by melting point and TLC in comparison with authentic samples prepared according to method A.

9: Yield: 63: [A]; 55: [B]; m.p. 101-103 ^{0}C. Rf x100 (solvent system) 78 (S_1); [α] : -38.666 (C, 0.02, MeOH). IR (cm^{-1}): (KBr): 3310 (NH stretching), 3064 (CH aromatic), 2933 (CH aliphatic), 1732 (C=O ester), 1697, 1655 and 1536 (C=O amide I, amide II and amide III). ^{1}H-NMR (500 MHz, δ, ppm, DMSO-d_6): δ : 7.98 (m, 4H, aromatic H), 7.95 (s, 6H, 6NH, D_2O exchangeable) (sec. amide), 7.89 (m, 10H, 2 aromatic H)(L-Phe-ala), 4.95 (t, 2H, $\mathrm{NH\underline{\mathbf{CH}}CH_2Phe}$), 4.68 (t, 1H, $\mathrm{NHCH_2CH_2CH_2CH_2\underline{\mathbf{CH}}NH}$)(methino), 4.32 (s , 4H, $\mathrm{2CH_2}$, $\mathrm{NH\underline{\mathbf{CH_2}}CO}$) (methylene), 4.11 (s, 3H, $\mathrm{COO\underline{\mathbf{CH_3}}}$), 3.21 (d, 4H, 2CH2, $\mathrm{\underline{\mathbf{CH_2}}Phe}$), 2.06 (m, 2H, $\mathrm{NH\underline{\mathbf{CH_2}}CH_2CH_2CH_2CHNH}$) (methylene), 1.86, 179 (m , 6H, 3CH2, $\mathrm{NHCH_2\underline{\mathbf{CH_2}}\underline{\mathbf{CH_2}}\underline{\mathbf{CH_2}}CHNH}$). MS (EI, 70 eV): m/z (%) =698 (M$^+$. 8%), 624 (10.39%), 547 (13.69), 429 (13.23%).365 (13.14%), 351 (10.03%), 308 (9%), 424 (1.69%), 278 (17.48%), 272 (19.89%), 244 (16.72%), 192 (26.59%), 177 (33.12), 156 (100%), 144 (41.55%), 132 (26.09%), 116 (22.69), 86 (10.78%), 70 (5.33%), 57 (23.39%), 51 (0.74%). Molecular formula (M.wt.), $C_{37}H_{42}N_6O_8$ (698.8). Calculated analysis; C: 63.60, H: 6.06, N: 12.03, Found; C 63.60, H 6.08, N 12.00.

10: Yield: 92: [A]; 65: [B]; m.p. oily ^{0}C. Rf x100 (solvent system) 66 (S_1); [α] : -5.35 (C, 0.02, MeOH). IR (cm^{-1}): (KBr): 3352 (NH stretching), 2944 (CH aromatic), 2832 (CH aliphatic), 1654 (C=O ester), 1452 and 1419 (C=O amide I and amide II). ^{1}H-NMR (500 MHz, δ, ppm, DMSO-d_6): δ : 8.31 (m, 4H, aromatic H), 7.95- 6.98 (s, 6H, 6NH, D_2O exchangeable) (sec. amide), 4.19 (t, 1H, $\mathrm{NHCH_2CH_2CH_2CH_2\underline{\mathbf{CH}}NH}$) (methino), 4.07, 3.81 (s , 8H, $\mathrm{4CH_2}$, $\mathrm{NH\underline{\mathbf{CH_2}}CO}$)(methylene), 3.73 (s, 3H, $\mathrm{COO\underline{\mathbf{CH_3}}}$), 3.59, 3.57(s, 6H, $\mathrm{2CH_3}$, $\mathrm{N\underline{\mathbf{CH_3}}}$), 3.21 (m, 2H, $\mathrm{NH\underline{\mathbf{CH_2}}CH_2CH_2CH_2CHNH}$)(methylene), 1.63, 1.57 (m , 6H, 3CH2, $\mathrm{NHCH_2\underline{\mathbf{CH_2}}\underline{\mathbf{CH_2}}\underline{\mathbf{CH_2}}CHNH}$). ***^{13}C-NMR*** (δ, ppm, DMSO-d_6): 15.1 ($\mathrm{CH_2CH_2\underline{\mathbf{C}}H_2CH_2CH}$), 22.9 ($\mathrm{CH_2CH_2CH_2\underline{\mathbf{C}}H_2CH}$), 23.1 ($\mathrm{CH_2\underline{\mathbf{C}}H_2CH_2CH_2CH}$), 29 ($\mathrm{NHCH_2CH_2CH_2CH_2\underline{\mathbf{C}}HNH}$), 38.6, 39.2 ($\mathrm{2NCH_3}$), 40.2, 40, 39.9 ($\mathrm{3NH\text{-}\underline{\mathbf{C}}H_2}$), 51.7($\mathrm{COO\underline{\mathbf{C}}H_3}$), 52.3 ($\mathrm{2NCH_3\underline{\mathbf{C}}H_2}$) , 126.9, 126.4, 126.3 (4C, aromatic C_2, C_3, C_5, C_6), 129.8 (2C, aromatic C_1, C_4), 168.8 ($\mathrm{2CH_2NH\underline{\mathbf{C}}O}$), 169.3 ($\mathrm{2\ NCH_3\underline{\mathbf{C}}O\ CH_2}$)173.6, 173($\mathrm{2\ NH\underline{\mathbf{C}}O\ CH_2}$), 174.1 ($\mathrm{\underline{\mathbf{C}}OOCH_3}$). MS (EI, 70 eV): m/z (%) = 548 (M$^+$ +2, 27.92%), 525 (28.93%), 509 (28.93%), 276 (26.14%), 239 (26.65%), 204 (2.03%), 177 (29.44%), 119 (26.14%), 109 (27.92%), 86 (30.96%), 73 (12.69%), 64 (100%), 52 (6.09%), 50 (1.27%). Molecular formula (M.wt.), $C_{25}H_{34}N_6O_8$ (546.6), Calculated analysis; C: 54.94, H: 6.27, N: 15.38, Found; C 45.90, H 9.22, N 15.35.

