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Synthesis of Hydroxylactams and Esters Derived from Thalidomide and Their Antitumor Activities

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A novel and convenient route for the synthesis of a series of thalidomide derivatives is described. Compound **2** was cyclized with different amines under alkaline condition to obtain 4-nitro substituted phthalimidines **3a-d**. Hydroxylactams **4a-d** were produced *via* bromination and hydroxylation. Different acyl chlorides were reacted with hydroxylactams to provide the desired esters **5a-d**. All compounds were evaluated by MTT assay for their inhibitory activities against HCT-116, MG-63, MCF-7, HUVEC and HMVEC cell lines *in vitro*. Most of them showed no obvious cytotoxic effect on normal human cells, compounds **4a-d**, **5a$_2$**, **5a$_4$**, **5a$_5$**, **5b$_2$**, **5c$_2$** and **5d$_2$** exhibited potent antitumor activities, among which compounds **5a$_2$** and **5b$_2$** were more effective than 5-FU.

Key Words : Thalidomide derivatives, Hydroxylactams, Synthesis, Antitumor activity

Introduction

Thalidomide was released into the market in 1957 prescribed as a sedative or hypnotic, which was used to alleviate morning sickness in pregnant women previously, but withdrawn from the market in 1960s because of its severe teratogenicity.[1-3] Despite the side effects, researches into thalidomide were not halted, and its derivatives have been designed to treat various diseases, including leprosy, myeloma, AIDS and so on.[2-5] The first commercially useful derivative was lenalidomide approved in 2005 by the FDA as a treatment for multiple myeloma.

It was reported that phthalimide analogues with nitro or amino exhibited potent antitumor activity.[6] Some researches showed that the teratogenicity of thalidomide was caused by double carbonyls in phthalimide,[7,8] and thalidomide derivatives obviously presented the anti-angiogenesis effect because of its metabolites containing hydroxyl.[9,10] Hydroxylactams have attracted a lot of attention from biological and synthetic chemists, which were regarded as very significant structural segments in sedatives, hypnotics, and muscle relaxants such as zopiclone, pazinaclone and desmethylzopiclone.[11,12] Based on the superposition principle of pharmacophore, introducing hydroxy and nitro on the phthalimidines ring could not only improve the bioactivity of these compounds, but also decrease the side effects on the human body.[13,14] Here a novel and convenient synthetic approach for 4-nitro substituted hydroxylactams **4a-d** and the corresponding esters **5a-d** with their antitumor activities is described, which are never reported.

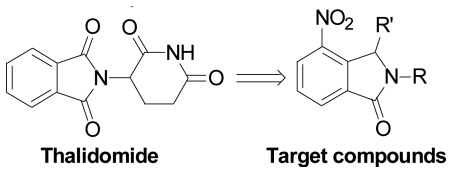


Figure 1. Thalidomide and its derivatives (R'=H, OH, or ester group).

Experimental

Chemicals. Melting points were determined using a SGW X-4 melting point instrument without calibration. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Advance 400 spectrometer with TMS as an internal standard. HRMS spectra were obtained by a Waters ACQUITY^{TM} UPLC & Q-TOF MS Premier system. Column chromatography was performed on silica gel (200-300 mesh) with the eluents indicated. All reactions were carried out in a nitrogen atmosphere unless otherwise specified and monitored by TLC using 0.25 mm silica gel plates with UV indicator. Solvents and liquid reagents were transferred using hypodermic syringes. All the solvents and chemicals were of analytical reagent and commercially available, and used without further purification.

General Procedure for the Synthesis of 2-Substituted 4-Nitro-2,3-dihydro-isoindol-1-ones (3a-d). A mixture of compound **2** (3.65 mmol) and K_2CO_3 (7.30 mmol) and the organic amines (4.38 mmol) in MeCN (80 mL) were stirred at room temperature for 5 h. The reaction mixture was filtrated and the filtrate was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with a CH_2Cl_2-MeOH (200:1) elution solvent to provide compounds **3a-d**.

