Research Article

Synthesis of New Bis-Phthalimide and Thalidomide Ester Derivatives, and Evaluation of their Cytotoxic Activity

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ABSTRACT

The esterification reaction of trimellitic anhydride chloride TMAC 1 with N-(hydroxymethyl) phthalimide NHMP 2, N-(2-hydroxyethyl) phthalimide NHEP 3 or N-(hydroxymethyl) thalidomide NHMT 4 afforded novel cyclic imide-ester derivatives; phthaloyl-phthalic anhydride methyl ester PPAME 5, phthaloyl-phthalic anhydride ethyl ester PPAEE 6 and thalidomide-phthalic anhydride methyl ester TPAME 7, respectively. These novel derivatives appeared very useful to prepare number of new hybrid structure of substituted N, N-bis-phthalimide and phthalimide-thalidomide moieties with different amino acids. The cytotoxicity of all synthesized compounds were *in vitro* evaluated against Hep-G2 and MCF-7, which showed that phthalimide derivatives 6, 9b, 10d, 11 and 12 possess significant antitumor potency.

Keywords: Amino acids, Antitumor, Phthalimide, Thalidomide, Cytotoxicity.

INTRODUCTION

Cancer is a leading cause of death worldwide, so treatment of cancer becomes a global challenge and an urgent need, the use of chemotherapy which targeting tumor progress is one of the most common techniques. The need of highly effective drug with low side effects, and inexpensive is vital and important in order to protect millions of patients around the world from certain death.

Manufacturing biologically active drugs is task expensive, which forced the generic manufacturers for the discovery of new drugs based on their economic value.[1] Thalidomide is a sedative simple teratogenic molecule with a wide range of biological activities, it is considered as a cornerstone for producing new biologically active compounds.[2,3] Thalidomide is used as racemic mixture and its mechanism of action was recently described in details.[4] Thalidomide has a broad spectrum of bioactivity i.e., anti-cachexia, anti-inflammatory, antitumor, antiviral, inhibitor of TNF-α, NFκB regulator, anxiolytic, anticonvulsant, against Whipple disease, treatment of type 2 leprosy reactions, treatment of various hematologic malignancies, solid tumors such as breast, colon and brain, prostate cancer, remission of advanced hepatocellular carcinoma in addition to autoimmune diseases.[2,5-23] Thalidomide has been used along with its analogues for the treatment of various diseases such as HIV-related ulcers, multiple myeloma, Kaposi's sarcoma etc.[24] The structure activity correlations of thalidomide analogues and their metabolites suggested that phthalimide is a fundamental pharmacophoric moiety.[2]

Phthalimides are compounds with a wide range of interesting applications, as it contains an imide ring [-CO-N(R)-CO-], which possesses hydrophobic and neutral characters, and helping it to cross the biological membranes *in vivo*.[25] Compounds containing the phthalimide moiety have been examined for their biological activity and proved to have anti-inflammatory,[26] antimicrobial,[27] antitumor,[28] antivirus,[29] angiogenesis inhibitor properties[30] and α-glucosidase inhibitors.[31] Moreover, various *N*-phenyl-phthalimides were reported to possess enhanced activity over thalidomide.[18,32-34] Interest in amino acids has increased enormously during recent years, mainly because of the application of these compounds in the nutritional and medicinal fields. The suitable merging for simple biologically active organic molecules together may results in producing of new molecules more effective, cheaper and safer for their use as a drug.[1,35,36]

In continuation of our strategic research program to develop several antitumor heterocyclic compounds,[37,38] based on our prior findings for the antitumor activity of thalidomide derivatives,[39-43] and following this line of our research on a series of thalidomide and phthalimide ester analogues linked to various biologically potent carboxylic acids,[44] the presented work illustrated the synthesis of novel thalidomide derivatives. Molecular modification via hybridization was applied to produce novel cyclic imide-ester hybrid structure of bis-phthalimide as well as phthalimide-thalidomide moieties with different amino acids which might be applicable as antitumor agents with potent activity and low toxicity.

MATERIALS AND METHODS

Chemistry

The progress of all reactions and synthesized product were monitored via analytical silica gel TLC plates 60 F254 (Merck), spots were located by UV light.1H- and 13C-NMR spectra for all synthesized compounds were

recorded on a JEOL JNM-AL-400MHzfor 1H- and 101 MHz for 13C- NMR, with TMS as an internal standard, Chemical shifts are reported in parts per million (ppm) relative to the respective deuterated solvent peak DMSO-d6 (δ 2.50 ppm) for 1H and (δ 39. 51 ppm) for 13C. MALDI mass spectral data of all compounds were obtained using JEOL JMS-700N for electron ionization and/or on JEOL JMS-T100TD for ESI using α-cyano- 4-hydroxy-cinnamic acid (CHCA) as a matrix (m/z 189.17) (Nagasaki University-Japan). Electrospray ionization high resolution mass spectra (ESIHRMS) were performed on PE SCIEX API Q-Star Pulsar Mass Spectrometer. For accurate ion mass determinations, the [MH^+] or [MNa^+] ion was peak matched by calibration with NaI (University of Southern Denmark-Denmark). IR spectra were recorded (KBr) on a Pye-Unicam Sp-883 Perkins-Elmer spectrometer at Cairo University. Melting points were determined on a Büchi melting point apparatus and uncorrected. Solvents were distilled prior to use, while reagents used as purchased.

General synthetic procedure for the preparation of compounds 5, 6 and 7

A mixture of 1,3-dioxo-1,3-dihydroisobenzofuran-5-carbonyl chloride **1** (1.05 g, 5 mmol) and 2-(hydroxymethyl) isoindoline-1,3-dione **2** (0.89 g, 5 mmol) or 2-(2-hydroxyethyl)isoindoline-1,3-dione **3** (0.96 g, 5 mmol) or 3-(1,3-dioxo-2,3-dihydro-1H-inden-2-yl)piperidine-2,6-dione **4** (1.06 g, 5 mmol) was dissolved in 10 ml of dry pyridine and stirred for 10 h at room temperature. The precipitated solid was filtered off, washed with diethyl ether, dried and recrystallized from toluene to yield the anhydride products **[5-7]** as white powder.