1.1.6. Synthesis of Cyclo-(N$^{\alpha}$-1, 4-benzenedicarbonyl)- *bis*-[dipeptide]-L-Lys-acid; (1, 4-benzenedicarbonyl cyclic pentapeptides); (11 and 12)

To a stirred and cold methanolic solution (-5 °C, 20 ml) of the corresponding cyclic pentapeptide methyl esters (**9** and **10**) (1 mmol), sodium hydroxide (1N, 25 ml) was dropwisely added. The reaction mixture was stirred for 3 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 1 N hydrochloric acid to pH≈3. The obtained solid was filtered off, washed with dist. water, dried and crystallized from ethanol/water to give the corresponding cyclic pentapeptides, (**11** and **12).**

11: Yield: 65; m.p. oily ^{0}C. Rf x100 (solvent system) 44 (S_1); [α] : -83.7 (C, 0.02, MeOH). IR (cm^{-1}): (KBr): 3383 (NH stretching) 2924 (CH aromatic) 2858 (CH aliphatic) 1724 (C=O acid) 1640, 1565 and 1451 (C=O amide I, amide II and amide III). ^{1}H-NMR (500 MHz, δ, ppm, DMSO-d_6): δ : 12.85 (s, 1H, OH, D_2O exchangeable), 8.61 (m, 4H, aromatic H), 8, 7.96 (s, 6H, 6NH, D_2O exchangeable) (sec. amide), 7.89, 7.87 (m, 4H, 2 aromatic H($C_3$and C_5)) (L-Phe-ala), 7.22, 7.18 (m, 4H, 2 aromatic H($C_2$and C_6))(L-Phe-ala), 7.15, 7.12 (m, 2H, aromatic H, C_4)(L-Phe-ala), 4.60, 4.32 (t, 2H, $\mathrm{NH\underline{\mathbf{CH}}CH_2Phe}$), 4.31-3.92 (t, 1H, $\mathrm{NHCH_2CH_2CH_2CH_2\underline{\mathbf{CH}}NH}$)(methino), 3.6-3.53 (s , 4H, $\mathrm{2CH_2}$, $\mathrm{NH\underline{\mathbf{CH_2}}CO}$)(methylene), 3.38-3.13 (dd, 6H, 3CH2, $\mathrm{\underline{\mathbf{CH_2}}Phe}$), 3.05 (m, 2H, $\mathrm{NH\underline{\mathbf{CH_2}}CH_2CH_2CH_2CHNH}$) (methylene), 2.4-1.3 (m , 6H, 3CH2, $\mathrm{NHCH_2\underline{\mathbf{CH_2}}\underline{\mathbf{CH_2}}\underline{\mathbf{CH_2}}CHNH}$). MS (EI, 70 eV): m/z (%) = 684(M$^+$. 22.68%), 655(39.50%), 636(35.51), 547(35.84%).492(100%), 457(76.63%), 431(47.56%), 367(60.68%), 293(51.33%), 276(52.32%), 254(68.89%), 233(58.41%), 204(59.73), 180(59.46%), 170(66.41), 137(80.47%), 125(68%), 98(68%), 78(66.07), 69(80.24%), 67(40.27%), 60(54.41%), 51(23.54%). Molecular formula (M.wt.), $C_{36}H_{40}N_6O_8$ (684.7), Calculated analysis; C: 63.15, H: 5.89, N: 12.27, Found; C 63.13, H 5.77, N 12.22.

12: Yield: 90; m.p. 128-131 ^{0}C. Rf x100 (solvent system) 40 (S_1); [α] : -7 (C, 0.02, MeOH). IR (cm^{-1}): (KBr): 3355 (NH stretching), 2945 (CH aromatic), 2832 (CH aliphatic), 1651(C=O acid), 1452 and 1418 (C=O amide I and amide II). ^{1}H-NMR (500 MHz, δ, ppm, DMSO-d_6): δ : 12.57 (s, 1H, OH, D_2O exchangeable), 7.91, 7.90 (m, 2H, aromatic H), 77.85, 7.83 (m, 2H, aromatic H), 7.37-7.20(s, 6H, 6NH, D_2O exchangeable)(sec. amide), 4.26 (t, 1H, $NHCH_2CH_2CH_2CH_2\underline{\mathbf{CH}}NH$) (methino), 3.9 (s , 8H, $4CH_2$ ($2NH\underline{\mathbf{CH_2}}CO$, $2NCH3\underline{\mathbf{CH_2}}CO$)) (methylene), 3.51, 3.47 (s, 6H, $2CH_3$, $N\underline{\mathbf{CH_3}}$), 3.82 (m, 2H,$NH\underline{\mathbf{CH_2}}CH_2CH_2CH_2CHNH$) (methylene), 2.49, 2.46 (m , 6H, 3CH2, $NHCH_2\underline{\mathbf{CH_2CH_2CH_2}}CHNH$). ***^{13}C-NMR*** (δ, ppm, DMSO-d_6): 15.1 ($CH_2CH_2\underline{\mathbf{C}}H_2CH_2CH$), 27.7 ($CH_2\underline{\mathbf{C}}H_2CH_2CH_2CH$), 28.3 ($CH_2CH_2CH_2\underline{\mathbf{C}}H_2CH$), 29.6 $N\underline{\mathbf{C}}H_3$, 39.8 ($NHCH_2CH_2CH_2CH_2\underline{\mathbf{C}}HNH$), 43.5 ($3NH$-$\underline{\mathbf{C}}H_2$), 51.7 ($2NCH_3\underline{\mathbf{C}}H_2$)126.7, 125.9 (4C, aromatic C_2, C_3, C_5, C_6), 129.02 (2C, aromatic C_1, aromatic C_4), 170.3 (6C, $4NH\underline{\mathbf{C}}$=O, 2 $NCH_3\underline{\mathbf{C}}O$), 171.1 ($\underline{\mathbf{C}}$=OOH). MS (EI, 70 eV): m/z (%) = 531(M^+ -1, 39.33%), 461(41.57%), 171(3.75%), 166(45.32%), 143(39.33%), 141(38.58%), 123(44.57%), 102(45.32%), 97(32.96%), 87(33.33%), 75(43.45%), 64(100%), 57(15.36%), 52(41.57%). Molecular formula (M.wt.), $C_{24}H_{32}N_6O_8$ (532.5), Calculated analysis; C: 54.13, H: 6.06, N: 15.78, Found; C 54.03, H 6.01, N 15.65.

