

Anti-proliferate Activity and 5 α -reductase Inhibitors of Chiral Macrocylic (N α -di-nicotinoyl)[L-phenylalaninyl-L-leucinyl]Pentapeptide Candidates Against LNCaP and PC-3 Prostate Cancer Cell Lines

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A series of macrocylic derivatives **2-6** were prepared using N⁶-dinicotinoyl-bis[L-phenylalaninyl-L-leucyl]hydrazide **1** and cyclo pentapeptide hydrazide **2** as starting materials. The hydrazide **1** was cyclized with diaminoalkanes to macrocylic hezaazahexamide derivatives **3a-c**, respectively. Finally, compound **2** was condensed with 3,5-diacetylpyridine gave the corresponding macrocylic Schiff base **4**. Finally, condensation of **2** with substituted aromatic or heterocyclic aldehyde derivatives gave the corresponding Schiff base derivatives **5a-f** and **6a-c**, respectively. The synthesized compounds were screened as 5 α -reductase inhibitors and anti-proliferative activity against prostate cancer cell lines.

Keywords: Macrocylic pentapeptide, Schiff bases, 5 α -reductase inhibitors and anti-proliferate activities.

Introduction

Peptides are formed from the chemical reaction between two or more amino acids by different methods¹ and the covalent chemical bonds are formed when the carboxyl group of one amino acid reacts with the amino group of another. Interestingly, some individual amino acids exemplified by valine, leucine, iso-leucine, and phenylalanine were reported to have biological and pharmacological properties in different experimental models². In previous work, we have found that certain substituted heterocyclic compounds show antimicrobial³, anti-viral⁴, and reductase inhibitors⁵. On the other hand, some heterocyclic pyridine derivatives have promising biological⁶ and anticancer activity⁷. Recently, in our previous work, some of peptidopyridine derivatives have been synthesized^{8,9} and tested as pharmacological activity¹⁰, analgesic and anti-inflammatory¹¹, antimicrobial^{12,13}, antiparkinsonian¹⁴, anticancer^{15,16} agents. In view of these observations and in continuation of our previous

work in pyridine and peptide chemistry, we synthesized some new macrocylic pyridine pentapeptide compounds and screened as 5 α -reductase inhibitors and anti-proliferative activity against prostate cancer cell lines.

Materials and methods

Melting points were determined in open glass capillary tubes with an Electro Thermal Digital melting point apparatus (model: IA9100) and are uncorrected. Elemental microanalysis for carbon, hydrogen and nitrogen (Microanalytical 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. Proton and carbon nuclear magnetic resonance (¹H- and ¹³C NMR) spectra were run in (DMSO-d₆) on Jeol 500 MHz instruments “500 MHz to ¹HNMR and 125 MHz to ¹³CNMR”. Mass spectra were run on a MAT Finnigan SSQ 7000 spectrometer, using the electron impact technique (EI). Analytical thin layer chromatography

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(TLC) was performed on silica gel aluminum sheets, 60F₂₅₄ (E. Merck).

Synthesis of cyclo-(N^o-dinicotinoyl)-bis[L-phenylalaninyl-L-leucyl]aliphatic diamines (3a-c)

To a stirred solution of hydrazide (**1**)^{8,17,18} (1 mmol) a mixture of 5N HCl (2 ml), AcOH (3 ml) and H₂O (10 ml) at -5°C for 15 min, sodium nitrite (0.2 g, 2 ml water) was added. The reaction mixture was stirred for 0.5 h, then extracted with cold dichloromethane (-5°C), which was added to aliphatic diamine, namely, 1,3-propanediamine, 1,4-butanediamine or 1,6-hexanediamine (1 mmol, 10 mL dichloromethane) with stirring for 3 h at -5°C, then at room temperature for 20 h. The mixture product washed with water, 0.5 N hydrochloric acid, water and dried over anhydrous calcium chloride. The solvent was evaporated to dryness, the crude residue were purified by preparative chromatography on silica gel by using chloroform/ethanol (9:1, v/v) as eluent to give the corresponding macrocyclic tetrapeptide derivatives **3a-c**, respectively.

