

## Electronic supplementary information

# Design and synthesis of 5-aminolaevulinic Acid / 3-hydroxypyridinone conjugates for photodynamic therapy: enhancement of protoporphyrin IX production and photo-toxicity in tumor cells

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## Experimental

**Instruments.** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded on a Bruker Avance 500 spectrometer (Bruker Corp., Germany) with TMS as an internal standard. Electrospray ionization (ESI) mass spectra were obtained by infusing samples into an LCQ Deca XP ion-trap instrument (ThermoFinnigan, San Jose, CA). High resolution mass spectra (HRMS) were determined on a QTOFMicro (Waters, U.S.) by direct infusing samples into the ESI source.

## Synthetic Procedures

**Compound 2:** Yield: 84%, <sup>1</sup>HNMR (400MHz, CDCl<sub>3</sub>) δ 2.08 (s, 3H, CH<sub>3</sub>), 5.14 (s, 2H, PhCH<sub>2</sub>O), 6.35 (d, *J* = 4 Hz, 1H, Pyridinone C5-H), 7.26-7.38 (m, 5H, Ar ), 7.57 (d, *J* = 4Hz,

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Pyridinone C6-H) , ESI-MS m/z 217 ([M+H]<sup>+</sup>).

General procedure for the preparation of compounds **3**:

A mixture of **2** (10g, 46.3mmol), amine RNH<sub>2</sub> (55mmol), sodium hydroxide (4g, 50mmol) in methanol/water (30mL/30mL) was refluxed. The reaction was monitored by TLC. After completion of the reaction (about 2h), the reactant was concentrated under reduced pressure to about half volume, adjust to pH 2 with concentrated HCl, washed with Et<sub>2</sub>O (40mL×2). The aqueous layer was then adjust to pH 10 with 10 M of NaOH, extracted with dichloromethane (50mL×3), the combined organic layers were washed with brine twice and dried over anhydrous sodium sulfate. After removal of the solvent, the crude product **3** was obtained as a brown oil.

**3a.** Yield: 88%. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 2.06 (s, 3H, CH<sub>3</sub>), 3.44 (s, 3H, NCH<sub>3</sub>), 5.13 (s, 2H, PhCH<sub>2</sub>), 6.28 (d, *J* = 8Hz, 1H, Pyridinone C5-H), 7.18 (d, *J* = 8Hz, 1H, Pyridinone C6-H), 7.23-7.39 (m, 5H, Ar). ESI-MS m/z 230 ([M+H]<sup>+</sup>).

**3b.** Yield: 86%. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 1.28 (t, *J* = 8Hz, 3H, CH<sub>3</sub>CH<sub>2</sub>), 2.08 (s, 3H, CH<sub>3</sub>), 3.80 (d, *J*=8Hz, NCH<sub>2</sub>), 5.18 (s, 2H, PhCH<sub>2</sub>), 6.44 (d,*J*=8Hz, Pyridinone C5-H), 7.20 (d, *J* = 8Hz, Pyridinone C6-H), 7.26-7.38 (m, 5H, Ar). ESI-MS m/z 244([M+H]<sup>+</sup>).

**3c.** Yield: 87%. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 0.89 (t, 3H, *J* = 4Hz, CH<sub>3</sub>), 1.24-1.30 (m, 2H, CH<sub>2</sub>), 1.57-1.62(m, 2H, CH<sub>2</sub>), 2.07 (s, 3H, CH<sub>3</sub>), 3.71 (t, *J* = 8Hz, 2H, NCH<sub>2</sub>), 5.21 (s, 2H, PhCH<sub>2</sub>), 6.41 (d, *J* = 8Hz, 1H, Pyridinone C5-H ), 7.16 (d, *J* = 8Hz, 1H, Pyridinone C6-H), 7.25-7.39 (m, 5H, Ar). ESI-MS m/z 272 ([M+H]<sup>+</sup>).