(1,3-Dioxoisoindolin-2-yl)methyl-1,3-dioxo-1,3-dihydroisobenzofuran-5-carboxylate (5)

Crystallized from Toluene as a White powder, m. p. 238-240°C, yield 71%, 1.25 g. Analysis: IR (KBr, υ_{max} cm^{-1}): 3492 ($C=O_{overtone}$), 1856, 1776 ($C=O_{anhydride}$), 1733 ($C=O_{ester}$), 1600 ($C=C_{aryl}$). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 5.89 (s, 2H, NCH_2O), 7.88-7.96 (m, 4H, aryl), 8.16 (d, 1H, J = 8 *Hz*, aryl), 8.42 (s, 1H, aryl), 8.44 (d, 1H, J = 8 *Hz*, aryl). ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 61.62 (NCH_2O), 123.87, 128.95, 129.54, 130.65, 131.53, 132.21, 132.43, 135.21, 138.19 (aryl), 163.65 ($C=O_{ester}$), 166.78 (2 × $C=O_{phthalimide}$), 167.50, 168.46 (2 × $C=O_{anhydride}$).

2-(1,3-Dioxoisoindolin-2-yl)ethyl1,3-dioxo-1,3-dihydroisobenzofuran-5-carboxylate (6)

Crystallized from Toluene as a White powder, m. p. 228-230 °C, yield 72%, 1.30 g. Analysis: IR (KBr, υ_{max} cm^{-1}): 3465 ($C=O_{overtone}$), 1861, 1783 ($C=O_{anhydride}$), 1720 ($C=O_{ester}$), 1612 ($C=C_{aryl}$); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 4.00 (t, 2H, NCH_2CH_2O), 4.51 (t, 2H, NCH_2CH_2O), 7.74 (d, 1H, J = *8 Hz*, aryl), 7.81-7.88 (m, 4H, , aryl), 8.05 (d, 1H, J = *8 Hz*, aryl), 8.16 (s, 1H, aryl). ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 36.72 (NCH_2CH_2O), 62.86 (NCH_2CH_2O), 123.25, 128.81, 129.47, 131.21, 131.69, 131.84, 132.31, 134.62, 137.93 (aryl), 164.59 ($C=O_{ester}$), 167.52 ($C=O_{anhydride}$), 167.97 (2 × $C=O_{phthalimide}$), 168.51 ($C=O_{anhydride}$). HR-MALDI-MS calcd for $C_{19}H_{12}NO_7^+$ $[M+H]^+$ m/z 366.0614; found m\z 366.0610.

(3-(1,3-Dioxoisoindolin-2-yl)-2,6-dioxopiperidin-1-yl)methyl 1,3-dioxo-1,3-dihydroisobenzo furan-5-carboxylate (7)

Crystallized from Toluene as a White powder, m. p. 230-232 °C, yield 91%, 1.12 g. Analysis: IR (KBr, υ_{max} cm^{-1}): 3494 ($C=O_{overtone}$), 1860, 1788 ($C=O_{anhydride}$), 1750 ($C=O_{ester}$), 1602 ($C=C_{aryl}$). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.15-2.18 (m, 1H, H-4'), 2.59-2.67 (m, 1H, H-5'), 2.87-2.91 (m, 1H, H-4'), 3.06-3.12 (m, 1H, H-5'CO), 5.39-5.44 (m, 1H, H-3'), 5.94-6.01 (m, 2H, NCH_2O), 7.88-7.94 (m, 4H, aryl), 8.11 (d, 1H, J = 8 Hz, aryl), 8.21 (s, 1H, aryl), 8.61 (d, 1H, J = 8 Hz, aryl). ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 21.0 ($C_{4'}$21.00 ($C_{4'}$), 31.10 ($C_{5'}$), 49.52 ($C_{3'}$), 64.03 (NCH_2O), 123.68, 124.43, 128.36, 130.70, 131.36, 132.01, 135.06, 135.13, 138.17 (aryl), 163.69 ($C=O_{ester}$), 167.26 (2 × $C=O_{anhydride}$), 167.61, 168.42 (2 × $C=O_{phthalimide}$), 169.60 ($C_{2'}$), 171.42 ($C_{6'}$). HR-ESI-MS calcd for $C_{23}H_{14}N_2O_9Na^+$ $[M+Na]^+$ m\z 485.0597; found m\z 485.0579.

General synthetic procedure for synthesis of *N*-substituted anhydrides of compounds 8(a-d), 9(a-d) and 10(a-d)

A mixture of phthalimide or thalidomide esters **5-7** (0.5 mmol) with different amino acids (0.5 mmol) in 7 ml dry DMF was refluxed for 3-6 h. The reaction mixture was poured onto dilute HCl (1%), the crude products were filtered off and recrystallized from ethanol to yield the products **8[a-d], 9[a-d] and 10[a-d]**.

2-(5-(((1,3-Dioxoisoindolin-2-yl)methoxy)carbonyl)-1,3-dioxoisoindolin-2-yl)acetic acid (8a)

Crystallized from ethanol as a Pale yellow powder, m. p. 220-221 °C, yield 76%, 0.16 g. Analysis: IR (KBr, υ_{max} cm^{-1}): 3433(OH_{acid}), 1782 ($C=O_{imide}$), 1736 ($C=O_{ester}$), 1727 ($C=O_{imide}$), 1717($C=O_{acid}$), 1615 ($C=C_{aryl}$), 1403, 1375(C-N-C). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 4.28 (s, 2H, NCH_2COOH), 5.88 (s, 2H, NCH_2O), 7.88-7.95 (m, 4H, aryl), 8.03 (d, 1H, J = 8 *Hz*, aryl), 8.28 (s, 1H, aryl), 8.38 (d, 1H, J = 8 *Hz*, aryl). ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 29.54 (NCH_2COOH), 61.79 (NCH_2O), 123.79, 123.84, 124.05, 131.59, 131.99, 134.65, 135.17, 135.36, 136.07 (aryl), 163.42 ($C=O_{ester}$), 166.44, 166.52 (2 × $C=O_{phthalimide}$), 166.75 (2 × $C=O_{phthalimide}$), 168.82 ($C=O_{acid}$). HR-MALDI-MS calcd for $C_{20}H_{12}N_2O_8Na^+$ $[M+Na]^+$ m/z 431.0491; found m\z 431.0483.