2-(*p*-Tolyl)-4-nitro-2,3-dihydro-isoindol-1-one (3a): 3a was obtained in 94% yield as a light yellow crystalline; mp 178-179 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, 1H, J = 8.0 Hz), 8.26 (d, 1H, J = 8.0 Hz), 7.73 (m, 3H), 7.27 (d, 2H, J = 8.4 Hz), 5.31 (s, 2H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.2, 143.0, 136.4, 136.2, 135.6, 133.9, 130.2, 129.7, 129.4, 127.2, 119.5, 51.4, 20.4. ESI-HRMS: calcd for

$C_{15}H_{13}N_2O_3$ $[M+H]^+$, 269.0944, found 269.0926.

2-(4-Chloro-phenyl)-4-nitro-2,3-dihydro-isoindol-1-one (3b): 3b was obtained in 90% yield as a yellow solid; mp 252-253 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (d, 1H, J = 8.0 Hz), 8.25 (d, 1H, J = 8.0 Hz), 7.99 (d, 2H, J = 9.0 Hz), 7.86 (t, 1H), 7.55 (d, 2H, J = 9.0 Hz), 5.46 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.4, 143.0, 138.7, 136.5, 135.5, 130.3, 130.0, 129.1, 127.3, 124.7, 119.6, 51.4. ESI-HRMS: calcd for $C_{14}H_{10}N_2O_3Cl$ $[M+H]^+$, 289.0404, found 289.0380.

2-Benzyl-4-nitro-2,3-dihydro-isoindol-1-one (3c): 3c was obtained in 95% yield as a white solid; mp 122-123 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, 1H, J = 8.0 Hz), 8.23 (d, 1H, J = 8.0 Hz), 7.69 (t, 1H), 7.31 (m, 5H), 4.85 (s, 2H), 4.76 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.2, 143.3, 137.2, 136.9, 135.2, 130.0, 129.5, 128.7, 127.8, 127.5, 126.6, 50.3, 45.3. ESI-HRMS: calcd for $C_{15}H_{13}N_2O_3$ $[M+H]^+$, 269.0922, found 269.0926.

4-Nitro-2-propyl-2,3-dihydro-isoindol-1-one (3d): 3d was obtained in 97% yield as a white solid; mp 104-105 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (d, 1H, J = 8.0 Hz), 8.20 (d, 1H, J = 8.0 Hz), 7.68 (t, 1H), 4.86 (s, 2H), 3.63 (t, 2H), 1.73 (m, 2H), 0.97 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.4, 143.2, 138.0, 134.6, 131.1, 128.2, 126.9, 50.7, 29.7, 20.3, 11.3. ESI-HRMS: calcd for $C_{11}H_{13}N_2O_3$ $[M+H]^+$, 221.0846, found 221.0875.

General Procedure for the Synthesis of 2-Substituted 3-Hydroxy-4-nitro-2,3-dihydro-isoindol-1-ones (4a-d): The solution of compounds **3a-d** (2.99 mmol) in MeCN (80 mL) were stirred at room temperature, NBS (4.48 mmol) and AIBN (1.49 mmol) were added little by little over 0.5 h. The mixture was heated under reflux for 2 h before cooling to room temperature. The reaction mixture was filtrated and the filtrate was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with a CH_2Cl_2-MeOH (100:1) elution solvent to give compounds **4a-d**.

2-(*p*-Tolyl)-3-hydroxy-4-nitro-2,3-dihydro-isoindol-1-one (4a): 4a was obtained in 76% yield as pale yellow powder; mp 201-202 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (d, 1H, J = 8.0 Hz), 8.16 (d, 1H, J = 8.0 Hz), 7.87 (t, 1H), 7.65 (d, 2H, J = 8.4 Hz), 7.27 (d, 2H, J = 8.4 Hz), 7.12 (d, 1H, J = 9.2 Hz), 6.92 (d, 1H, J = 9.2 Hz), 2.34 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.1, 143.7, 138.1, 134.7, 134.3, 134.1, 131.8, 129.2, 128.9, 127.6, 122.6, 81.7, 20.5. ESI-HRMS: calcd for $C_{15}H_{13}N_2O_4$ $[M+H]^+$, 285.0846, found 285.0875.