**3d.** Yield: 90%. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 0.90 (t, *J* = 8Hz, 3H, CH<sub>3</sub>), 1.21-1.30 (m, 6H, CH<sub>2</sub>), 1.58-1.62 (m, 2H, CH<sub>2</sub>), 2.07 (s, 3H, CH<sub>3</sub>), 3.75 (t, *J* = 8Hz, 2H, NCH<sub>2</sub>), 5.23 (s, 2H, PhCH<sub>2</sub>), 6.43(d, *J* = 8Hz, 1H, Pyridinone C5-H), 7.17(d, *J* = 8Hz, 1H, Pyridinone C6-H), 7.27-7.40 (m, 5H, Ar). ESI-MS m/z 300 ([M+H]<sup>+</sup>).

**3e.** Yield: 84%. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) 2.09 (s, 3H, CH<sub>3</sub>), 3.16 (s, 3H, OCH<sub>3</sub>),

3.48 (t,  $J= 4\text{Hz}$ , 2H, OCH<sub>2</sub>), 3.80 (t,  $J= 4\text{Hz}$ , 2H, NCH<sub>2</sub>), 5.15 (s, 2H, PhCH<sub>2</sub>), 6.35 (d,  $J= 8\text{Hz}$ , 1H, Pyridinone C5-H), 7.22-7.34 (m, 5H, Ar), 7.37(d,  $J= 8\text{Hz}$ , 1H, Pyridinone C6-H), ESI-MS m/z 274 ([M+H]<sup>+</sup>).

General procedure for the preparation of compounds **4**:

A mixture of **3** (10mmol), SeO<sub>2</sub> (30mmol) in acetic acid/acetic anhydride (25mL/25mL) was heated at 90-100°C for 4-6h. After removal of the solvent, the residue was purified by silica gel column chromatography using ethyl acetate/methanol (50:1~20:1) as an eluent to provide aldehyde **4** as a brown oil.

**4a.** Yield: 77%, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 400MHz) δ 3.78(s, 3H, NCH<sub>3</sub>), 5.50(s, 2H, CH<sub>2</sub>), 7.30- 7.36(m, 5H, Ar), 6.49(d,  $J= 8\text{Hz}$ , 1H, C5-H in pyridinone), 7.16(d,  $J= 8\text{Hz}$ , 1H, C6-H in pyridinone), 10.05(s, 1H, CHO). ESI-MS m/z 244([M+H]<sup>+</sup>).

**4b.** Yield: 79%, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 400MHz) δ 1.26(t,  $J= 8\text{Hz}$ , 3H, CH<sub>3</sub>), 4.19(q,  $J= 8\text{Hz}$ , 2H, NCH<sub>2</sub>), 5.50(s, 2H, PhCH<sub>2</sub>), 6.54(d,  $J= 8\text{Hz}$ , 1H, C5-H in pyridinone), 7.20(d,  $J= 8\text{Hz}$ , 1H, C6-H in pyridinone), 7.30- 7.35(m, 5H, Ar), 10.03(s, 1H, CHO). ESI-MS m/z 258([M+H]<sup>+</sup>).

**4c.** Yield: 78%, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 400MHz) δ 0.88 (t,  $J= 8\text{Hz}$ , 3H, CH<sub>3</sub>), 1.33(m, 2H, CH<sub>2</sub>), 1.77(m, 2H, CH<sub>2</sub>), 4.12(t,  $J= 8\text{Hz}$ , 2H, NCH<sub>2</sub>), 5.48(s, 2H, PhCH<sub>2</sub>), 6.50(d,  $J= 8\text{Hz}$ , 1H, C5-H in pyridinone), 7.20 (d,  $J= 8\text{Hz}$ , 1H, C6-H in pyridinone), 7.29- 7.34(m, 5H, Ar), 10.01(s, 1H, CHO). ESI-MS m/z 286([M+H]<sup>+</sup>).

**4d.** Yield: 79%, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 400MHz) δ 0.89 (t,  $J= 8\text{Hz}$ , 3H, CH<sub>3</sub>), 1.33(m, 6H, CH<sub>2</sub>), 1.77(m, 2H, CH<sub>2</sub>), 4.13(t,  $J= 8\text{Hz}$ , 2H, NCH<sub>2</sub>), 5.49(s, 2H, PhCH<sub>2</sub>), 6.51(d,  $J= 4\text{Hz}$ , 1H, C5-H in pyridinone), 7.19 (d,  $J= 4\text{Hz}$ , 1H, C6-H in pyridinone), 7.30- 7.34(m, 5H, Ar), 10.01(s, 1H, CHO). ESI-MS m/z 314 ([M+H]<sup>+</sup>).