1.1.7. Synthesis of Cyclo-(N^α-1, 4-benzenedicarbonyl)-*bis*-[dipeptide]-L-Lys-$NHNH_2$; (1, 4-benzenedicarbonyl cyclic pentapeptide hydrazides); (13 and 14)

To a stirred methanolic solution (50ml) of the corresponding cyclic pentapeptide methyl esters (**9** and **10**) (1 mmol), anhydrous hydrazine hydrate (0.35ml, 10 mmol) was added. The reaction mixture was refluxed for 3 hours, after which the solvent was evaporated. The obtained residue was triturated with ether, filtered off and crystallized from methanol/ether to afford the corresponding cyclic hydrazides **(13** and **14)**.

13: Yield: 53; m.p. 130-133 ^{0}C. Rf x100 (solvent system) 75 (S); [α] : -3.8 (C, 0.02, MeOH). IR (cm^{-1}): (KBr): 3296(NH stretching), 3065(CH aromatic), 22933(CH aliphatic), 1693(C=O Hyd.), 1648, 1538 and 1448(C=O amide I, amide II and amide III). MS (EI, 70 eV): m/z (%) = 698(M^+. 0.97%), 622(4.40), 566(5.07%), 500(3.58%).451(7.97%), 420(14.32%), 352(13.65%), 346(11.75%), 316(11.01%), 300 (11.22%), 278(10.38%), 225(23.99%), 192(100%), 173(10.67%), 148(12.38), 110(6.26%), 77(6.42%), 62(4.95%), 55(5.24), 51(1.10%). Molecular formula (M.wt.), $C_{36}H_{42}N_8O_7$ (698.8), Calculated analysis; C: 61.88, H: 6.06, N: 16.04, Found; C 61.83, H 5.95, N 15.98.

14: Yield: 58; m.p. oily ^{0}C. Rf x100 (solvent system) 65 (S); [α] :-5.6 (C, 0.02, MeOH). IR (cm^{-1}): (KBr): 3384(NH stretching), 2943(CH aromatic), 2835(CH aliphatic), 1682(C=O Hyd.), 1453 and 1236(C=O amide I and amide II). ^{1}H-NMR (500 MHz, δ, ppm, DMSO-d_6): δ : 8.62 (s, 1H, $CO\underline{\mathbf{NH}}NH_2$), 817-7.87 (m, 4H, aromatic H), 7.57-7.40(s, 6H, 6NH, D_2O exchangeable)(sec. amide), 4.41 (t, 1H, $NHCH_2CH_2CH_2CH_2\underline{\mathbf{CH}}NH$) (methino), 4.28 (s , 8H, $4CH_2$ ($2NH\underline{\mathbf{CH_2}}CO$, $2NCH3\underline{\mathbf{CH_2}}CO$)) (methylene), 4.09 (s, 2H, $CONH\underline{\mathbf{NH_2}}$), 3.58, 3.54 (s, 6H, $2CH_3$, $N\underline{\mathbf{CH_3}}$), 3.44, 3.38 (m, 2H,$NH\underline{CH_2}CH_2CH_2CH_2$-CHNH) (methylene), 1.94- 1.10 (m , 6H, 3CH2, $NHCH_2\underline{\mathbf{CH_2CH_2CH_2}}CHNH$). ***^{13}C-NMR*** (δ, ppm, DMSO-d_6): 22.8 ($NHCH_2CH_2\underline{\mathbf{C}}H_2CH_2CHNH$), 29.2 ($NHCH_2\underline{\mathbf{C}}H_2CH_2$-$CH_2CHNH$), 31 ($NHCH_2CH_2CH_2$-$\underline{\mathbf{C}}H_2CHNH$), 34.8, 34.7 ($2N\underline{\mathbf{C}}H_3CH_2$), 38.8, ($NH\underline{\mathbf{C}}H_2CH_2CH_2CH_2$-CHNH), 47.5, 47.4 ($2NH\underline{\mathbf{C}}H_2$-CO), 51.5, 48.4 ($2NCH_3\underline{\mathbf{C}}H_2$), 60.4 ($NHCH_2CH_2$-$CH_2CH_2\underline{\mathbf{C}}HNH$), 127.4, 127.3, 126.3 (aromatic 6C), 157.8, 157.6 ($CONHCH_2$), 170 ($2NCH_3\underline{\mathbf{C}}O$), 172 ($\underline{\mathbf{C}}ONHNH_2$), 173.8 ($2CH_2\underline{\mathbf{C}}ONH$). MS (EI, 70 eV): m/z (%) = 548(M^+ +1, 90.98%), 236(86.06%), 222 (83.61%), 199(95.08%), 185(35.25%), 184(86.07%), 174(84.43%), 148(86.07%), 145(99.18%), 130 (83.61%), 124(92.62%), 117(92.62%), 92(99.18%), 88(86.07%), 76(100%), 75(86.89%), 70(25.41%), 58 (9.02%). Molecular formula (M.wt.), $C_{24}H_{34}N_8O_7$ (546.6), Calculated analysis; C: 52.74, H: 6.27, N: 20.50, Found; C 62.70, H 6.26, N 20.42.