3a: Yield 65%; m.p. 206-208°C. $[\alpha]_D^{25} = -106$ ($c = 0.5$, DMF). IR (film): $\nu = 3448-3345$ (NH), 1654, 1534, 1254 (3C=O, amides) cm^{-1} . ¹H NMR: $\delta = 0.88-0.95$ (m, 12H, 4CH₃), 1.72-1.80 (m, 6H, 3CH₂), 2.22-2.36 (m, 2H, 2CH), 3.30 (t, 4H, 2CH₂), 3.44 (d, 4H, 2CH₂), 4.25-4.32 (m, 2H, 2CH), 4.68-4.72 (m, 2H, 2CH), 6.95-7.75 (m, 10H, Ar-H), 8.36 s, 9.05 (s, 3H, pyr-H), 8.66, 8.86, 9.20 (3s, 6H, 6NH, D₂Oexchangeable). ¹³C NMR: $\delta = 18.90, 19.40, 24.00, 32.65, 38.80, 39.40, 42.18, 52.90, 54.24, 125.80, 127.50, 128.32, 131.50, 138.75, 141.12, 152.35, 166.72, 169.85, 172.96$ (40 C). MS (EI, 70 eV): m/z (%) = 726 (13) [M]⁺. C₄₀H₅₁N₇O₆ (725.87): Calcd. C 66.19, H 7.08, N 13.51; found C 66.05, H 7.00, N 13.40.

3b: Yield 74%; m.p. 196-198°C. $[\alpha]_D^{25} = -118$ ($c = 0.5$, DMF). IR (film): $\nu = 3424-3365$ (NH), 1658, 1536, 1240 (3C=O, amides) cm^{-1} . ¹H NMR: $\delta = 0.86-0.95$ (m, 12H, 4 CH₃), 1.28-1.36 (m, 4H, 2 CH₂), 1.40-1.45 (m, 4H, 2 CH₂), 1.68-1.74 (m, 4H, 2 CH₂), 2.26-2.34 (m, 2H, 2CH), 3.36 (d, 4H, 2CH₂), 4.15-4.24 (m, 4H, 4 CH), 6.98-7.54 (m, 10H, 2Ph-H), 8.48, 9.05 (s, 3H, pyr-H), 8.80, 8.92, 9.16 (3s, 6H, 6NH, D₂Oexchangeable). ¹³C NMR: $\delta = 19.20, 19.45, 23.95, 28.82, 32.68, 39.42, 42.24, 52.86, 54.36, 125.82, 127.55, 128.36, 131.65, 138.74, 141.18, 152.34, 166.75, 169.80, 172.84$ (41C). MS (EI, 70 eV): m/z (%) = 740 (13) [M]⁺. C₄₁H₅₃N₇O₆ (739.90): Calcd. C 66.55, H 7.22, N 13.25; found C 66.45, H 7.16, N 13.22.

3c: Yield 70%; m.p. 232-234°C. $[\alpha]_D^{25} = -98$ ($c = 0.5$, DMF). IR (film): $\nu = 3410-3355$ (NH), 1660, 1534, 1245 (3C=O, amides) cm^{-1} . ¹H NMR: $\delta = 0.86-0.92$ (m, 12H, 4 CH₃), 1.24-1.26 (m, 4H, 2 CH₂), 1.32-1.35 (m, 4H, 2CH₂), 1.42-1.46 (m, 4H, 2 CH₂), 1.62-1.70 (m, 4H, 2 CH₂), 2.26-2.35 (m, 2H, 2CH), 3.44 (d, 4H, 2CH₂), 4.18-4.24 (m, 4H, 4 CH), 7.00-7.65 (m, 10H, 2Ph-H), 8.42, 9.08 (2s, 3H, pyridyl-H), 8.75, 8.93, 9.16 (3s, 6H, 6NH, D₂Oexchangeable). ¹³C NMR: $\delta = 18.95, 19.32, 23.90, 27.82, 32.68, 40.56, 39.48, 42.56, 52.80, 54.34, 125.80, 127.58, 128.34, 138.75, 131.64, 141.24, 152.35, 166.74, 168.98, 172.80$ (43C). MS (EI, 70 eV): m/z (%) = 768 (16) [M]⁺. C₄₃H₅₇N₇O₆ (767.95): Calcd. C 67.25, H 7.48, N 12.77; found C 67.14, H 7.41, N 12.68.