(1,3-Dioxoisoindolin-2-yl)methyl2-(2-ethoxy-2-oxoethyl)-1,3-dioxoisoindoline-5-carboxylate (8b)

Crystallized from ethanol as a White powder, m. p. 181-182 °C, yield 75%, 0.17 g. Analysis: IR (KBr, υ_{max} cm^{-1}): 1786 ($C=O_{imide}$), 1735 ($C=O_{ester}$), 1730 ($C=O_{ester}$), 1727 ($C=O_{imide}$), 1616 ($C=C_{aryl}$), 1410, 1380 (C-N-C). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 1.19 (t, 3H, CH_3), 4.12-4.17 (m, 2H, OCH_2CH_3), 4.44 (s, 2H, CH_2CO), 5.91 (s, 2H, NCH_2O), 7.90-7.99 (m, 4H, aryl), 8.07 (d, 1H, J = 8 *Hz*, aryl),

8.32 (s, 1H, aryl), 8.42 (d, 1H, *J* = 8 *Hz*, aryl). ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 13.91 (CH_3), 25.31 (NCH_2CO), 61.54 (OCH_2CH_3), 61.78 (NCH_2O), 123.83, 123.92, 124.15, 131.58, 131.88, 134.83, 135.17, 136.15 (aryl), 163.38 ($C=O_{ester}$), 166.37, 166.41 (2 × $C=O_{phthalimide}$), 166.74 (2 × $C=O_{phthalimide}$), 167.48 ($C=O_{ester}$). HR-MALDI-MS calcd for $C_{22}H_{17}N_2O_8^+$ $[M+H]^+$ m/z 437.0985; found m\z 437.0970.

2-(5-(((1,3-Dioxoisoindolin-2-yl)methoxy)carbonyl)-1,3-dioxoisoindolin-2-yl)propanoic acid (8c)

Crystallized from ethanol as a White powder, m. p. 177-179 °C, yield 72%, 0.16 g. Analysis: IR (KBr, υ_{max} cm^{-1}): 3450 (OH_{acid}), 1781($C=O_{imide}$), 1735 ($C=O_{ester}$), 1727 ($C=O_{imide}$), 1718 ($C=O_{acid}$), 1615 ($C=C_{aryl}$), 1393, 1370 (C-N-C). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 1.55 (d, 3H, CH_3), 4.86-4.92 (m, 1H, NC*H*COOH), 5.91 (s, 2H, NCH_2O), 7.91-7.99 (m, 4H, aryl), 8.03 (d, 1H, *J* = 8 *Hz*, aryl), 8.29 (s, 1H, aryl), 8.40 (d, 1H, *J* = 8 *Hz*, aryl), 13.19 (br s, 1H, COOH). ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 14.67 (CH_3), 47.27 (NCHCOOH), 61.76 (NCH_2O), 123.58, 123.70, 123.84, 131.43, 131.72, 134.44, 135.04, 135.12, 135.87 (aryl), 163.25 ($C=O_{ester}$), 166.23, 166.30 (2 × $C=O_{phthalimide}$), 166.56 (2 × $C=O_{phthalimide}$), 170.79 ($C=O_{acid}$). HR-ESI-MS calcd for $C_{21}H_{14}N_2O_8Na^+$ $[M+ Na]^+$ m\z 445.0648; found m\z 445.0652.

2-(5-(((1,3-Dioxoisoindolin-2-yl)methoxy)carbonyl)-1,3-dioxoisoindolin-2-yl)-3 phenylpropanoic acid (8d)

Crystallized from ethanol as a Orange crystal, m. p. 126-127 °C, yield 70%, 0.18 g. Analysis: IR (KBr, υ_{max} cm^{-1}): 3445 (OH_{acid}), 1783 ($C=O_{imide}$), 1736 ($C=O_{ester}$), 1727 ($C=O_{imide}$), 1720 ($C=O_{acid}$), 1610 ($C=C_{aryl}$), 1415, 1388 (C-N-C). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 3.30-3.36 (m, 1H, C*H*HPh), 3.46-3.52 (m, 1H, CH*H*Ph), 5.11-5.16 (m ,1H, NC*H*COOH), 5.89 (s, 1H, NCH_2O), 7.09-7.15 (m, 5H, aryl), 7.90-7.98 (m, 4H, aryl), 8.08 (d, 1H, *J* = 8 *Hz*, aryl), 8.22 (s, 1H, aryl), 8.34 (d, 1H, *J* = 8 *Hz*, aryl). ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 35.78 (CH_2Ph), 53.30 (NCHCOOH), 61.67 (NCH_2O), 123.69, 123.71, 123.90, 126.59, 128.33, 128.71, 131.23, 131.46, 134.32, 134.76, 135.01, 135.07, 137.23 (aryl), 162.31 ($C=O_{ester}$), 166.18, 166.29 (2 × $C=O_{phthalimide}$), 166.54 (2 × $C=O_{phthalimide}$), 169.86 ($C=O_{acid}$). HR-ESI-MS calcd for $C_{27}H_{18}N_2O_8Na^+$ $[M+Na]^+$ m\z 521.0961; found m\z 521.0962.

2-(5-((2-(1,3-Dioxoisoindolin-2-yl)ethoxy)carbonyl)-1,3-dioxoisoindolin-2-yl)acetic acid (9a)

Crystallized from ethanol as a Pale yellow powder, m. p. 156-160 °C, yield 88%, 0.19 g. Analysis: IR (KBr, υ_{max} cm^{-1}): 3460 (OH_{acid}), 1776 ($C=O_{imide}$), 1736 ($C=O_{ester}$), 1725 ($C=O_{imide}$), 1718 ($C=O_{acid}$), 1610 ($C=C_{aryl}$), 1394, 1375 (C-N-C). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 4.02 (t, 2H, NCH_2CH_2O), 4.34 (s, 2H, NCH_2COOH), 4.54 (t, 2H, NCH_2CH_2O), 7.82-7.89 (m, 4H, aryl), 8.06 (d, 1H, *J* = 8 *Hz*, aryl), 8.23 (s, 1H, aryl), 8.33 (d, 1H, *J* = 8 *Hz*, aryl). ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 28.36 (NCH_2COOH), 36.69 (NCH_2CH_2O), 63.34 (NCH_2CH_2O), 123.28, 123.53, 124.05, 131.74, 131.97, 134.65, 135.10, 135.31, 135.76 (aryl), 164.36 ($C=O_{ester}$), 166.46 (2 × $C=O_{phthalimide}$), 168.05 (2 × $C=O_{phthalimide}$), 168.87 ($C=O_{acid}$) ppm. HR-MALDI-MS calcd for $C_{21}H_{14}N_2O_8^+$ $[M]^+$ m/z 422.0750; found m\z 422.0740.