2.2. In-vitro cytototoxic activity against some selected human cancer cell lines

The potential cytotoxicity of the cylcopentapeptides, (**9 – 14)** was determined according to the colorimetric method, described by Skehan et al., for anticancer, "Drug Screening" [38].

In total, seven human cancer cell lines, namely, breast (MCF-7), liver (HEPG2), colon (CaCo-2), prostate (PC-3), cervical (HeLa), larynx (Hep-2) and breast (T47D) cancer cells were used. Two common reference anticancer drugs Doxorubicin and Tamoxifen were used as positive controls. A 96-well micro titer plate was handled using ELISA-reader Sunrise-TECAN. Sulforhodamine® (SRB) was used as the staining dye.

Cancer cells were placed in a 96-well micro-titer plate (10^4 cells/ well) for 24 hours before treatment with the candidates, to allow attachment of cells to the wall of the plate. Different concentrations of the compounds under test (0, 10, 25, 50 and 100 µg/ml) were added to the cell monolayer triplicates wells, (three wells for each dose). Monolayer cells were incubated with the compounds for 48 hours at 37 ^{0}C and in atmosphere of 5% CO_2. After 48 hrs, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain. After 48 hrs, cells were fixed, washed and stained with

10% trichloroacetic acid (to allow attachment of the cells to the wall of the plates). Cells were then washed with water to remove the tri-chloroacetic acid, growth medium, low-molecular weight metabolites and serum proteins, and were finally air dried.

Cells were stained for 30 minutes with 0.4 % (wt/vol, SRB, 1% acetic acid solution). At the end of the staining period, the dye was removed and cultures were quickly rinsed 4 times with 1% acetic acid solution, to remove the unbound dye. Bound dye was recovered by solubilization with Tris / EDTA (ethylene diamine tetra-acetic acid buffer). Color intensity was then, measured in an ELISA reader. For a specified candidate, the relation between surviving fraction and drug concentration was plotted to get the survival curve of each cancer cell line.

IC_{50} (the concentration of the compound needed to kill 50% of the initial cells) was determined for each compound. Compounds with no practical IC_{50} (> 100 µg/ml) were considered as inactive. All results were expressed throughout as means +/- S.E.M.

3. Results and discussion

3.1. Chemistry

We have successfully synthesized, chemically characterized, and biologically evaluated many bis-amino acid and peptide conjugates of N-isophthaloyl acid, N-phthaloyl acid, and 2,6-dipicolinic acid in our earlier research [24–37]. Here, a molecular structural comparison was carried out using 1,4-benzene dicarboxylic acid bridging pentapeptide analogues as an extrapolation of the realized anticancer outcomes.

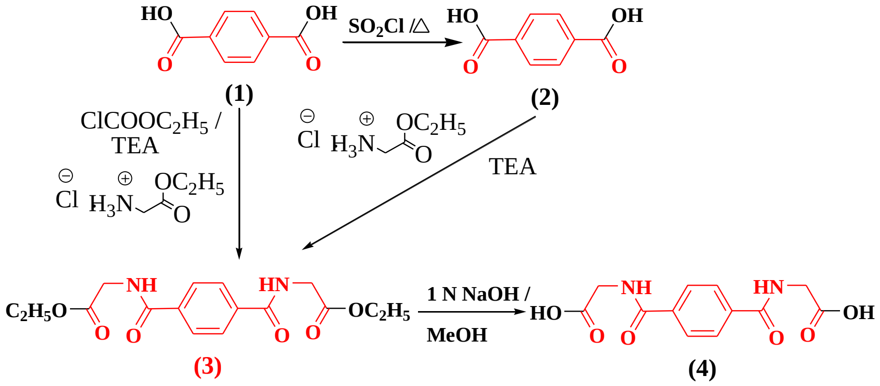
In a synthetic process, the initial linear tetra peptide bis-esters, (**5** and **6**), were hydrolyzed to yield the corresponding free acids (**7** and **8**), and then they were cyclized with L-lysine methyl ester to yield the cyclopeptide esters (**9** and **10**), respectively. These cyclopeptide esters underwent hydrolysis or hydrazinolysis, yielding the cyclopeptide acids (**11** and **12**) or hydrazides (**13** and **14**).

Thus, Cyclo-[N^{α}-1, 4-benzenedicarbonyl-*bis*-(Gly-L-Phe)-L–Lys]-OMe, **9**, Cyclo-(N^{α}-1, 4-benzenedicarbonyl-*bis*-(Gly-Gly)-L-Lys]-OMe, **10**, Cyclo-[N^{α}-1, 4-benzenedicarbonyl-*bis*-(Gly-L-Phe)-L-Lys]-OH, **11**, Cyclo-[N^{α}-1, 4-benzenedicarbonyl-*bis*-(Gly-Gly)-L-Lys]-OH, **12**, Cyclo-[N^{α}-1, 4-benzenedicarbonyl-*bis*-(Gly-L-Phe)-L-Lys]-$NHNH_2$, **13**, Cyclo-[N^{α}-1, 4-benzenedicarbonyl-*bis*-(Gly-Gly)-L-Lys]-$NHNH_2$ **14**, were rendered available, *via* conventional peptide synthesis, in solution.