Synthesis of compound 4

A mixture of 3,5-bis-hydrazide **1** (1 mmol) and 3,5-diacetylpyridine (0.163 g, 1 mmol) in AcOH (25 mL) was refluxed for 4 h. The reaction mixture was poured into ice water, the obtained solid was collected by filtration, washed with water and crystallized from AcOH/H₂O to give bicyclic product **4**. Yield 70%; m.p. 248-250°C. $[\alpha]_D^{25} = -98$ ($c = 0.5$, DMF). IR (film): $\nu = 3430-3355$ (NH), 1654, 1534, 1252 (3C=O, amides) cm^{-1} . ¹H NMR: $\delta = 0.90-0.96$ (m, 18H, 6CH₃), 1.68-1.74 (m, 4H, 2CH₂), 2.22-2.30 (m, 2H, 2CH), 3.44 (d, 4H, 2CH₂), 4.20-4.30 (m, 2H, 2CH), 4.65-4.75 (m, 2H, 2CH), 6.96-7.70 (m, 10H, 2Ph-H), 8.20-8.25 (m, 3H, pyr-H), 8.46, 9.08 (2s, 3H, pyr-H), 8.75, 8.85, 9.16 (3s, 6H, 6NH, D₂Oexchangeable). ¹³C NMR: $\delta = 13.55, 17.96, 18.45, 23.72, 40.70, 42.42, 52.62, 53.18, 124.28, 128.44, 129.45, 138.80, 155.56, 126.12, 131.74, 135.88, 140.52, 152.45, 154.55, 163.85, 169.35, 178.55$ (46 C). MS (EI, 70 eV): m/z (%) = 842 (34) [M-1]⁺. C₄₆H₅₄N₁₀O₆ (842.98): Calcd. C 65.54, H 6.46, N 16.62; found C 65.40, H 6.38, N 16.50.

Synthesis of Schiff base derivatives 5a-c and 6a-c

A mixture of **2** (1 mmol) and substituted aromatic aldehydes "benzaldehyde, *p*-methyl-, *o*-*p*-methoxybenzaldehyde" or heterocyclic aldehydes, "2-, 3-, or 4-pyridinecarbaldehydes" (1 mmol) in absolute ethanol (25 mL) in the presence of a mixture of TEA/DEA (4 ml, 2:2) was refluxed for 4-6 h. The solvent was concentrated to dryness and the obtained residue was solidified with ether, filtered off, washed with ether and crystallized from the proper solvents to afford pentapeptide hydrazone derivatives **5a-c** and **6a-c**, respectively.

5a: Yield 65%; m.p. 210-212°C. $[\alpha]_D^{25} = -118$ ($c = 0.5$, DMF). IR (film): $\nu = 3425-3356$ (NH), 1655, 1533, 1234 (3C=O, amides) cm^{-1} . $^1\text{H NMR}$: $\delta = 0.86-0.92$ (m, 12H, 4CH₃), 1.28-1.40 (m, 4H, 2CH₂), 1.55-1.65 (m, 4H, 2 CH₂), 2.22-2.30 (m, 2H, 2CH), 3.15-3.25 (m, 2H, CH₂), 3.45 (d, 4H, 2CH₂), 4.00-4.10 (m, 4H, 4CH), 4.42-4.46 (m, 1H, CH), 6.96-7.60 (m, 16H, 3Ph-H + CH=N), 8.38, 9.02 (2s, 3H, pyr-H), 8.56, 8.75, 8.86, 9.25 (3s, 7H, 7 NH, D₂Oexchangeable). $^{13}\text{C NMR}$: $\delta = 17.75, 18.12, 28.32, 30.36, 38.05, 23.75, 40.92, 41.74, 52.25, 52.85, 58.45, 124.35, 128.35, 128.65, 129.15, 129.45, 131.05, 133.55, 138.71, 142.75, 131.44, 140.14, 152.22, 163.80, 169.18, 170.63, 176.74$ (49C). MS (EI, 70 eV): m/z (%) = 886 (16) [M]⁺. C₄₉H₅₉N₉O₇ (886.04): Calcd. C 66.42, H 6.71, N 14.23; found C 66.28, H 6.58, N 14.12.