2-(4-Chloro-phenyl)-3-hydroxy-4-nitro-2,3-dihydro-isoindol-1-one (4b): 4b was obtained in 83% yield as a yellow green solid; mp 202-203 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (d, 1H, J = 8.0 Hz), 8.19 (d, 1H, J = 8.0 Hz), 7.89 (t, 1H), 7.85 (d, 2H, J = 8.8 Hz), 7.54 (d, 2H, J = 8.8 Hz), 7.21 (d, 1H, J = 9.2 Hz), 6.99 (d, 1H, J = 9.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.3, 143.7, 138.0, 135.7, 134.0, 132.0, 129.2, 129.1, 128.7, 127.9, 123.7, 81.7. ESI-HRMS: calcd for $C_{14}H_{10}N_2O_4Cl$ $[M+H]^+$, 305.0292, found 305.0329.

2-Benzyl-3-hydroxy-4-nitro-2,3-dihydro-isoindol-1-one (4c): 4c was obtained in 87% yield as a yellow solid; mp 185-186 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (d, 1H, J = 8.0 Hz), 8.18 (d, 1H, J = 8.0 Hz), 7.73-7.77 (t, 1H), 7.30 (m, 5H), 6.23 (s, 1H), 5.27 (d, 1H, J = 15 Hz), 4.41 (d, 1H, J = 15 Hz), 3.72 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.9, 143.9, 138.5, 137.1, 134.3, 131.7, 128.7, 128.6, 127.8, 127.3, 127.2, 79.7, 42.2. ESI-HRMS: calcd for $C_{15}H_{13}N_2O_4$ $[M+H]^+$, 285.0874, found 285.0875.

3-Hydroxy-4-nitro-2-propyl-2,3-dihydro-isoindol-1-one (4d): 4d was obtained in 64% yield as a white solid; mp 108-109 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (d, 1H, J = 8.0 Hz), 8.06 (d, 1H, J = 8.0 Hz), 7.81 (t, 1H), 6.94 (s, 1H), 6.33 (s, 1H), 3.26-3.61 (m, 2H), 1.60 (m, 2H), 0.88 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.8, 143.8, 138.6, 134.6, 131.6, 128.4, 126.9, 80.1, 29.5, 20.9, 11.4. ESI-HRMS: calcd for $C_{11}H_{13}N_2O_4$ $[M+H]^+$, 237.0542, found 237.0544.

General Procedure for the Synthesis of the Corresponding Esters (5a-d). A mixture of compounds **4a-d** (0.35 mmol) and Et_3N (0.70 mmol) and the acetyl chlorides (0.70 mmol) in MeCN (12 mL) were stirred at room temperature for 3 h. The reaction mixture was filtrated and the filtrate was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with a CH_2Cl_2-MeOH (200:1) elution solvent to afford compounds **5a-d**.

Acetic Acid 7-Nitro-3-oxo-2-(*p*-tolyl)-2,3-dihydro-1*H*-isoindol-1-yl ester (5a$_1$): 5a$_1$ was obtained in 61% yield as a yellow solid, 70 mg; mp 139-140 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (d, 1H, J = 8.0 Hz), 8.26 (d, 1H, J = 8.0 Hz), 8.12 (s, 1H), 7.83 (t, 1H), 7.38 (d, 2H, J = 8.4 Hz), 7.25 (d, 2H, J = 8.4 Hz), 2.38 (s, 3H), 1.95 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0, 164.3, 143.6, 137.7, 135.4, 135.2, 132.2, 131.7, 129.9, 129.4, 128.0, 125.7, 79.8, 21.2, 20.3. ESI-HRMS: calcd for $C_{17}H_{15}N_2O_5$ $[M+H]^+$, 327.0941, found 327.0981.

Benzoic Acid 7-Nitro-3-oxo-2-(*p*-tolyl)-2,3-dihydro-1*H*-isoindol-1-yl ester (5a$_2$): 5a$_2$ was obtained in 52% yield as a light yellow solid; mp 208-209 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (d, 1H, J = 8.0 Hz), 8.37 (s, 1H), 8.31 (d, 1H, J = 8.0 Hz), 7.85 (t, 3H), 7.51 (t, 1H), 7.38-7.43 (t, 2H), 7.36 (d, 2H, J = 8.4 Hz), 7.21 (d, 2H, J = 8.4 Hz), 2.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.8, 164.5, 143.6, 137.7, 135.5, 135.3, 133.7, 132.3, 131.8, 130.1, 130.0, 129.9, 128.5, 128.4, 128.0, 125.7, 80.5, 21.1. ESI-HRMS: calcd for $C_{22}H_{17}N_2O_5$ $[M+H]^+$, 389.1127, found 389.1137.