Based on the acylation of glycine ester with 1, 4-benzenedicarbonyl dichloride **2**, which was created by converting 1, 4-benzenedicarboxylic acid **1** by its interaction with thionyl chloride, Nα-1, 4-benzenedicarbonyl-bis-(Glycine ethyl ester) **3** was obtained. Then the ester and acid chloride were combined at a low temperature while trimethylamine was present as an organic base. In the same way, Nα-1, 4-benzenedicarboxylic acid **1**, and glycine ethyl ester were produced, with ethyl-chloroformate acting as a mixed anhydride partner, to yield bis-Gly ethyl ester **3**. Nα-1, 4-benzenedicarbonyl-bis-(Glycine) **4** was obtained by hydrolyzing **3** with methanolic NaOH, Scheme 1.

In addition to the ester group, the presence of an aromatic ring, aliphatic hydrogens, and an amide linkage was confirmed by the IR spectrum of **3**. The two classic distinctive infrared bands of the amide, amide I and II, located in the areas of 1660 and 1555 cm^{-1}, respectively, proved the amide linkage. The strong band in the areas 1766 cm-1 (ν C=O, ester) confirmed the presence of the ester group. Furthermore, the absorption band detected at 3485 cm^{-1} is ascribed to ν NH, or hydrogen bound amide. Furthermore, the (ν C=O, ester) was absent from the IR spectrum of **4**, and a band corresponding to (ν C=O, acid) appeared at 1675 cm-1, Scheme 1.

Treatment of **4** with free amino acid esters namely, L-Phenylalanine methyl ester or Glycine ethyl ester, in the presence of ethyl chloroformate, afforded the corresponding N^{α}-1, 4-benzenedicarbonyl-*bis*-(dipeptide)-esters, (**5** and **6**). Hydrolysis with ethanolic sodium hydroxide afforded the corresponding N^{α}-1, 4-benzenedicarbonyl-*bis*-(dipeptides), (**7** and **8**) (Scheme 2).



Scheme 1. Synthetic routes for N^{α}-1, 4-benzenedicarbonyl-*bis*-(Glycine ethyl ester) **3** and the corresponding carboxylic acid **4**. Red color represents 1,4-benzenedicarbonyl-*bis*-Glycine.

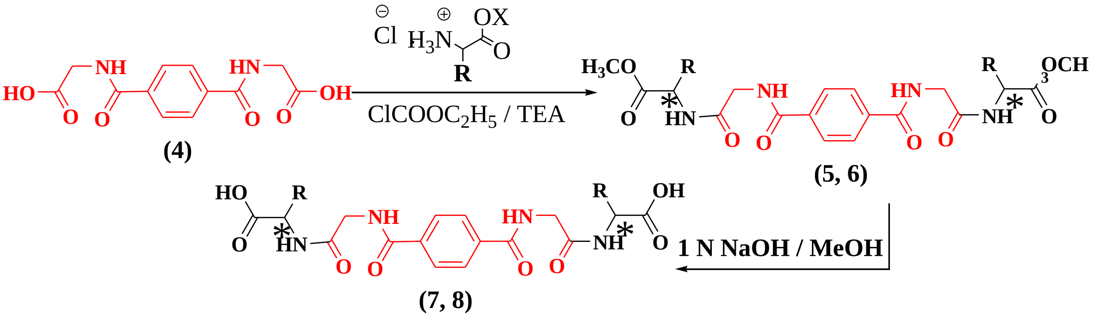
The presence of an aromatic ring, aliphatic hydrogens, and an amide linkage in addition to the ester groups was confirmed by the infrared spectra of compounds **5** and **6**. Its three distinctive infrared bands (1749, 1642, and 1543 cm^{-1}, representing amide I, II, and III, respectively) verified the amide linkage. The ester group's band in the areas of 1752 cm^{-1} (ν C=O, ester) confirms its presence, Scheme 2.

Furthermore, an absorption band at 3318 cm^{-1} was detected, which was identified as hydrogen-bonded NH amide. Likewise, the infrared spectra of **7** and **8** revealed the presence of a band at 1749 cm^{-1} (ν C=O, acid) in place of (ν C=O, ester), Scheme 2.

Cyclization of the *bis*-dipeptides **7** and **8** with L-lysine methyl ester, *via* different peptide coupling methods, afforded the corresponding cyclopentapeptide esters, (**9** and **10**), respectively, with acceptable yield and chemical purity (Scheme 3).

Ultimately, the methyl groups of the cylcopentapeptides' L-Lys-OMe esters (9 and 10) were changed into hydrazide or carboxylic acid functionalities. Consequently, the corresponding acid analogues were obtained by hydrolyzing the cyclopentapeptide methyl esters (9 and 10) with 1N methanolic NaOH (**11** and **12**). Equally, hydrazinolysis of cyclopentapeptide methyl esters, (**9** and **10**), respectively, with methanolic hydrazine hydrate, afforded the corresponding cyclopentapeptide hydrazides, (**13** and **14**), Scheme 3.