5b: Yield 70%; m.p. 232-234°C. $[\alpha]_D^{25} = -98$ ($c = 0.5$, DMF). IR (film): $\nu = 3455-3325$ (NH), 1652, 1531, 1233 (3C=O, amides) cm^{-1} . $^1\text{H NMR}$: $\delta = 0.90-0.95$ (m, 12H, 4CH₃), 1.22-1.40 (m, 4H, 2CH₂), 1.56-1.68 (m, 4H, 2 CH₂), 2.18-2.30 (m, 2H, 2CH), 2.40 (s, 3H, CH₃), 3.15-3.25 (m, 2H, CH₂), 3.50 (d, 4H, 2CH₂), 4.02-4.16 (m, 4H, 4CH), 4.46-4.50 (m, 1H, CH), 7.00-7.64 (m, 15H, 3Ph-H + CH=N), 8.50, 9.10 (2s 3H, pyr-H), 8.60, 8.82, 8.90, 9.24 (4s, 7H, 7 NH, D₂Oexchangeable). $^{13}\text{C NMR}$: $\delta = 17.84, 18.54, 24.18, 28.37, 30.36, 38.15, 23.74, 40.94, 41.74, 52.30, 52.80, 58.35, 126.00, 127.48, 128.35, 129.06, 129.24, 130.16, 138.90, 140.43, 142.70, 131.46, 140.34, 152.25, 163.80, 169.18, 170.60, 176.90$ (50C). MS (EI, 70 eV): m/z (%) = 900 (22) [M]⁺. C₅₀H₆₁N₉O₇ (900.07): Calcd. C 66.72, H 6.83, N 14.01; found C 66.64, H 6.70, N 13.94.

5c: Yield 72%; m.p. 244-246°C. $[\alpha]_D^{25} = -106$ ($c = 0.5$, DMF). IR (film): $\nu = 3418-3350$ (NH), 1654, 1532, 1235 (3C=O, amides) cm^{-1} . $^1\text{H NMR}$: $\delta = 0.90-0.96$ (m, 12H, 4CH₃), 1.20-1.34 (m, 4H, 2CH₂), 1.55-1.60 (m, 4H, 2 CH₂), 2.22-2.34 (m, 2H, 2CH), 3.16-3.24 (m, 2H, CH₂), 3.44 (d, 4H, 2CH₂), 3.72 (s, 3H, OCH₃), 4.02-4.15 (m, 4H, 4CH), 4.40-4.46 (m, 1H, CH), 6.98-7.60 (m, 15H, 3Ph-H + CH=N), 8.44, 9.12 (2s, 3H, pyr-H), 8.56, 8.76, 8.85, 9.24 (4s, 7H, 7 NH, D₂Oexchangeable). $^{13}\text{C NMR}$: $\delta = 17.86, 18.42, 28.30, 30.30, 38.00, 23.72, 40.90, 41.80, 52.26, 52.84, 55.74, 58.40, 114.45, 124.45, 125.45, 128.36, 129.34, 129.70, 138.75, 162.46, 142.65, 131.50, 140.20, 152.18, 163.82, 169.20, 170.62, 176.34$ (50C). MS (EI, 70 eV): m/z (%) = 916 (20) [M]⁺. C₅₀H₆₁N₉O₈ (916.07): Calcd. C 65.56, H 6.71, N 13.76, found C 65.46, H 6.60, N 13.68.