2-(1,3-Dioxoisoindolin-2-yl)ethyl2-(2-ethoxy-2-oxoethyl)-1,3-dioxoiso indoline-5-carboxylate (9b)

Crystallized from ethanol as a White powder, m. p. 136-138 °C, yield 74 %, 0.17 g. Analysis: IR (KBr, υ_{max} cm^{-1}): 1779 ($C=O_{imide}$), 1743 ($C=O_{ester}$), 1733 ($C=O_{ester}$), 1720 ($C=O_{imide}$), 1605 ($C=C_{aryl}$), 1410, 1385 (C-N-C). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 1.20 (t, 3H, CH_3), 4.02 (t, 2H, NCH_2CH_2O), 4.13-4.18 (m, 2H, OCH_2CH_3), 4.45 (s, 2H, NCH_2CO), 4.53 (t, 2H, NCH_2CH_2O), 7.82-7.85 (m, 4H, aryl), 8.05 (d, 1H, *J* = 8 *Hz*, aryl), 8.22 (s, 1H, aryl), 8.32 (d, 1H, *J* = 8 *Hz*, aryl). ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 13.94 (CH_3), 23.94 (NCH_2CO), 36.66 (NCH_2CH_2O),61.57 (OCH_2CH_3), 63.33 (NCH_2CH_2O), 123.24, 123.58, 124.09, 131.72, 131.87, 134.61, 135.02, 135.33, 135.78 (aryl), 164.30 ($C=O_{ester}$), 166.32, 166.40 (2 × $C=O_{phthalimide}$), 167.52 ($C=O_{ester}$), 168.01 (2 × $C=O_{phthalimide}$). HR-MALDI-MS calcd for $C_{23}H_{18}N_2O_8Na^+$ $[M+Na]^+$ m/473.0961; found m\z 473.0970.

2-(5-((2-(1,3-Dioxoisoindolin-2-yl)ethoxy)carbonyl)-1,3-dioxoisoindolin-2-yl)propanoic acid (9c)

Crystallized from ethanol as a White powder, m. p. 136-137 °C, yield 84 %, 0.19 g. Analysis: IR (KBr, υ_{max} cm^{-1}): 3458 (OH), 1779 ($C=O_{imide}$), 1735 ($C=O_{ester}$), 1726 ($C=O_{imide}$), 1716 ($C=O_{acid}$), 1610 ($\upsilon C=C_{aryl}$), 1392, 1370 (C-N-C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.56 (d, 3H, CH_3), 4.02 (t, 2H, NCH_2CH_2O), 4.54 (t, 2H, NCH_2CH_2O), 4.87-4.92 (m, 1H, NC*H*COOH), 7.83-7.88 (m, 4H, aryl), 8.03 (d, 1H, *J* = 8 *Hz*, aryl), 8.21 (s, 1H, aryl), 8.32 (d, 1H, *J* = 8 *Hz*, aryl), 13.17 (br s, 1H, COOH). ^{13}C NMR (101 MHz, DMSO-d_6 , δ ppm): 14.67 (CH_3), 36.66 (NCH_2CH_2O), 47.23 (NCHCOOH), 63.24 (NCH_2CH_2O), 123.11, 123.27, 123.77, 131.59, 131.73, 134.47, 134.86, 135.06, 135.49 (aryl), 164.14 ($C=O_{ester}$), 166.17 (2 × $C=O_{phthalimide}$), 167.82 (2 × $C=O_{phthalimide}$), 170.81 ($C=O_{acid}$). HR-ESI-MS calcd for $C_{22}H_{16}N_2O_8Na^+$ $[M+Na+2H]^+$ m\z 461.0961; found m\z 461.0962.

2-(5-((2-(1,3-Dioxoisoindolin-2-yl)ethoxy)carbonyl)-1,3-dioxoisoindolin-2-yl)-3-phenylpropanoic acid (9d)

Crystallized from ethanol as a Orange crystal, mp: 80-82 °C, yield 81%, 0.21 g. Analysis: IR (KBr, υ_{max} cm^{-1}): 3481 (OH_{acid}), 1780 ($C=O_{imide}$), 1736 ($C=O_{ester}$), 1727 ($C=O_{imide}$), 1715 ($C=O_{acid}$), 1610 ($C=C_{aryl}$), 1394, 1371 (C-N-C). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 3.29-3.36 (m, 1H, C*H*HPh), 3.47-3.52 (m, 1H, CH*H*Ph), 4.00 (m, 2H, NCH_2CH_2O), 4.51 (m, 2H, NCH_2CH_2O), 5.12-5.16 (m, 1H, NC*H*COOH), 7.09-7.14 (m, 5H, aryl), 7.81-7.84 (m, 4H, aryl), 7.95 (d, 1H, *J* = 8 *Hz*, aryl), 8.13 (s, 1H, aryl), 8.26 (d, 1H, *J* = 8 *Hz*, aryl). ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 33.95 (CH_2Ph), 36.63 (NCH_2CH_2O), 53.34 (NCHCOOH), 63.33 (NCH_2CH_2O), 123.23, 123.62, 124.07, 126.74, 128.47, 128.84, 131.17, 131.72, 134.23, 134.59, 135.49, 135.94, 137.35 (aryl), 164.21 ($C=O_{ester}$), 166.27 (2 × $C=O_{phthalimide}$), 168.01 (2 × $C=O_{phthalimide}$), 170.03 ($C=O_{acid}$). HR-MALDI-MS calcd for

$C_{28}H_{21}N_2O_8Na^+$ [M+Na+H]$^+$ m/z 536.1196; found m\z 536.1186.