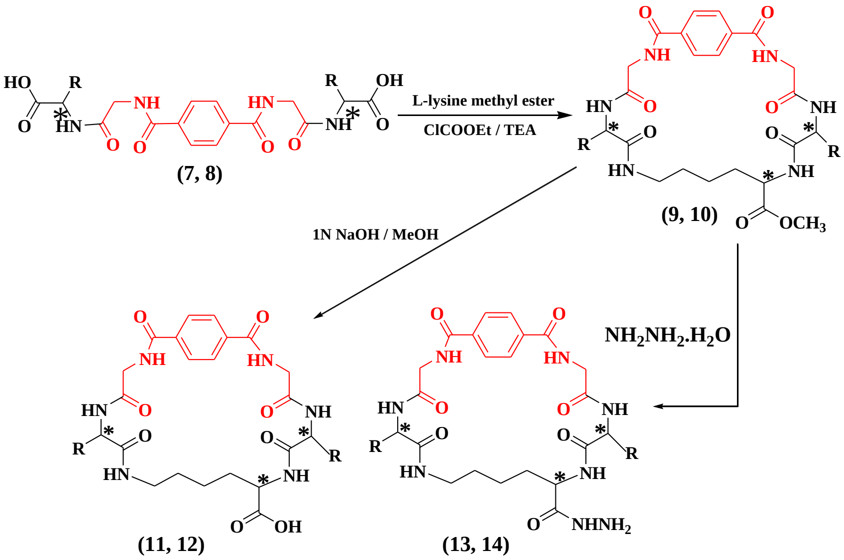
The infrared spectra of samples 11 and 12 revealed the existence of a band at 1706 cm^{-1} for (μ C=O, acid) and the absence of (ν C=O, ester). The amide and hydrazide groups' NH vibrations were visible in the IR spectra of compounds **13** and **14**, where they were centered at 3435 cm^{-1}, Scheme 3.



[(5, 7): R= CH_2Phe]; [(6, 8): R= H]

5: N^{α}-1, 4-benzenedicarbonyl-bis-(Gly-L-Phe)-OMe; **7**: N^{α}--1, 4-benzenedicarbonyl-bis-(Gly-L-Phe)-OH
6: N^{α}-1, 4-benzenedicarbonyl-bis-(Gly-Gly)-OMe; **8**: N^{α}-1, 4-benzenedicarbonyl-bis-(Gly-Gly)-OH

Scheme 2. Synthetic routes for compounds **5 - 8**. Red color represents 1,4-benzenedicarbonyl-*bis*-Glycine.



[(9, 11, 13): R= CH_2Phe]; [(10, 12, 14): R= H]

9: Cyclo-(N^{α} -1, 4-benzenedicarbonyl-bis-[Gly-L-Phe]-L-Lys)-OMe
10: Cyclo-(N^{α} -1, 4-benzenedicarbonyl-bis-[Gly-GLy]-L-Lys)-OMe
11: Cyclo-(N^{α} -1, 4-benzenedicarbonyl-bis-[Gly-L-Phe]-L-Lys)-OH
12: Cyclo-(N^{α} -1, 4-benzenedicarbonyl-bis-[GlyGLy]-L-Lys)-OH
13: Cyclo-(N^{α} -1, 4-benzenedicarbonyl-bis-[Gly-L-Phe]-L-Lys)-$NHNH_2$
14: Cyclo-(N^{α} -1, 4-benzenedicarbonyl-bis-[Gly-GLy]-L-Lys)-$NHNH_2$

Scheme 3. Synthetic routes for compounds **9–14.** Red color represents 1,4-benzenedicarbonyl-*bis*-Glycine.

3.2. Cytotoxic investigations

Determination of the potential cytotoxicity data (IC_{50}) for the cyclopeptides (**9-14**) was realized, by the Egyptian National Cancer Institute, Cairo, Egypt. The cytotoxic activity of the six compounds **9–14** was assessed using two human cancer cell lines: breast (MCF-7) and liver (HEPG2). Two reference drugs, Tamoxifen and Doxorubicin were, concomitantly assayed. IC_{50} (the concentration of the compound needed to kill 50% of the initial cells) was determined for each compound. The results are shown in table 1.

Table 1. IC_{50} of the tested compounds 9-14 against the MCF-7 and HEPG-2 cell lines

	IC_{50} (µg/ml)	
Compound	MCF-7	HEPG-2
9	4.04	2.82
10	16.4	11.7
11	20.3	20.5
12	6.02	10.1
13	19.1	17.2
14	10.9	15.5
Tamoxifen	8.31	10.9
Doxorubicin	2.97	3.73

All compounds showed concentration-dependent effects on both cell lines. Interestingly, compound **9** showed potent activity against both cancer cell lines, being almost twice more potent and 3.87 times more potent than Tamoxifen in MCF-7 and HEPG-2 cell lines respectively, while being almost 75% as potent as Doxorubicin and 1.3 times more potent than the same control in both MCF-7 and HEPG-2 cell lines respectively. On the other hand, compound **12** showed moderate activity against both cell lines, being more potent than Tamoxifen in MCF-7 cell line, as potent as Tamoxifen in HEPG-2 cell line, but less effective than Doxorubicin in both cell lines. The remaining compounds **10, 11 13** and **14** displayed weak to moderate activity against both cell lines, with compounds **11** and **13** being the least effective, suggesting than replacing the methyl group in compound **9** with either an acid group or a hydrazine hydrate group had a significant negative impact on biological activity.

Based on the promising results obtained with compound **9**, additional five cell lines, namely colon (CaCo-2), prostate (PC-3), cervical (HeLa), larynx (Hep-2) and breast (T47D) cancer cells were used for further investigation of the antiproliferative activity of this cyclic pentapeptide. Table 2 summarizes all the IC_{50} values for compound **9** in all seven cancer cell lines, using Doxorubicin as a reference positive control drug. For comparison purposes between compound 9 and doxorubicin, the % relative cytotoxicity was obtained from the formula:

% Relative Cytotoxicity =

[100 - (IC_{50} cyclopeptide candidate) × 100] / [100 - (IC_{50} reference drug)].

Table 2. IC_{50} of compound 9 compared to Doxorubicin reference drug against several cancer cell lines.