6a: Yield 65%; m.p. 246-248°C. $[\alpha]_D^{25} = -104$ ($c = 0.5$, DMF). IR (film): $\nu = 3410-3340$ (NH), 1655, 1535, 1235 (3C=O, amides) cm^{-1} . $^1\text{H NMR}$: $\delta = 0.86-0.95$ (m, 12H, 4CH₃), 1.22-1.34 (m, 4H, 2CH₂), 1.55-1.70 (m, 4H, 2 CH₂), 2.24-2.32 (m, 2H, 2CH), 3.15-3.26 (m, 2H, CH₂), 3.45 (d, 4H, 2CH₂), 4.00-4.12 (m, 4H, 4CH), 4.40-4.46 (m, 1H, CH), 7.12-7.29 (m, 10H, 2Ph-H), 7.39-8.20 (m, 5H, pyr-H + CH=N), 8.45, 9.12 (2s, 3H, pyr-H), 8.58, 8.74, 8.86, 9.45 (4s, 7H, 7 NH, D₂Oexchangeable). $^{13}\text{C NMR}$: $\delta = 17.64, 17.94, 28.38, 30.36, 38.08, 23.86, 40.95, 41.74, 52.35, 52.85, 58.45, 119.34, 125.12, 135.65, 148.65, 152.98, 124.98, 127.60, 128.68, 136.16, 143.82, 131.55, 140.15, 152.15, 165.54, 169.45, 170.76, 175.12$ (48C). MS (EI, 70 eV): m/z (%) = 886 (35) [M]⁺. C₄₈H₅₈N₁₀O₇ (887.06): Calcd. C 64.99, H 6.59, N 15.79; found C 64.90, H 6.52, N 15.72.

6b: Yield 60%; m.p. 225-227°C. $[\alpha]_D^{25} = 118$ ($c = 0.5$, DMF). IR (film): $\nu = 3424-3345$ (NH), 1652, 1533, 1234 (3C=O, amides) cm^{-1} . $^1\text{H NMR}$: $\delta = 0.90-0.96$ (m, 12H, 4CH₃), 1.24-1.32 (m, 4H, 2CH₂), 1.52-1.67 (m, 4H, 2 CH₂), 2.25-2.30 (m, 2H, 2CH), 3.18-3.25 (m, 2H, CH₂), 3.48 (d, 4H, 2CH₂), 4.04-4.10 (m, 4H, 4CH), 4.38-4.45 (m, 1H, CH), 7.15-7.32 (m, 10H, 2Ph-H), 7.54-8.40 (m, 3H, pyr-H), 8.65 (s, 1H, CH=N), 8.48, 9.02, 9.14 (3s, 4H, pyr-H), 8.56, 8.78, 8.82, 9.46 (4s, 7H, 7 NH, D₂Oexchangeable). $^{13}\text{C NMR}$: $\delta = 17.74, 17.90, 28.50, 30.64, 38.19, 23.85, 40.95, 41.52, 52.76, 52.80, 58.56, 123.08, 129.34, 133.12, 148.75, 150.68, 124.66, 127.74, 128.80, 136.10, 142.72, 131.70, 140.56, 152.26, 165.90, 169.60, 171.00, 175.34$ (48C). MS (EI, 70 eV): m/z (%) = 887 (16) [M]⁺. C₄₈H₅₈N₁₀O₇ (887.06): Calcd. C 64.99, H 6.59, N 15.79; found C 64.88, H 6.52, N 15.68.