2,2-Dimethyl-propionic acid 7-nitro-3-oxo-2-(*p*-tolyl)-2,3-dihydro-1*H*-isoindol-1-yl ester (5a$_3$): 5a$_3$ was obtained in 49% yield as a yellow solid; mp 140-141 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, 1H, J = 8.0 Hz), 8.27 (d, 1H, J = 8.0 Hz), 8.12 (s, 1H), 7.83 (t, 1H), 7.34 (d, 2H, J = 8.4 Hz), 7.24 (d, 2H, J = 8.4 Hz), 2.37 (s, 3H), 1.28 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.3, 175.0, 164.3, 143.6, 137.7, 135.6, 135.4, 132.0, 129.9, 129.8, 127.8, 126.0, 79.7, 39.0, 26.7, 21.2. ESI-HRMS: calcd for $C_{20}H_{21}N_2O_5$ $[M+H]^+$, 369.1393, found 369.1450.

4-Chloro-benzoic Acid 7-nitro-3-oxo-2-(*p*-tolyl)-2,3-di-

hydro-1*H*-isoindol-1-yl ester (5a$_4$): 5a$_4$ was obtained in 38% yield as a yellow solid; mp 197-198 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (d, 1H, *J* = 8.0 Hz), 8.35 (s, 1H), 8.31 (d, 1H, *J* = 8.0 Hz), 7.85 (t, 1H), 7.80 (d, 2H, *J* = 8.4 Hz), 7.40 (d, 2H, *J* = 8.4 Hz), 7.33 (d, 2H, *J* = 8.4 Hz), 7.21 (d, 2H, *J* = 8.4 Hz), 2.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.4, 164.0, 143.6, 140.3, 137.8, 135.5, 135.1, 132.4, 131.3, 130.1, 130.0, 129.5, 128.9, 128.1, 126.8, 125.7, 80.7, 21.1. ESI-HRMS: calcd for $C_{22}H_{16}N_2O_5Cl$ $[M+H]^+$, 423.0736, found 423.0748.

4-Nitro-benzoic Acid 7-Nitro-3-oxo-2-(*p*-tolyl)-2,3-dihydro-1*H*-isoindol-1-yl ester (5a$_5$): 5a$_5$ was obtained in 13% yield as a yellow brown solid; mp 71-72 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, 1H, *J* = 8.0 Hz), 8.38 (s, 1H), 8.33 (d, 1H, *J* = 8.0 Hz), 8.21 (d, 2H, *J* = 8.8 Hz), 8.03 (d, 2H, *J* = 8.8 Hz), 7.89 (t, 1H), 7.40 (d, 2H, *J* = 8.4 Hz), 7.22 (d, 2H, *J* = 8.4 Hz), 2.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.3, 163.1, 144.1, 143.6, 138.0, 136.7, 135.6, 132.6, 131.1, 130.3, 130.1, 129.9, 128.2, 125.6, 123.6, 119.9, 81.1, 29.7. ESI-HRMS: calcd for $C_{22}H_{16}N_3O_7$ $[M+H]^+$, 434.0945, found 434.0988.

Acetic Acid 2-(4-Chloro-phenyl)-7-nitro-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl ester (5b$_1$): 5b$_1$ was obtained in 93% yield as a yellow brown solid; mp 153-154 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, 1H, *J* = 8.0 Hz), 8.27 (d, 1H, *J* = 8.0 Hz), 8.16 (s, 1H), 7.85 (t, 1H), 7.50 (m, 2H), 7.42 (m, 2H), 1.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0, 164.1, 143.6, 135.1, 134.9, 133.2, 132.9, 132.4, 130.1, 129.4, 128.3, 126.6, 79.5, 20.3. ESI-HRMS: calcd for $C_{16}H_{12}N_2O_5Cl$ $[M+H]^+$, 347.0388, found 347.0435.

Benzoic Acid 2-(4-Chloro-phenyl)-7-nitro-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl ester (5b$_2$): 5b$_2$ was obtained in 76% yield as a yellow needle crystal; mp 153-154 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, 1H, *J* = 8.0 Hz), 8.41 (s, 1H), 8.32 (d, 1H, *J* = 8.0 Hz), 7.86 (m, 3H), 7.52 (m, 3H), 7.35 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.8, 164.3, 143.6, 135.1, 135.0, 133.9, 133.2, 133.1, 132.5, 130.2, 130.0, 129.5, 128.6, 128.3, 128.1, 126.7, 80.2. ESI-HRMS: calcd for $C_{21}H_{14}N_2O_5Cl$ $[M+H]^+$, 409.0575, found 409.0591.