2-(5-(((3-(1,3-Dioxoisoindolin-2-yl)-2,6-dioxopiperidin-1-yl)methoxy)carbonyl)-1,3-dioxoisoindolin-2-yl)acetic acid (10a)

Crystallized from ethanol as a White powder, m. p. 208-210 °C, yield 89%, 0.27 g. Analysis: IR (KBr, υ_{max} cm^{-1}): 3223 (OH_{acid}), 1780 ($C=O_{imide}$), 1745 ($C=O_{ester}$), 1728 ($C=O_{imide}$), 1718 ($C=O_{acid}$), 1615 ($C=C_{aryl}$), 1401, 1375 (C-N-C). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.15-2.18 (m, 1H, H-4'), 2.60-2.65 (m, 1H, H-5'), 2.86-2.90 (m, 1H, H-4'), 3.05-3.11 (m, 1H, H-5'CO), 4.34 (s, 2H, NC*H*$_2$COOH), 5.36-5.40 (m, 1H, H-3'), 5.94-6.03 (m, 2H, NCH_2O), 7.86-7.94 (m, 4H, aryl), 8.09 (d, 1H, *J* = 8 *Hz*, aryl), 8.26 (s, 1H, aryl), 8.37 (d, 1H, *J* = 8 *Hz*, aryl). ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 21.39 ($C_{4'}$), 31.49 ($C_{5'}$), 34.63 (NCH_2COOH), 49.87 ($C_{3'}$), 64.56 (NCH_2O), 123.94, 123.96, 124.49, 131.60, 132.35, 134.89, 135.42, 135.68, 136.26 (aryl), 163.61 ($C=O_{ester}$), 166.71, 166.77 (2 × $C=O_{phthalimide}$), 167.49 (2 × $C=O_{phthalimide}$), 169.09 ($C_{2'}$), 169.83 ($C_{6'}$), 171.62 ($C=O_{acid}$). HR-ESI-MS calcd for $C_{25}H_{18}N_3O_{10}^+$ [M+H]$^+$ m\z 520.0992; found m\z 520.0971.

(3-(1,3-Dioxoisoindolin-2-yl)-2,6-dioxopiperidin-1-yl)methyl-2-(2-ethoxy-2-oxoethyl)-1,3-dioxoisoindoline-5-carboxylate (10b)

Crystallized from ethanol as a White powder, m. p. 120-122 °C, yield 95%, 0.26 g. Analysis: IR (KBr, υ_{max} cm^{-1}): 1778 ($C=O_{imide}$), 1750 ($C=O_{ester}$), 1735($C=O_{ester}$), 1727 ($C=O_{imide}$), 1610 ($C=C_{aryl}$), 1394, 1365 (C-N-C). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 1.18 (t, 3H, CH_3), 2.14-2.16 (m, 1H, H-4'), 2.60-2.68 (m, 1H, H-5'), 2.85-2.89 (m, 1H, H-4'), 3.04-3.10 (m, 1H, H-5'CO), 4.14 (q, 2H, OCH_2CH_3), 4.45 (s, 2H, NCH_2CO), 5.36-5.41 (m, 1H, H-3'), 5.94-6.02 (m, 2H, NCH_2O), 7.87-7.94 (m, 4H, aryl), 8.09 (d, 1H, *J* = 8 *Hz*, aryl), 8.26 (s, 1H, aryl), 8.37 (d, 1H, *J* = 8 *Hz*, aryl). ^{13}C NMR (101 MHz, DMSO-d_6, δ ppm): 13.97 (CH_3), 20.96 ($C_{4'}$), 24.02 (NCH_2CO), 31.07 ($C_{5'}$), 49.44 ($C_{3'}$), 61.53 (OCH_2CH_3), 64.13 (NCH_2O), 123.47, 123.53, 124.15, 131.2, 132.48, 134.54, 134.99, 135.29, 135.87 (aryl), 163.15 ($C=O_{ester}$), 166.23, 167.05 (2 × $C=O_{phthalimide}$), 167.12 (2 × $C=O_{phthalimide}$), 167.33 ($C=O_{ester}$), 169.39 ($C_{2'}$), 171.17 ($C_{6'}$) HR-ESI-MS calcd for $C_{27}H_{21}N_3O_{10}Na^+$ [M+Na]$^+$ m\z 570.1125; found m\z 570.1109.

2-(5-(((3-(1,3-Dioxoisoindolin-2-yl)-2,6-dioxopiperidin-1-yl)methoxy)carbonyl)-1,3- dioxoisoindolin-2-yl) propanoic acid (10c)

Crystallized from ethanol as a White powder, m. p. 139-141 °C, yield 83%, 0.22 g. Analysis: IR (KBr, υ_{max} cm^{-1}): 3235 (OH_{acid}), 1784 ($C=O_{imide}$), 1745 ($C=O_{ester}$), 1720 ($C=O_{imide}$), 1700 ($C=O_{acid}$), 1600 ($C=C_{aryl}$), 1395, 1367 (C-N-C). ^{1}H- NMR (400 MHz, DMSO-d_6, δ ppm): 1.54 (d, 3H, CH_3), 2.15-2.18 (m, 1H, H-4'), 2.59-2.67 (m, 1H, H-5'), 2.84-2.90 (m, 1H, H-4'), 3.04-3.13 (m, 1H, H-5'CO), 4.87-4.92 (m, 1H, NC*H*COOH), 5.36-5.40 (m, 1H, H-3'), 5.93-6.02 (m, 2H, NCH_2O), 7.86-7.94 (m, 4H, aryl), 8.05 (d, 1H, *J* = 8 *Hz*, aryl), 8.23 (s, 1H, aryl), 8.35 (d, 1H, *J* = 8 *Hz*, aryl). ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 15.13 (CH_3), 21.40 ($C_{4'}$), 31.50 ($C_{5'}$), 47.74 (*C*HCOOH), 49.88 ($C_{3'}$), 64.56 (NCH_2O), 123.86, 123.95, 124.37, 131.60, 132.25, 134.81, 135.41, 135.60, 136.17 (aryl), 163.63 ($C=O_{ester}$), 166.63, 166.69 (2 × $C=O_{phthalimide}$), 167.48 (2 × $C=O_{phthalimide}$), 169.82 ($C_{2'}$), 171.27 ($C_{6'}$), 171.6 ($C=O_{acid}$). HR-ESI-MS calcd for $C_{26}H_{19}N_3O_{10}Na^+$ [M+Na]$^+$ m\z 556.0968; found m\z 556.0948.