Cancer cell line	Compound 9 IC_{50} (µg/ml)	Doxorubicin IC_{50} (µg/ml)	% Relative Cytotoxicity
MCF-7	4.04	2.97	98
HEPG-2	2.82	3.73	101
CaCo-2	4.43	3.58	99.6
PC-3	3.8	4.8	101
Hela	3.4	4.2	100.8
Hep-2	8.4	3.73	95
T47D	3.38	7.8	104.8

Compound **9** was found to be more potent than doxorubicin in three cell lines, (almost 2.5 times inT47D, 1.2 times in HeLa and PC-3 cell lines), and almost as potent in CaCo-2 cell line, consequently representing a very promising anticancer candidate. Overall, the values of relative cytotoxicity show that compound 9 surpasses the potency of the reference Doxorubicin in four cell lines out of the seven tested, is almost as potent in a fifth one, and is only less effective in two cell lines but still with significant potency.

4. Conclusion

The goal of this work was to synthesize novel cyclic pentapeptides based on1,4-benzenedicarbonyl chloride. Compound 9, namely Cyclo-[N^{α}-1, 4-benzenedicarbonyl-bis-(Gly-L-Phe)-L–Lys]-OMe, showed exceptionnally high potent anticancer activity in all the cell lines tested for cytotoxic activity.Assessment of its possible mechanism of action as multi-targeted kinase inhibitor will be investigated. We suggest, based on our previous work in macrocyclic peptide-based compounds, performing molecular docking studies within the active site of a kinase inhibitor in order to better understand the potential binding mode and possible interactions. Additionnally, profound biological investigations, including inhibitory evaluations against several kinase enzymes, seem worthy to be realized in the near future.

5. Conflict of interest

The authors declare that they have no confict of interest.

6. Acknowledgements

The authors express their great gratitude to the project fund received from the National Research Centre (NRC, Cairo, Egypt) for the internal grant (No. 13010105).

7. References

1. International Agency for Research on Cancer, World health Organization, 2023
2. Marqus S., Pirogova E. and Piva T.J., Evaluation of the use of therapeutic peptides for cancer treatment. *J. Biomed. Sci*., 24(21), 1-15 (2017)
3. Debela, D. T., Muzazu, S. G., Heraro, K. D., Ndalama, M. T., Mesele, B. W., Haile, D. C., ... & Manyazewal, T. (2021). New approaches and procedures for cancer treatment: Current perspectives. *SAGE open medicine*, *9*, 20503121211034366.
4. Sharma, P., Jhawat, V., Mathur, P., & Dutt, R. (2022). Innovation in cancer therapeutics and regulatory perspectives. *Medical Oncology*, *39*(5), 76.
5. McGregor D.P., Discovering and improving novel therapeutics. Curr. Opin. Pharmacol., 8(5), 616-619 (2008).
6. Renu, K., Pureti, L. P., Vellingiri, B., & Valsala Gopalakrishnan, A. (2022). Toxic effects and molecular mechanism of doxorubicin on different organs–an update. *Toxin Reviews*, *41*(2), 650-674.
7. Shi, S., Chen, Y., Luo, Z., Nie, G., & Dai, Y. (2023). Role of oxidative stress and inflammation-related signaling pathways in doxorubicin-induced cardiomyopathy. *Cell Communication and Signaling*, *21*(1), 1-20.
8. Cicero A.F.G., Fogacci F. and Colletti A., Potential role of bioactive peptides in prevention and treatment of chronic diseases: a narrative review. Br. J. Pharmacol. 174(11),1378-1394 (2017).
9. Moreno-Aspitia, A., & Perez, E. A. (2009, June). Treatment options for breast cancer resistant to anthracycline and taxane. In *Mayo Clinic Proceedings* (Vol. 84, No. 6, pp. 533-545). Elsevier.
10. Edwardson, D., Chewchuk, S., & Parissenti, A. M. (2013). Resistance to anthracyclines and taxanes in breast cancer. *Breast Cancer Metastasis and Drug Resistance: Progress and Prospects*, 227-247.
11. Mason, J. M. (2010). Design and development of peptides and peptide mimetics as antagonists for therapeutic intervention. *Future medicinal chemistry*, *2*(12), 1813-1822.
12. Lee, A. C. L., Harris, J. L., Khanna, K. K., & Hong, J. H. (2019). A comprehensive review on current advances in peptide drug development and design. *International journal of molecular sciences*, *20*(10), 2383.
13. Blanco-Miguez A., Gutierrez-Jacome A., Perez-Perez M., Perez-Rodriguez G., Catalan-Garcia S., fdez-Riverola F., Loureco A. and Sanchez B.,From amino acid sequence to bioactivity: The biomedical potential of antitumor peptides. Protein Sci., 25 (6), 1084-1095 (2016).
14. Shinde, S. D., Atpadkar, P., Swain, P., Apparao, C. V., Sandhya, V., & Sahu, B. (2024). Peptide and protein in therapeutics. In *Peptide and Protein Drug Delivery Using Polysaccharides* (pp. 1-24). Academic Press.
15. Lamers, C. (2022). Overcoming the shortcomings of peptide-based therapeutics. *Future Drug Discovery*, *4*(2), FDD75.
16. Marqus, S., Pirogova, E., & Piva, T. J. (2017). Evaluation of the use of therapeutic peptides for cancer treatment. *Journal of biomedical science*, *24*(1), 1-15.
17. Yavari, B., Mahjub, R., Saidijam, M., Raigani, M., & Soleimani, M. (2018). The potential use of peptides in cancer treatment. *Current Protein and Peptide Science*, *19*(8), 759-770.
18. Lau J.L. and Dunn M.K., Therapeutic peptides: Historical perspectives, current development trends, and future directions. Bioorganic & Medicinal Chemistry, 26(10), 2700-2707 (2018).
19. Haggag, Y. A., Donia, A. A., Osman, M. A., & El-Gizawy, S. A. (2018). Peptides as drug candidates: limitations and recent development perspectives. *Biomed J*, *1*(3).
20. Fosgerau, K., & Hoffmann, T. (2015). Peptide therapeutics: current status and future directions. *Drug discovery today*, *20*(1), 122-128.
21. Diao, L., & Meibohm, B. (2013). Pharmacokinetics and pharmacokinetic–pharmacodynamic correlations of therapeutic peptides. *Clinical pharmacokinetics*, *52*, 855-868.
22. Feliu L., Oliveras G., Cirac A., Besalu E., Roses C., Colomer R., Bardaji E., Planas M. and Puig T., Antimicrobial cyclic decapeptides with anticancer activity, Peptides, 31(11),2017-2026 (2010).
23. Gaspar D., Veiga A.S. and Castanho M.A.R.B., From antimicrobial to anticancer peptides. A review., Front. Microbiol., 4, 294 (2013).
24. Moustafa G.O., El-Sawy A.A. and Abo-Ghalia M.H., Synthesis of Novel Cyclopeptide Candidates: I-Cyclo-[N^{α}-isophthaloyl-bis-Glycine-Amino Acid-L-Lysine] Derivatives with Expected Anticancer Activity, Egypt. J. Chem., 56(5),473-494 (2013).
25. Amr A.E.E., Abo-Ghalia M.H., Moustafa G.O., Al-Omar M.A., Nossier E.S. and Elsayed E.A., Design, synthesis and docking studies of novel mac®ocyclic pentapeptides as anticancer multi-targeted kinase inhibitors., Molecules, 23, 2416 (2018).