**4e.** Yield: 80%, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz) δ 3.23(s, 3H, OCH<sub>3</sub>), 3.47(t, *J*= 4Hz, 2H, OCH<sub>2</sub>), 4.31(t, *J*= 4Hz, 2H, NCH<sub>2</sub>), 5.50(s, 2H, PhCH<sub>2</sub>), 6.49(d, *J*= 8Hz, 1H, C5-H in pyridinone), 7.25(d, *J*= 8Hz, 1H, C6-H in pyridinone), 7.31- 7.35(m, 5H, Ar), 10.00(s, 1H, CHO). ESI-MS m/z 288([M+H]<sup>+</sup>).

General procedure for the preparation of compounds **5**:

To a solution of **4** (10mmol) in acetone/water (20mL/20mL) was added sodium chlorite (12mmol) and sulfamic acid (15mmol). The mixture was stirred at room temperature overnight. After filtration, compounds **5** were obtained as white solids.

**5a.** Yield: 65%, <sup>1</sup>H NMR (DMSO, 400MHz) δ 3.60 (s, 3H, NCH<sub>3</sub>), 5.03(s, 2H, CH<sub>2</sub>), 6.31(d, *J*= 4Hz, 1H, C5-H in pyridinone), 7.24- 7.36(m, 5H, Ar), 7.65(d, *J*= 8Hz, 1H, C6-H in pyridinone), ESI-MS m/z 260 ([M+H]<sup>+</sup>).

**5b.** Yield: 62%, <sup>1</sup>H NMR (DMSO, 400MHz) δ 1.30(t, *J*= 8Hz, 3H, CH<sub>3</sub>), 4.31(q, *J*= 8Hz, 2H, NCH<sub>2</sub>), 5.35(s, 2H, PhCH<sub>2</sub>), 6.59(d, *J*= 8Hz, 1H, C5-H in pyridinone), 7.28(d, *J*= 8Hz, 1H, C6-H in pyridinone), 7.22- 7.35 (m, 5H, Ar). ESI-MS m/z 274([M+H]<sup>+</sup>).

**5c.** Yield: 55%, <sup>1</sup>H NMR (DMSO, 400MHz) δ 0.86(t, *J*= 8Hz, 3H, CH<sub>3</sub>), 1.42(m, 2H, CH<sub>2</sub>), 1.79(m, 2H, CH<sub>2</sub>), 4.25(t, *J*= 8Hz, 2H, NCH<sub>2</sub>), 5.45(s, 2H, PhCH<sub>2</sub>), 6.55(d, *J*= 8Hz, 1H, C5-H in pyridinone), 7.20(d, *J*= 8Hz, 1H, C6-H in pyridinone), 7.25- 7.33(m, 5H, Ar). ESI-MS m/z 302([M+H]<sup>+</sup>).

**5d.** Yield: 54%, <sup>1</sup>H NMR (DMSO, 400MHz) δ 0.84(t, *J*= 8Hz, 3H, CH<sub>3</sub>), 1.20- 1.26(m, 6H, CH<sub>2</sub>), 1.66- 1.72(m, 2H, CH<sub>2</sub>), 3.88(t, *J*= 8Hz, 2H, NCH<sub>2</sub>), 5.04(s, 2H, PhCH<sub>2</sub>), 6.36(d, *J*= 8Hz, 1H, C5-H in pyridinone), 7.27- 7.42(m, 5H, Ar), 7.72(d, *J*= 8Hz, 1H, C6-H in pyridinone). ESI-MS m/z 330 ([M+H]<sup>+</sup>).