2-(5-(((3-(1,3-Dioxoisoindolin-2-yl)-2,6-dioxopiperidin-1-yl)methoxy)carbonyl)-1,3-dioxoisoindolin-2-yl)-3-phenylpropanoic acid (10d)

Crystallized from ethanol as a Pale orange crystal, m. p. 88-89 °C, yield 90%, 0.27g g. Analysis: IR (KBr, υ_{max} cm^{-1}): 3245 (OH_{acid}), 1782 ($C=O_{imide}$), 1740 ($C=O_{ester}$), 1728 ($C=O_{imide}$), 1718 ($C=O_{acid}$), 1600 ($C=C_{aryl}$), 1410, 1377 (C-N-C). ^{1}H- NMR (400 MHz, DMSO-d_6, δ ppm): 2.15-2.18 (m, 1H, H-4'), 2.59-2.66 (m, 1H, H-5'), 2.82-2.86 (m, 1H, H-4'), 3.04-3.13 (m, 1H, H-5'CO), 3.27-3.34 (m, 1H, C*H*HPh), 3.44-3.47 (m, 1H, CH*H*Ph), 5.08-5.12 (m, 1H, NC*H*COOH), 5.36-5.40 (m, 1H, H-3'), 5.90-5.96 (m, 2H, NCH_2O), 7.06-7.10 (m, 5H, aryl), 7.83-7.88 (m, 4H, aryl), 7.92 (d, 1H, *J* = 8 *Hz*, aryl), 8.11 (s, 1H, aryl), 8.26 (d, 1H, *J* = 8 *Hz*, aryl). ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 20.97 ($C_{4'}$), 31.03 ($C_{5'}$), 35.85 (CH_2Ph), 49.44 ($C_{3'}$), 53.51 (NCHCOOH), 64.08 (NCH_2O), 123.49, 123.61, 124.01, 126.61, 128.34, 128.65, 131.07, 131.12, 134.36, 134.60, 134.97, 135.97, 137.21 (aryl), 163.05 ($C=O_{ester}$), 166.14, 166.19 (2 × $C=O_{phthalimide}$), 167.07 (2 × $C=O_{phthalimide}$), 169.37 ($C_{2'}$), 169.89 ($C_{6'}$), 171.19 ($C=O_{acid}$). HR-ESI-MS calcd for $C_{32}H_{23}N_3O_{10}Na^+$ [M+Na]$^+$ m\z 632.1281; found m\z 632.1268.

General synthetic procedure for the synthesis of *N*-substituted anhydrides of compounds 11, 12 and 13

A mixture of phthalimide and thalidomide esters **5-7** (0.5 mmol) with tryptamine hydrochloride (0.5 mmol) in 10 ml dry DMF in the presence of 0.07 ml of TEA was refluxed for 5-10 h. The reaction mixture was poured onto dilute HCl (1%), the crude products were filtered off and recrystallized from ethanol to yield the products **[11-13]**.

(1,3-Dioxoisoindolin-2-yl)methyl2-(2-(1H-indol-3-yl)ethyl)-1,3-dioxoisoindoline-5-carboxylate (11)

Crystallized from ethanol as a Brownish yellow powder, m. p. 225-226 °C, yield 74%, 0.19 g. Analysis: IR (KBr, υ_{max} cm^{-1}): 3253 (NH), 1770 ($C=O_{imide}$), 1735 ($C=O_{ester}$), 1715 ($C=O_{imide}$), 1610 ($C=C_{aryl}$), 1402, 1373 (C-N-C), 1303 ($C-N_{aryl}$). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 3.04 (t, 2H, *J* = 8 *Hz*, NCH_2CH_2-indole), 3.86 (t, 2H, *J* = 8 *Hz*, NCH_2CH_2-indole), 5.91 (s, 2H, NCH_2O), 6.97 (t, 1H, *J* = 8 *Hz*, aryl), 7.04 (t, 1H, *J* = 8 *Hz*, aryl), 7.19 (s, 1H, C*H*NH), 7.33 (d, 1H, *J* = 8 *Hz*, aryl), 7.55 (d, 1H, *J* = 8 *Hz*, aryl), 7.92-7.98 (m, 4H, aryl), 8.07 (d, 1H, *J* = 8 *Hz*, aryl), 8.21 (s, 1H, aryl), 8.34 (d, 1H, *J* = 8 *Hz*, aryl), 10.83 (br s, 1H, NH). ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 23.77 (NCH_2CH_2-indole), 38.49 (NCH_2CH_2-indole), 61.71 (NCH_2O), 110.51, 111.44, 117.94, 118.35, 120.98, 123.00, 123.21, 123.32, 123.70, 127.01, 131.33, 131.44, 132.05, 134.12, 135.03, 135.20, 136.18 (aryl), 163.33 ($C=O_{ester}$), 166.56 (2 × $C=O_{Phthalimide}$), 166.93,

167.07 (2 × $C=O_{phthalimide}$). HR-MALDI-MS calcd for $C_{28}H_{21}N_3O_6^+$ $[M+2H]^+$ m/z 495.1430; found m\z 495.1433.