2,2-Dimethyl-propionic Acid 2-(4-Chloro-phenyl)-7-nitro-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl ester (5b$_3$): 5b$_3$ was obtained in 44% yield as a pale yellow fan needle crystal; mp 171-172 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, 1H, *J* = 8.0 Hz), 8.27 (d, 1H, *J* = 8.0 Hz), 8.17 (s, 1H), 7.85 (t, 1H), 7.48 (d, 2H, *J* = 8.8 Hz), 7.42 (d, 2H, *J* = 8.8 Hz), 1.01 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.4, 164.2, 143.6, 135.2, 135.1, 133.2, 132.9, 132.2, 130.0, 129.3, 128.2, 126.9, 79.4, 39.0, 26.7. ESI-HRMS: calcd for $C_{19}H_{18}N_2O_5Cl$ $[M+H]^+$, 389.0877, found 389.0904.

Acetic Acid 2-Benzyl-7-nitro-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl ester (5c$_1$): 5c$_1$ was obtained in 70% yield as a white crystal; mp 134-135 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (d, 1H, *J* = 8.0 Hz), 8.21 (d, 1H, *J* = 8.0 Hz), 7.78 (t, 1H), 7.61 (s, 1H), 7.28-7.39 (m, 5H), 4.82 (d, 1H, *J* = 15.0 Hz), 4.67 (d, 1H, *J* = 15.0 Hz), 1.85 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 165.4, 143.5, 136.5, 135.6, 135.2, 132.0, 129.7, 128.6, 128.3, 127.8, 127.7, 79.5, 44.8, 20.1. ESI-HRMS: calcd for $C_{17}H_{15}N_2O_5$ $[M+H]^+$, 327.0970, found 327.0981.

Benzoic Acid 2-Benzyl-7-nitro-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl ester (5c$_2$): 5c$_2$ was obtained in 59% yield as a white solid; mp 168-169 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, 1H, *J* = 8.0 Hz), 8.25 (d, 1H, *J* = 8.0 Hz), 7.81 (m, 4H), 7.53 (t, 1H), 7.40 (t, 2H), 7.36 (d, 1H, *J* = 8.0 Hz), 7.23 (t, 2H), 7.15 (t, 1H), 5.02 (d, 1H, *J* = 15.0 Hz), 4.53 (d, 1H, *J* = 15.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 165.4, 143.5, 136.4, 135.8, 135.3, 133.8, 132.1, 130.0, 129.7, 128.7, 128.5, 128.4, 128.3, 127.8, 127.7, 79.8, 44.6. ESI-HRMS: calcd for $C_{22}H_{17}N_2O_5$ $[M+H]^+$, 389.1105, found 389.1137.

Acetic Acid 7-Nitro-3-oxo-2-propyl-2,3-dihydro-1*H*-isoindol-1-yl ester (5d$_1$): 5d$_1$ was obtained in 68% yield as a pale yellow crystal; mp 74-75 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, 1H, *J* = 8.0 Hz), 8.16 (d, 1H, *J* = 8.0 Hz), 7.78 (t, 1H), 7.61 (s, 1H), 3.75-3.83 (m, 1H), 3.18 (m, 1H), 2.14 (s, 1H), 1.73 (m, 2H), 0.94 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 165.3, 143.4, 135.5, 135.4, 132.0, 129.5, 127.4, 79.2, 42.2, 21.5, 20.4, 11.3. ESI-HRMS: calcd for $C_{13}H_{15}N_2O_5$ $[M+H]^+$, 279.0998, found 279.0981.

Benzoic Acid 7-Nitro-3-oxo-2-propyl-2,3-dihydro-1*H*-isoindol-1-yl ester (5d$_2$): 5d$_2$ was obtained in 49% yield as a yellow crystal; mp 162-163 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, 1H, *J* = 8.0 Hz), 8.21 (d, 1H, *J* = 8.0 Hz), 7.97 (d, 2H, *J* = 7.2 Hz), 7.90 (s, 1H), 7.81 (t, 1H), 7.57 (t, 1H), 7.41 (t, 2H), 3.80 (m, 1H), 3.25 (m, 1H), 1.69 (m, 2H), 0.93 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 165.4, 143.5, 135.6, 135.5, 133.9, 132.1, 130.1, 129.6, 128.6, 128.3, 127.5, 79.7, 42.4, 21.5, 11.3. ESI-HRMS: calcd for $C_{18}H_{17}N_2O_5$ $[M+H]^+$, 341.2257, found 341.1137.