**5e.** Yield: 60%, <sup>1</sup>H NMR (DMSO, 400MHz) δ 3.22 (s, 3H, OCH<sub>3</sub>), 3.59(t, *J*= 4Hz, 2H, OCH<sub>2</sub>), 4.07(t, *J*= 4Hz, 2H, NCH<sub>2</sub>), 5.04(s, 2H, PhCH<sub>2</sub>), 6.34(d, *J*= 8Hz, 1H, C5-H in pyridinone), 7.28- 7.41(m, 5H, Ar), 7.61(d, *J*= 8Hz, 1H, C6-H in pyridinone). ESI-MS m/z

304 ( $[M+H]^+$ ) .

General procedure for the preparation of compounds **6**:

To a solution of **5** (5mmol) and hydrochloride salt of amino acid ester (5mmol) in DMF (30mL) was added HCTU (5.5mmol) and Et<sub>3</sub>N (15mmol). The mixture was stirred at room temperature overnight. The reactant was concentrated and the residue was dissolved in dichloromethane (50mL). The resulting solution was washed with 5% NaHCO<sub>3</sub> and brine successively. After removal of the solvent, residue was purified by column chromatography (MeOH/EtOAc, 1:20 to 1:3) to obtain the product **6**.

**6a.** Yield: 78%, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 400MHz) δ 1.21 (t, *J*=7.2Hz, 3H, CH<sub>3</sub>), 3.04- 3.15 (m, 2H, CH<sub>2</sub>), 3.39 (s, 3H, CH<sub>3</sub>), 4.13 (q, *J*= 7.2Hz, 2H, COOCH<sub>2</sub>), 4.83 (q, *J*=6.8Hz, 1H, CH), 5.05-5.14 (m, 2H, CH<sub>2</sub>), 6.21 (d, *J*= 7.2Hz, 1H, C5-H in pyridinone), 6.93 (d, *J*=7.2Hz, 1H, C6-H in pyridinone), 7.13-7.30 (m, 10H, Ph), 7.91 (d, *J*= 7.6Hz, 1H, NH). ESI-MS: *m/z* 435 ( $[M+H]^+$ ).

**6b.** Yield: 75%, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 500MHz) δ 1.14 (t, *J*= 6.0Hz, 3H, CH<sub>3</sub>), 1.24 (t, *J*= 5.6Hz, 3H, CH<sub>3</sub>), 3.09-3.20 (m, 2H, CH<sub>2</sub>), 3.57-3.73 (m, 2H, CH<sub>2</sub>), 4.09 (q, *J*= 5.6Hz, 2H, CH<sub>2</sub>), 4.83 (q, *J*=5.6Hz, 1H, CH), 4.97-5.08(m, 2H, CH<sub>2</sub>), 6.13 (d, *J*=6.0 Hz, 1H, C5-H in pyridinone), 6.93 (d, *J*= 6.0Hz, 1H, C6-H in pyridinone), 7.14- 7.34 (m, 10H, Ph), 8.88 (br, 1H, NH). ESI-MS: *m/z* 449 ( $[M+H]^+$ ).

**6c.** Yield: 72%, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 500MHz) δ 0.81 (t, *J*= 6.0 Hz, 3H, CH<sub>3</sub>), 1.08 (m, 2H, CH<sub>2</sub>), 1.12 (t, *J*= 6.0 Hz, 3H, CH<sub>3</sub>), 1.50 (m, 2H, CH<sub>2</sub>), 2.94 and 3.06 (m, 2H, CH<sub>2</sub>), 3.42 and 3.68 (m, 2H, CH<sub>2</sub>), 4.01 (t, *J*= 5.6Hz, 2H, CH<sub>2</sub>), 4.84 (q, *J*= 5.6Hz, 1H, CH), 4.97- 5.10 (m, 2H, CH<sub>2</sub>), 6.76 (d, *J*= 6.0Hz, 1H, C5-H in pyridinone), 6.99 (d, *J*= 6.0Hz, 1H, C6-H in pyridinone), 7.13-7.21 (m, 10H, Ph). ESI-MS: *m/z* 477 ( $[M+H]^+$ ).