2-(1,3-Dioxoisoindolin-2-yl)ethyl2-(2-(1H-indol-3-yl)ethyl)-1,3-dioxoisoindoline-5-carboxylate (12)

Crystallized from ethanol as a Brownish yellow powder, m. p. 66-68 °C, yield 88%, 0.22 g. Analysis: IR (KBr, υ_{max} cm^{-1}): 3412 (NH), 1778 ($C=O_{imide}$), 1735 ($C=O_{ester}$), 1727 ($C=O_{imide}$), 1600 ($C=C_{aryl}$), 1397, 1380 (C-N-C), 1287 ($C-N_{aryl}$). ^{1}H- NMR (400 MHz, DMSO-d_6, δ ppm): 3.02 (t, 2H, *J* = 8 *Hz*, NCH_2CH_2-indole), 3.84 (t, 2H, *J* = 8 *Hz*, NCH_2CH_2-indole), 4.02 (t, 2H, NCH_2CH_2O), 4.53 (t, 2H, NCH_2CH_2O), 6.96 (t, 1H, *J* = 8 *Hz*, aryl), 7.05 (t, 1H, *J* = 8 *Hz*, aryl), 7.18 (s, 1H, CHNH), 7.32 (d, 1H, *J* = 8 *Hz*, aryl), 7.54 (d, 1H, *J* = 8 *Hz*, aryl), 7.82-7.85 (m, 4H, aryl), 7.93 (d, 1H, *J* = 8 *Hz*, aryl), 8.13 (s, 1H, aryl), 8.25 (d, 1H, *J* = 8 *Hz*, aryl), 10.84 (br s, 1H, NH). ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 23.76 (NCH_2CH_2-indole), 36.67 (NCH_2CH_2O), 38.54 (NCH_2CH_2-indole), 63.23 (NCH_2CH_2O), 110.62, 111.58, 118.05, 118.48, 121.13, 123.00, 123.11, 123.22, 123.48, 127.14, 131.69, 132.20, 134.60, 134.80, 135.30, 135.41, 136.43 (aryl), 164.43 ($C=O_{ester}$), 167.06, 167.10 (2 × $C=O_{phthalimide}$), 167.99 (2 × $C=O_{phthalimide}$). HR-MALDI-MS calcd for $C_{29}H_{22}N_3O_6Na^+$ $[M+Na+H]^+$ m/z 531.1406; found m\z 531.1430.

(3-(1,3-Dioxoisoindolin-2-yl)-2,6-dioxopiperidin-1-yl)methyl 2-(2-(3a,7a-dihydro-1H-indol-3-yl)ethyl)-1,3-dioxoisoindoline-5-carboxylate (13)

Crystallized from ethanol as a Brownish yellow powder, m. p. 134-136 °C, yield 92%, 0.28 g. Analysis: IR (KBr, υ_{max} cm^{-1}): 3417 (NH), 1775 ($C=O_{imide}$), 1745 ($C=O_{ester}$), 1726 ($C=O_{imide}$), 1612 ($C=C_{aryl}$), 1397, 1363 (C-N-C), 1285 ($C-N_{aryl}$). ^{1}H- NMR (400 MHz, DMSO-d_6, δ ppm): 2.13-2.18 (m, 1H, H-4'), 2.59-2.67 (m, 1H, H-5'), 2.84-2.90 (m, 1H, H-4'), 3.01 (t, 2H, *J* = 8 *Hz*, NCH_2CH_2-indole), 3.05-3.11 (m, 1H, H-5'CO), 3.84 (t, 2H, *J* = 8 *Hz*, NCH_2CH_2-indole), 5.36-5.41 (m, 1H, H-3'), 5.94-6.02 (m, 2H, NCH_2O), 6.95 (t, 1H, *J* = 8 *Hz*, aryl), 7.04 (t, 1H, *J* = 8 *Hz*, aryl), 7.17 (s, 1H, CHNH), 7.31 (d, 1H, *J* = 8 *Hz*, aryl), 7.53 (d, 1H, *J* = 8 *Hz*, aryl), 7.87-7.96 (m, 4H, aryl), 8.00 (d, 1H, *J* = 8 *Hz*, aryl), 8.18 (s, 1H, aryl), 8.32 (d, 1H, *J* = 8 *Hz*, aryl), 10.82 (br s, 1H, NH). ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 21.02 ($C_{4'}$), 24.23 (NCH_2CH_2-indole), 31.12 ($C_{5'}$), 38.94 (NCH_2CH_2-indole), 49.88 ($C_{3'}$), 64.10 (NCH_2O), 110.96, 111.92, 118.40, 118.82, 121.45, 123.40, 123.45, 123.79, 123.96, 127.46, 131.62, 132.49, 134.60, 135.35, 135.41, 135.67, 136.66 (aryl), 163.71 ($C=O_{ester}$), 166.28, 167.47 (2 × $C=O_{phthalimide}$), 167.49 (2 × $C=O_{phthalimide}$), 169.81 ($C_{2'}$), 171.30 ($C_{6'}$)HR-ESI-MS calcd for $C_{33}H_{27}N_4O_8^+$ $[M+H]^+$ m\z 607.1829; found m\z 607.1842.

Biological Evaluation

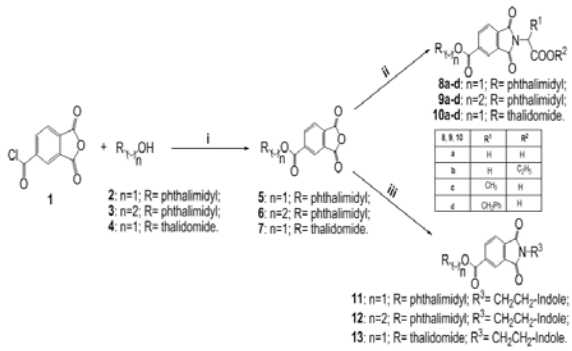
Cytotoxicity toward different cancer cell

The *in vitro* cytotoxic effect against Human hepatocellular carcinoma cell line Hep-G2 and Human Caucasian breast adenocarcinoma MCF-7 using MTT method was performed based on the previously reported procedures.[45-47]

RESULTS AND DISCUSSION

Chemistry

The esterification reaction of commercially available trimellitic anhydride chloride (TMAC) **1** with 2-(hydroxymethyl)isoindoline-1,3-dione (NHMP) **2** 2,2-(hydroxyethyl)isoindoline-1,3-dione (NHEP) **3** and 2-(1-(hydroxymethyl)-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (NHMT) **4** in dry pyridine at room temperature afforded the ester products (1,3-dioxoisoindolin-2-yl)methyl 1,3-dioxo-1,3-dihydroisobenzofuran-5-carboxylate (PPAME) **5**, (1,3-dioxoisoindolin-2-yl) ethyl 1,3-dioxo-1,3-dihydroisobenzofuran-5-carboxylate (PPAEE) **6** and (3-(1,3-dioxoisindolin-2-yl)-2,6-dioxopiperidin-1-yl)methyl 1,3-dioxo-1,3-dihydroiso-benzofuran-5-carboxylate (TPAME) **7**, respectively (Scheme 1).