6c: Yield 72%; m.p. 212-214°C. $[\alpha]_D^{25} = -96$ ($c = 0.5$, DMF). IR (film): $\nu = 3458-3350$ (NH), 1652, 1532, 1233 (3 C=O, amides) cm^{-1} . $^1\text{H NMR}$: $\delta = 0.88-0.94$ (m, 12H, 4CH₃), 1.20-1.30 (m, 4H, 2CH₂), 1.55-1.68 (m, 4H, 2 CH₂), 2.23-2.30 (m, 2H, 2CH), 3.22-3.26 (m, 2H, CH₂), 3.44 (d, 4H, 2CH₂), 4.00-4.12 (m, 4H, 4CH), 4.40-4.46 (m, 1H, CH), 7.10-7.34 (m, 10H, 2Ph-H), 7.86-8.52 (m, 5H, pyr-H + CH=N), 8.56, 9.16 (2s, 3H, pyr-H), 8.56, 8.74, 8.80, 9.42 (4s, 7H, 7 NH, D₂Oexchangeable). $^{13}\text{C NMR}$: $\delta = 17.60, 17.92, 28.46, 30.50, 38.12, 23.90, 40.96, 41.50, 52.40, 52.56, 58.52, 119.78, 144.00, 149.05, 124.87, 127.68, 128.75, 136.18, 144.65, 131.70, 140.56, 152.26, 165.60, 169.24, 170.56, 175.42$ (48C). MS (EI, 70 eV):

m/z (%) = 886 (42) [M-1]⁺. C₄₈H₅₈N₁₀O₇ (887.06): Calcd. C 64.99, H 6.59, N 15.79; found C 64.90, H 6.50, N 15.70.

Biological Activities

5 α -Reductase inhibitors

The experimental method which was used in 5 α -reductase inhibitors assay has been adopted from Farghaly *et al.*, 2012¹⁹ (Fig. 1)

Anti-prostate cancer screening anti-androgenic bioassay in human prostate cancer cells

The experimental method which was used in 5 α -reductase inhibitors assay has been adopted from Yuet *et al.*, 2018²⁰.

Results and Discussion

Chemistry

In the present work, some of newly synthesized macrocyclic pentapeptide derivatives **2-6** were obtained by using cyclo penta-peptide hydrazide **2** and N^o-dinicotinoyl-bis[L-phenylalaninyl-L-leucyl] hydrazide **1** as starting materials, which were synthesized from 3,5-pyridinedicarbonyl chloride, according to the previous reported procedures¹⁷. Cyclization of **1** with diaminoalkanes by azide method or with 3,5-diacetylpyridine in refluxing acetic acid afforded the corresponding cyclohexaazahexacarboxamide pyridines **3a-c** and bicyclopripyridine derivative **4**. Condensation of hydrazide **2** with substituted aromatic or heterocyclic aldehydes afforded the corresponding Schiff base derivatives **5a-c** and **6a-c**, respectively (Scheme 1).

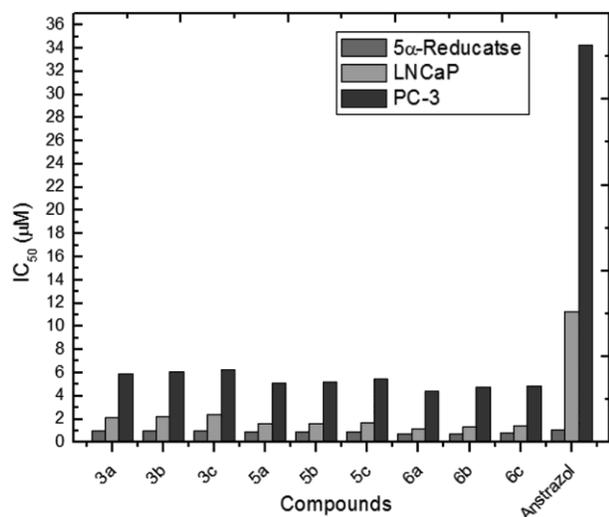
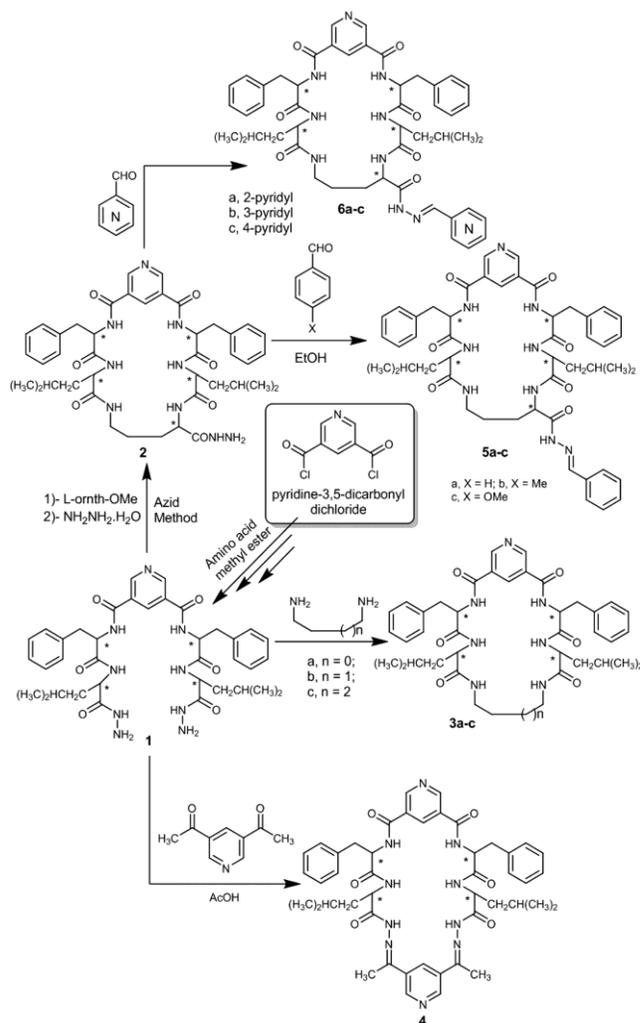