**6d.** Yield: 77%, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 400MHz) δ 0.85 (t, *J*= 7.2Hz, 3H, CH<sub>3</sub>), 1.07- 1.26

(m, 9H, 3CH<sub>2</sub> and CH<sub>3</sub>), 1.54 (m, 2H, CH<sub>2</sub>), 2.92- 3.07 (m, 2H, CH<sub>2</sub>), 3.46 and 3.69 (m, 2H, CH<sub>2</sub>), 4.02 (m, 2H, CH<sub>2</sub>), 4.83 (q, *J*=7.2Hz, 1H, CH), 5.01-5.10(m, 2H, CH<sub>2</sub>), 6.69 (d, *J*= 7.2Hz, 1H, C5-H in pyridinone), 7.00 (d, *J*= 8.0Hz, 1H, C6-H in pyridinone), 7.10- 7.21 (m, 10H, Ph). ESI-MS: *m/z* 505 ([M+H]<sup>+</sup>).

**6e.** Yield: 81%, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz) δ 1.11 (t, *J*= 7.2Hz, 3H, CH<sub>3</sub>), 3.01- 3.13 (m, 2H, CH<sub>2</sub>), 3.26 (s, 3H, OCH<sub>3</sub>), 3.47 and 3.62 (m, 2H, CH<sub>2</sub>), 3.78 and 3.92 (m, 2H, CH<sub>2</sub>), 4.02-4.10(m, 2H, CH<sub>2</sub>), 4.69 (q, *J*= 7.2Hz, 1H, CH), 4.94-5.15(m, 2H, CH<sub>2</sub>), 6.13(d, *J*= 7.2Hz, 1H, C5-H in pyridinone), 7.08- 7.32 (m, 11H, 2Ph and buried C6-H in pyridinone), 8.94 (d, *J*= 6.8Hz, 1H, NH). ESI-MS: *m/z* 479 ([M+H]<sup>+</sup>).

**6f.** Yield: 76%, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz) δ 0.71 (d, *J*= 6.0Hz, 3H, CH<sub>3</sub>), 0.75 (d, *J*= 6.0Hz, 3H, CH<sub>3</sub>), 1.27 (t, *J*= 7.2Hz, 3H, CH<sub>3</sub>), 1.63-1.77 (m, 3H, CH<sub>2</sub> and CH), 3.66 (s, 3H, CH<sub>3</sub>), 4.18 (m, 2H, CH<sub>2</sub>), 4.55 (m, 1H, CH), 4.91 and 5.30 (m, 2H, CH<sub>2</sub>), 6.16 (d, *J*= 7.6Hz, 1H, C5-H in pyridinone), 6.98 (d, *J*= 7.6Hz, 1H, C6-H in pyridinone), 7.21-7.33 (m, 5H, Ph), 8.83 (d, *J*= 7.2Hz, 1H, NH). ESI-MS: *m/z* 401 ([M+H]<sup>+</sup>).

**6g.** Yield: 77%, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500MHz) δ 0.69 and 0.74 (br, 6H, CH<sub>3</sub>), 1.26 (m, 3H, CH<sub>3</sub>), 1.42 (m, 3H, CH<sub>3</sub>), 1.64-1.75 (m, 3H, CH<sub>2</sub> and CH), 3.93 and 4.01 (m, 2H, CH<sub>2</sub>), 4.18 (m, 2H, CH<sub>2</sub>), 4.56 (m, 1H, CH), 4.91 and 5.30 (m, 2H, CH<sub>2</sub>), 6.22 (d, *J*=7.5Hz, 1H, C5-H in pyridinone), 7.11 (d, *J*=7.5 Hz, 1H, C6-H in pyridinone), 7.22 (m, 3H, Ph), 7.34 (m, 2H, Ph), 8.98 (br, 1H, CONH). ESI-MS: *m/z* 415 ([M+H]<sup>+</sup>).