Scheme 1: Synthesis of bis-phthalimide and thalidomide ester derivatives linked to amino acids. Reagents and conditions: (i) Dry Pyridine, room temperature. (ii) Different amino acids, dry DMF, reflux. (iii) Tryptamine hydrochloride, TEA, dry DMF, reflux.

The structures of the ester derivatives were elucidated based on their spectral analysis; IR spectra revealed the presence of two sets of absorption bands around 1856-1861 and 1776-1788 cm^{-1} resulting from the symmetrical and asymmetrical stretching of C=O anhydride functions. Absorption band around 1720-1750 cm^{-1} characteristics for stretching C=O ester function. Moreover, a weak absorption overtone band for the C=O stretching appeared around 3470 cm^{-1}. Furthermore, 1H-NMR spectra illustrated a downfield shift of a singlet signal at δ 5.89 ppm corresponding to the methylenic protons (N–CH2 –O) of compound 5, as multiplet at δ 5.94-6.01 ppm for compound 7 and (N–CH2CH2–O) as triplet at δ 4.00, 4.51 ppm for compound 6. The appearing of a multiplet signal around δ 7.81–7.96 ppm characteristic for the four aromatic protons of the isoindole-1,3-dione fragment, and confirmed that it were not affected by esterification. 13C-NMR for the synthesized ester showed signals at δ 61.6, 64.0 ppm (N–CH2–O) for compounds 5, 7 respectively and δ 36.7, 62.9 ppm (N–CH2CH2–O) for compound **6**. An ester C=O signal appeared at δ 163.7 ppm for compounds **5**, **7** and at δ 164.6 ppm for compound **6**, two anhydride signals appeared from

166.3–168.5 ppm. Mass spectral fragmentation data of thalidomide and phthalimide esters **5, 6** and **7** respectively, revealed the presence of molecular ions (M^+) which confirmed their molecular weights as shown in the experimental part.

The newly cyclic imide-ester derivatives **5**, **6** and **7** appeared very useful for preparation of new hybrid structure of bis-*N*-substituted phthalimide and phthalimide-thalidomide moieties. Further reactions of the resulted esters PPAME 5, PPAEE 6 or TPAME 7 with different amino acids i.e., glycine for producing (**8-10a**) derivatives, glycine ethyl ester gave (**8-10b**), alanine gave (**8-10c**) and phenylalanine gave (**8-10d**) under reflux in dry DMF yielded the phthaloyl derivatives in more than 70% yield, phthaloylation by this method was found to took place without racemization.[48]

The reaction of tryptamine hydrochloride with compounds **5**, **6** or **7** in base catalyzed reaction using TEA under reflux in dry DMF afforded *N*-tryptamino-bis phthalimide methyl ester (NTPME) **11**, *N*-tryptamino-bis phthalimide ethyl ester (NTPEE) **12** and *N*-tryptamino-phthalimide thalidomide methyl ester (NTPTME) **13** respectively. IR spectra for the above mentioned derivatives **8a-d**, **9a-d**, **10a-d**, **11**, **12** and **13** illustrated the disappearance of the two absorption bands of C=O anhydride functions. Reagents and analytical data (IR, ^{1}H-NMR, ^{13}C-NMR, HR-ESI/MS and MALDI mass spectrometry) of all synthesized derivatives are summarized in the Experimental part.

Biological Evaluation

Cytotoxic activity against Hep-G2 and MCF-7 cell lines

The *in-vitro* anti-tumor activity of all newly synthesized compounds was examined against Hep-G2 and MCF-7 cell lines using MTT (3-[4,5-Dimethylthiazol]-2,5-Diphenyltetrazolium bromide) assay[45]. The assay depends on the active mitochondrial dehydrogenase in vital cells to cleave the MTT tetrazolium rings to form blue insoluble formazan crystals. The anti-tumor activity of compounds was measured and expressed as median lethal concentration (LC_{50}) (μM), as mentioned before[45]. Doxorubicin was used as a positive control and a probit analysis using SPSS 11 program was used to determine LC_{50}.

LC_{50} was measured and only LC_{50} values of the most potent compounds were shown in Table 1. Results revealed that PPAEE **6** exhibited the highest anti-tumor activity against MCF-7 and Hep-G2 cell lines with LC_{50} values of 45.3 and 38.3 µM, respectively compared to **9b**, **10d, 11** and **12** as shown in Table 1. The activity might be interpreted through the lack of amino acids as the derivatives containing amino acids possess lower anti-tumor activity than that without amino acids. Consequently, the absence of amino acid in derivative PPAEE **6** might be facilitate its hydrolysis to the corresponding metabolites containing carboxylic group, which is an ionizable group leading to increase the solubility and membrane permeability [49].

On the other hand, anti-tumor activity of derivatives PPAEE **6**, NTPEE **12,** and **9b** might be attributed to the length of alkyl linker (CH_2) and this was completely compatible with the previously reported results which stated that the increase in the length of alkyl chain enhances the anti-tumor activity by modulating the physicochemical properties and increasing the folding and flexibility of the drug[50-52].

Further investigations of novel thalidomide and phthalimide analogs are now under considerations in order to predict a proper structure-activity relationship.

Table 1: Median lethal concentration (LC_{50}) induced by the most potent compounds.

Compounds	LC_{50}(µM)	
	MCF-7	HeP-G2
6	45.3	38.3
9b	63.9	73.1
10d	–	77.4
11	67.4	–
12	54.9	67.9
Doxorubicin	45.0	37.8

CONCLUSION

In this study, the newly cyclic imide-ester derivatives 5-7 seems to be effective starting materials for preparing novel three series of phthalimide and thalidomide moieties as a core and skeleton linked to different amino acids 8a-d, 9a-d and 10a-d. These new hybrid derivatives were biologically investigated for their antitumor activity against MCF-7 and Hep-G2 cells. The obtained results indicated that, these derivatives 6, 11, 12, 9b and 10d possessed significant antitumor activity. Phthalimide derivatives have antitumor activity higher than that of thalidomide derivatives, which supported the concept that phthalimide moiety is an essential pharmacophoric fragment in thalidomide structure.

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