2-Bromomethyl-3-nitro-benzoic Acid Methyl Ester (2): A mixture of raw material **1** (25.64 mmol) NBS (30.77 mmol) and AIBN (5.03 mmol) in CCl_4 (60 mL) were stirred at room temperature for 1 h, followed by heating under reflux for 25 h. The reaction mixture was cooled to room temperature and filtrated. The filtrate was evaporated under reduced pressure. The crude product was purified by recrystallization to afford compound **2** as a pale yellow crystal in 96% yield; mp 69-70 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, 1H, *J* = 8.0 Hz), 7.95 (d, 1H, *J* = 8.0 Hz), 7.52 (t, 1H), 5.16 (s, 2H), 2.26 (s, 3H). ESI-HRMS: calcd for $C_{21}H_{21}N_2O_5$ $[M+H]^+$, 275.0741, found 381.0746.

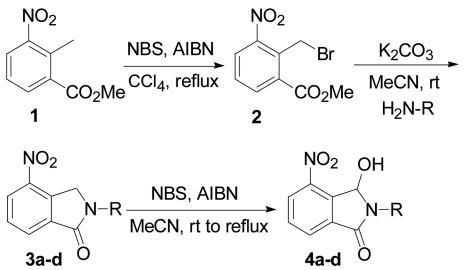
Biological Evaluation. MTT assay was used to evaluate the inhibitory activities of each compound against HCT-116, MG-63, MCF-7, HUVEC and HMVEC.[15,16] All cells were cultured in RPMI-1640 or DMEM medium supplemented with 10% fetal bovine serum at 37 ºC in a humidified atmosphere with 5% CO_2. The exponentially growing cells were used throughout the experiments. Then the cells, treated with Trypsin-EDTA solution, were dissolved to 10^5 cells/mL by culture medium and seeded into 96-well plates at 100 μL/well with 4 replicates for each drug concentration and maintained in a 5% CO_2 incubator at 37 ºC for 24 h. Control cells were treated with DMSO equal to the highest percentage of solvent used in the experimental conditions. 5-FU was used as a positive control. Thalidomide and lenalidomide were

used as the lead compounds. Then the cells were treated with synthetic compounds at different concentrations (10, 50, 100, 500, 1000 μM) for 24 h. MTT (20 μL, 5 mg/mL) was added to each well and incubation was continued for 4 h. The crystals formed were dissolved by adding DMSO (100 μL) to each well. The optical density (OD) was measured at 570 nm with a microplate reader, and then the IC_{50} value of each test compound was worked out.

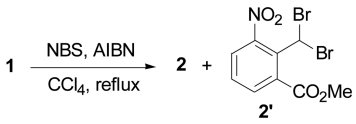
Results and Discussion

Chemistry. The synthetic approach for hydroxylactams is shown in Scheme 1. In order to explore cyclization and hydroxylation method, a series of compounds were prepared. 2-Bromomethyl-3-nitro-benzoic acid methyl ester (**2**) was prepared from 2-methyl-3-nitro-benzoic acid methyl ester (**1**) by the free radical reaction with *N*-bromosuccinimide (NBS) and 2,2-azobisisobutyronitrile (AIBN) at reflux.[17] Different amines (**a-d**) were cyclized with compound **2** under alkaline condition to provide 4-nitro substituted phthalimidines (**3a-d**).[18-21] Compounds **4a-d** were obtained in high yield by hydroxylation of compounds **3a-d** (1.0 equiv) when NBS (1.5 equiv) was used as the bromide reagent and AIBN (0.5 equiv) as the initiation. As expected, the bromination took place at the 3-position methene group of phthalimidines.[22,23] The intermediate bromides (**6**) formed were so unstable that they were rapidly hydrolyzed to hydroxylactams.