**6h.** Yield: 68%, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500MHz) δ 0.76 (d, *J*= 6.0Hz, 3H, CH<sub>3</sub>), 0.79 (d, *J*= 6.0Hz, 3H, CH<sub>3</sub>), 0.92 (t, *J*= 7.5Hz, 3H, CH<sub>3</sub>), 1.27 (t, *J*= 7.5Hz, 3H, CH<sub>3</sub>), 1.31 (m, 2H, CH<sub>2</sub>), 1.61 (m, 3H, CH<sub>2</sub> and CH), 1.74 (m, 2H, CH<sub>2</sub>), 3.80- 3.97 (m, 2H, CH<sub>2</sub>), 4.16 (q, *J*= 7.0Hz, 2H, COOCH<sub>2</sub>), 4.57 (q, *J*= 7.5Hz, 1H, CH), 4.99 and 5.30 (m, 2H, CH<sub>2</sub>), 6.42 (d, *J*=7.5Hz, 1H, C5-H in pyridinone), 7.12 (d, *J*=7.0Hz, 1H, C6-H in pyridinone), 7.23-7.34 (m, 5H, Ph), 7.74 (d, *J*= 5.0Hz, 1H, CONH). ESI-MS: *m/z* 443 ([M+H]<sup>+</sup>).

**6i.** Yield: 74%, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500MHz) δ 0.71 (d, *J*= 6.0Hz, 3H, CH<sub>3</sub>), 0.74 (d, *J*= 6.0Hz, 3H, CH<sub>3</sub>), 0.85 (t, *J*= 7.0Hz, 3H, CH<sub>3</sub>), 1.22-1.29 (m, 9H, CH<sub>3</sub> and 3CH<sub>2</sub>), 1.61-1.70 (m, 3H, CH<sub>2</sub> and CH), 1.77 (m, 2H, CH<sub>2</sub>), 3.77 and 3.96 (m, 2H, CH<sub>2</sub>), 4.15 (q, *J*= 7.0Hz, 2H, CH<sub>2</sub>), 4.56 (q, *J*=7.5Hz, 1H, CH), 4.90 and 5.28 (m, 2H, CH<sub>2</sub>), 6.24 (d, *J*= 7.5Hz, 1H, C5-H in pyridinone), 7.05 (d, *J*=7.5Hz, 1H, C6-H in pyridinone), 7.21 (m, 3H, Ph), 7.32 (m, 2H, Ph), 8.64 (br, 1H, NH). ESI-MS: *m/z* 471 ([M+H]<sup>+</sup>).

**6j.** Yield: 72%, <sup>1</sup>H NMR(CDCl<sub>3</sub>, 500MHz) δ 0.58 (t, *J*= 6.0Hz, 3H, CH<sub>3</sub>), 0.64 (d, *J*= 6.0Hz, 3H, CH<sub>3</sub>), 1.20 (t, *J*= 7.0Hz, 3H, CH<sub>3</sub>), 1.54- 1.66 (m, 3H, CH<sub>2</sub>CH), 3.27 (s, 3H, OCH<sub>3</sub>), 3.59- 3.72 (m, 2H, NCH<sub>2</sub>), 3.97- 4.10 (m, 2H, OCH<sub>2</sub>), 4.12- 4.16 (m, 2H, COOCH<sub>2</sub>), 4.43 (m, 1H, CHCOO), 4.81 and 5.31 (m, 2H, OCH<sub>2</sub>Ph), 6.09 (d, *J*= 7.5Hz, 1H, C5-H in pyridinone), 7.11-7.17 (m, 4H, 3H from Ph and C6-H in pyridinone), 7.27 (m, 2H, Ph), 8.95 (d, *J*= 6.5Hz, 1H, CONH). ESI-MS: *m/z* 445 ([M+H]<sup>+</sup>).

General procedure for the preparation of compounds **7**:

To a solution of **6** (1mmol) in methanol (15mL) cooled with ice-bath was add dropwise 0.2M LiOH (15mL). The stirring was continued for 1h. Amberlite® IR120 (H form) ion exchange resin was added to the resulting solution until pH 3-4. After filtration, the filtrate was concentrated to provide **7**.

**7a.** Yield: 93%, <sup>1</sup>H NMR (DMSO, 500MHz) δ 3.06- 3.19 (m, 2H, CH<sub>2</sub>Ph), 3.41 (s, 3H, NCH<sub>3</sub>), 4.78- 4.85 (m, 1H, CHCOOH), 5.04- 5.12 (m, 2H, OCH<sub>2</sub>Ph), 6.22 (d, *J*= 6.5Hz, 1H, C5-H in pyridinone), 7.10-7.33(m, 10H, Ph), 7.74 (d, *J*=6.5Hz, 1H, C6-H in pyridinone), 8.97 (d, *J*=7.5Hz, 1H, NH). ESI-MS: *m/z* 407 ([M+H]<sup>+</sup>).