Fig. 1 — 5 α -Reductase inhibitors activities and anti-proliferative activity against prostate different cancer cell lines

Biological Screening

A pentapeptide (Ile-Leu-Tyr-Met-Pro; ILYMP) was isolated from the protein hydrolysate of *Cyclina sinensis* was named *Cyclina sinensis* pentapeptide (CSP) and it was found to inhibit DU-145 cell proliferation at a half-maximal inhibitory concentration of 11.25 mM at a 72 h time interval²⁰. ILX651 is a pentapeptide has potential for activity across a wide number of solid tumors. ILX651 in vitro cytotoxicity was recently assessed against the NCI panel consisting of 60 human tumor cell lines²¹⁻²³. Cytotoxicity observed across the breadth of tumors tested (melanoma, NSCLC, prostate, breast, colon, CNS, and leukemia) with GI₅₀'s ranging from <10 nM to >1mM. The in vivo antitumor efficacy of ILX651 was assessed against human MX-1 breast tumor



Scheme 1 — Synthetic routes for compounds **2**, **3a-c**, **4**, **5a-c** and **6a-c**

with different dosages, treatment schedules, and administration routes.

5 α -Reductase inhibitors

The newly synthesized compounds were screened for their 5 α -reductase inhibitors activities in vivo and the obtained results showed potent 5 α -reductase inhibitors activities. The descending order of 5 α -Reductase inhibitor activities was as follow: **6a**, **6b**, **6c**, **5a**, **5b**, **5c**, **3a**, **3b**, **3c**.

Anti-proliferate activity against prostate cancer cell lines

The obtained high potent 5 α -reductase inhibitors activities encouraged the authors to identify their anti-prostate cancer in vitro using two prostate cell lines namely, LNCaP and PC-3 aiming at reaching a drug that combine both 5 α -reductase inhibitors activities and anti-prostate cancer activities. All the tested compounds were screened as anti-tumor activities in two prostate cell lines namely, LNCaP and PC-3. The descending order of anti-tumor activities in two prostate cell lines was as follow: **6a**, **6b**, **6c**, **5a**, **5b**, **5c**, **3a**, **3b**, **3c**.

Conclusion

All the tested compounds showed potent 5 α -reductase inhibitors activities and 5 α -reductase inhibitors activities. The descending order of activities was as follow: **6a**, **6b**, **6c**, **5a**, **5b**, **5c**, **3a**, **3b**, **3c**.

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