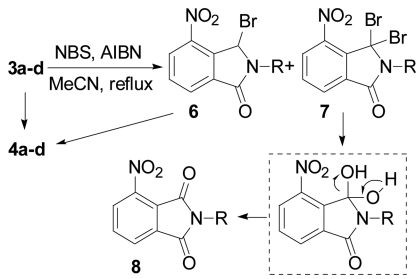
As shown in Table 1, compounds **3a-d** were obtained in very high yields. As for the hydroxylation of compound **3a**, it could be a free radical substitution at the 3-position methene group of phthalimidines or an electrophilic substitution on the phenyl ring to form compound **4a** in a moderate yield, which was due to the electron-donating effect of methyl group. Therefore, the ortho position of methyl group on the phenyl ring was as active as the 3-position methene group of phthalimidines. Compound **4d** was obtained in somewhat lower yield due to the recovery of partial substrate **3d**. Further investigation on the *N*-benzyl phthalimidine **3c** indicated that the benzyl group was intact in the process of bromination reaction. The NMR spectra of compound **4c** were identical with those of authentic samples. It suggested that the hydroxylation of compound **3c** was regiospecific.



Scheme 1. (H_2N-R was **a**: *p*-toluidine, **b**: *p*-chloroaniline, **c**: benzylamine, **d**: *n*-propylamine, respectively corresponding to compounds **3a-d**).



Scheme 2

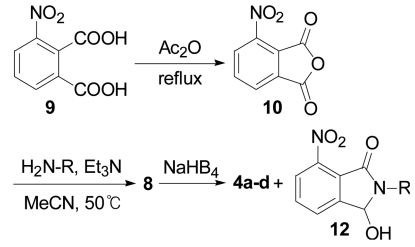


Scheme 3

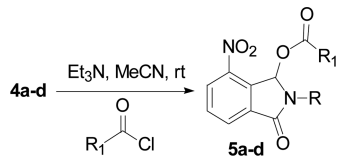
As a result of over bromination the byproduct **2'** could be produced as shown in Scheme 2, fortunately only a small amount of byproducts existed due to steric hindrance from the nitro, which could be removed by recrystallization.

As shown in Scheme 3, the formation of phthalimides **8** could lower the yield of compounds **4a-d**. The possible mechanism was proposed as follows: the unstable dibromides **7** were formed by over bromination, instead of the desired intermediates **6**. The forming process of dihydroxylactams was similar to the target compounds'. Then the byproduct **8** was formed after dehydration. In order to avoid this competitive side reaction, we changed the quantity of NBS (1.0 equiv or 2.0 equiv) at reflux. Unfortunately, most of the reactants were intact or a lot of byproduct **8** were still formed. It suggested that the right amount of NBS (1.5 equiv) was necessary for this reaction. Other reagents such as Br_2/CF_3COOAg and *N*-chlorosuccinimide (NCS)/benzoyl peroxide (BPO) were also tried to improve the yield, but the result was not good.

Before we found the new route, compounds **4a-d** were synthesized by the conventional method as shown in



Scheme 4



Scheme 5. (R_1COCl was acetyl chloride, pivaloyl chloride, benzol chloride, *p*-nitrobenzoyl chloride, *p*-chlorobenzoyl chloride, respectively corresponding to compounds **5a-d**).

Table 1. The yields of compounds **3a-d**, **4a-d** and **5a-d**

Entry	R	**3a-d** (%)	**4a-d** (%)	R_1	**5a-d**	Yield (%)
a	*p*-MeC_6H_4	94	76	Me	**5a$_1$**	61
				Ph	**5a$_2$**	52
				t-Bu	**5a$_3$**	49
				p-ClC_6H_4	**5a$_4$**	38
				p-$NO_2C_6H_4$	**5a$_5$**	13
b	*p*-ClC_6H_4	90	83	Me	**5b$_1$**	93
				Ph	**5b$_2$**	76
				t-Bu	**5b$_3$**	44
c	Bn	95	87	Me	**5c$_1$**	70
				Ph	**5c$_2$**	59
d	*n*-Pr	97	64	Me	**5d$_1$**	68
				Ph	**5d$_2$**	49