26. Moustafa G.O., Younis A., Al-Yousef S.A. and Mahmoud S.Y., Design, synthesis of novel cyclic pentapeptide derivatives based on 1,2-benzene dicarbonyl chloride with expected anticancer activity, J. Comput. Theor. Nanosci., 16 (5-6),1733-1739 (2019).
27. F.H. Mohamed, A.M. Shalaby, H. A. Soliman, A.Z. Abdelazem;,M.M. Mounier, E.S. Nossier and G.O.Moustafa. Design, Synthesis and Molecular Docking Studies of Novel Cyclic Pentapeptides Based on Phthaloyl Chloride with Expected Anticancer Activity. Egypt. J. Chem., 63, 1723-1736 (2020).
28. M.H. Abo-Ghalia, G. O. Moustafa, A. E. Amr, A.M. Naglah, E.A.Elsayed and A. H. Bakheit. Anticancer Activities of Newly Synthesized Chiral Macrocyclic Heptapeptide Candidates. Molecules, 25,1253 (2020).
29. Kalmouch, A.; Radwan, M.A.A.; Omran, M.M.; Sharaky, M.; Moustafa, G.O. Synthesis of novel 2, 3'-bipyrrole derivatives from chalcone and amino acids as antitumor agents. Egypt. J. Chem. 2020, 63, 11, 4409 – 4421
30. Moustafa, G.O., Al-Wasidi, A.S., Naglah, A.M., Refat, M.S. Isolation and Synthesis of Dibenzofuran Derivatives Possessing Anticancer Activities: A Review. Egyptian Journal of Chemistry, 2020, 63 (6), 2355-2367.
31. Elsherif, M.A.; Hassan, A.S.; Moustafa, G.O.; Awad, H.M.; Morsy, N.M., Antimicrobial Evaluation and Molecular Properties Prediction of Pyrazolines Incorporating Benzofuran and Pyrazole Moieties. J Appl Pharm Sci, 2020, 10 (02), 037-043.
32. Hassan, A.S.; Moustafa, G.O.; Morsy, N.M.; Abdou, A.M.; Hafez, T.S. Design, synthesis and antibacterial activity of N-aryl-3-(arylamino)-5-(((5-substituted furan-2-yl)methylene)amino)-1H-pyrazole-4-carboxamide as Nitrofurantoin® analogues. Egypt. J. Chem. 2020, 63, 11, 4485 - 4497.
33. Khalaf, H.S., Naglah, A.M., Al-Omar, M.A., Moustafa, G.O.; Awad, H.M., Bakheit, A.H. Synthesis, docking, computational studies, and antimicrobial evaluations of new dipeptide derivatives based on nicotinoylglycylglycine hydrazide. Molecules, 2020, 25 (16), 3589.
34. Naglah, A.M., Moustafa, G.O., Elhenawy, A.A., Mounier, M.M., El-Sayed, H.,. Al-Omar, M.A Almehizia, A.A., Bhat, M.A. Nα-1, 3-Benzenedicarbonyl-bis-(Amino Acid) and Dipeptide Candidates: Synthesis, Cytotoxic, Antimicrobial, Antifungal and Molecular Docking Investigation. Drug Design, Development and Therapy, 2021, 15, 1315-1332.
35. Moustafa, G.O., Therapeutic Potentials of Cyclic Peptides as Promising Anticancer Drugs. Egyptian Journal of Chemistry, 2021, 64 (4), 1777-1787.
36. Eman A. Abd El-Meguid, Gaber O. Moustafa, Hanem M. Awad, Eman R. Zaki, Eman S. Nossier, Novel benzothiazole hybrids targeting EGFR: Design, synthesis, biological evaluation and molecular docking studies, journal of molecular structure, 2021, 1240, 130595
37. Abd El-Meguid, E. A., Naglah, A. M., Moustafa, G. O., Awad, H. M. and El Kerdawy, A. M. Novel benzothiazole-based dual VEGFR-2/EGFR inhibitors targeting breast and liver cancers: Synthesis, cytotoxic activity, QSAR and molecular docking studies. Bioorganic & Medicinal Chemistry Letters, 2022, 58, 128529.
38. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J.T.; Bokesch, H.; Kenney, S. and Boyd, M.R.; J. Natl.Can. Ins., 82, 1107 (1990).