Table 2. Cytotoxic activity of compounds **4a-d** and **5a-d**

Compounds	IC_{50} (μM)				
	[a]HCT-116	[b]MG-63	[c]MCF-7	[d]HUVEC	[e]HMVEC
[f]5-FU	12	46	37	61	101
[g]T	625	717	796	845	766
[h]L	–	792	–	739	612
4a	90	81	167	976	–
4b	84	103	115	–	–
4c	127	274	201	–	–
4d	142	209	421	–	–
5a$_1$	912	-	825	–	–
5a$_2$	5	39	20	–	938
5a$_3$	887	-	902	–	–
5a$_4$	776	511	553	392	575
5a$_5$	225	278	393	753	896
5b$_1$	–	–	–	–	–
5b$_2$	11	63	34	–	–
5b$_3$	–	–	–	–	–
5c$_1$	–	–	–	–	–
5c$_2$	87	224	88	–	–
5d$_1$	–	–	–	–	–
5d$_2$	655	441	225	–	–

[a]HCT-116: human colon carcinoma cell line. [b]MG-63: human osteosarcoma cell line. [c]MCF-7: human breast adenocarcinoma cell line. [d]HUVEC: human umbilical vein endothelial cell line. [e]HMVEC: human microvascular endothelial cell line. [f]5-FU: 5-fluorouracil, the positive control. [g]T: thalidomide, the lead compound. [h]L: lenalidomide, the lead compound; "–" means "$IC_{50} > 1000$".

Scheme 4. Phthalandione **9** served as the starting material to provide phthalanhydride **10**. Phthalimides **8** was obtained by treating compound **10** with different amines. Compounds **8** was converted to compound **4a-d** by reduction reaction with sodium borohydride ($NaBH_4$). However, $NaBH_4$ used as the reductant usually produced a mixture of isomers (**4a-d** and **12**),[24] which resulted in bad yields (all the yields < 60%).

Compounds **5a-d** were synthesized as shown in Scheme 5. Compounds **4a-d** were reacted with different acyl chlorides and Et_3N (or *i*-Pr_2NEt) in MeCN to provide the corresponding esters **5a-d**.[25]

It was noticed that the yields of **5a-d** were relevant to the groups R and the acyl chlorides used. Experiments proved that the acetyl chloride was reacted with compounds **4a-d** more easily than other acyl chlorides. Pivaloyl chloride was nearly impossible to be reacted with compounds **4c-d**. Even worse, *p*-nitrobenzoyl chloride and *p*-chlorobenzoyl chloride could only be reacted with compound **4a**. It was supposed that the steric effect and the electronic effect from the group R and R_1 caused the different yields.

Antitumor Activities. The results were summarized in table 2. It suggested that compounds **4a-d** exhibited superior antitumor activities (against HCT-116, MG-63 and MCF-7) to the lead compounds thalidomide and lenalidomide, but inferior to 5-FU. Fortunately, they showed no obvious cytotoxic effect on normal human cells (HUVEC and HMVEC).

Compounds **5a-d** showed almost no cytotoxic effect on normal human cells, except that compounds **5a$_4$** and **5a$_5$** presented inhibitory activities against the five kinds of cell lines. Compounds **5a$_2$**, **5b$_2$**, **5c$_2$** and **5d$_2$** obtained by the acylation of aryl chloride demonstrated potent antitumor activities, among which compounds **5a$_2$** and **5b$_2$** exhibited the highest antitumor potency, more effective than 5-FU. However, compounds **5a$_1$**, **5a$_3$**, **5b$_1$**, **5b$_3$**, **5c$_1$** and **5d$_1$** showed no inhibitory effect on the proliferation of all cells.

Conclusion

In summary, a novel and facile synthetic approach for a series of thalidomide derivatives has been designed, which is better than the conventional methods. The structures of all compounds never reported were confirmed by ^1H NMR, ^{13}C NMR and HRMS techniques. Their cytotoxic activity was evaluated against HCT-116, MG-63, MCF-7, HUVEC and HMVEC cell lines *in vitro*. The results indicated that most of them presented no obvious cytotoxic effect on normal human cells. Compounds **4a-d**, **5a$_2$**, **5b$_2$**, **5c$_2$** and **5d$_2$** showed more potent antitumor activities than the lead compounds. What's more, compounds **5a$_2$** and **5b$_2$** exhibited superior antitumor activity to 5-FU in micromolar scale. In addition, the aromatic esters showed better antitumor activity than the aliphatic eaters. Further studies including the cell migration and lumen formation experiments are being undertaken in order to explore their angiogenesis inhibitory activity.

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