**7b.** Yield: 94%, <sup>1</sup>H NMR (DMSO, 500MHz) δ 1.18 (t, *J*= 7.0Hz, 3H, CH<sub>3</sub>), 3.05- 3.19 (m, 2H, CH<sub>2</sub>Ph), 3.60- 3.72 (m, 2H, NCH<sub>2</sub>), 4.79- 4.83 (m, 1H, CHCOOH), 4.99-5.10 (m, 2H, OCH<sub>2</sub>Ph), 6.15 (d, *J*= 6.5 Hz, 1H, C5-H in pyridinone), 7.15- 7.35(m, 10H, Ph), 7.54(d, *J*=

6.5Hz, 1H, C6-H in pyridinone), 9.08 (d,  $J=7.5$ Hz, 1H, NH). ESI-MS:  $m/z$  421 ([M+H]<sup>+</sup>).

**7c.** Yield: 93%, <sup>1</sup>HNMR (DMSO, 500MHz)  $\delta$  0.83 (t,  $J=6.5$ Hz, 3H, CH<sub>3</sub>), 1.13- 1.29 (m, 2H, CH<sub>2</sub>), 1.44- 1.53(m, 2H, CH<sub>2</sub>), 2.92- 3.07(m, 2H, CH<sub>2</sub>Ph), 3.43- 3.72 (m, 2H,NCH<sub>2</sub>), 4.88 (m, 1H, CHCOOH), 4.95-5.10 (m, 2H, OCH<sub>2</sub>Ph), 6.48 (d,  $J=6.0$ Hz, 1H, C5-H in pyridinone), 6.77 (d,  $J=6.0$ Hz, 1H, C6-H in pyridinone), 7.10-7.23 (m, 10H, Ph), 9.12 (d,  $J=7.5$ Hz, 1H, NH). ESI-MS:  $m/z$  449 ([M+H]<sup>+</sup>).

**7d.** Yield: 92%, <sup>1</sup>HNMR(DMSO, 500MHz)  $\delta$  0.84 (t,  $J=7.0$ Hz, 3H,CH<sub>3</sub>), 1.09- 1.24 (m, 6H, CH<sub>2</sub>), 1.50- 1.59 (m, 2H, CH<sub>2</sub>), 2.85- 3.16 (m, 2H, CH<sub>2</sub>Ph), 3.44- 3.55 (m, 2H, NCH<sub>2</sub>), 4.52- 4.58 (m, 1H, CHCOOH), 4.87-5.01 (m, 2H, OCH<sub>2</sub>Ph), 6.22 (d,  $J=6.5$ Hz, 1H,C5-H in pyridinone), 7.12-7.34 (m, 10H, Ph), 7.63 ( d,  $J=6.5$ Hz, 1H, C6-H in pyridinone), 9.17 (d,  $J=7.5$ Hz, 1H, NH). ESI-MS:  $m/z$  477 ([M+H]<sup>+</sup>).

**7e.** Yield:92%, <sup>1</sup>HNMR (DMSO, 500MHz)  $\delta$  3.01- 3.14 (m, 2H, CH<sub>2</sub>Ph), 3.27 (s, 3H, OCH<sub>3</sub>), 3.42-3.59 (m, 2H, NCH<sub>2</sub>), 3.77- 3.95 (m, 2H, OCH<sub>2</sub>), 4.58- 4.65 (m, 1H, CHCOOH), 4.90- 5.14 (m, 2H, OCH<sub>2</sub>Ph), 6.21 (d,  $J=6.5$ Hz, 1H, C5-H in pyridinone), 7.07-7.25 (m, 10H, Ph), 7.30 (d,  $J=6.5$ Hz, 1H, C6-H in pyridinone), 9.15 (d,  $J=7.5$ Hz, 1H, NH). ESI-MS:  $m/z$  451 ([M+H]<sup>+</sup>).

**7f.** Yield: 92%, <sup>1</sup>HNMR (DMSO, 500MHz)  $\delta$  0.73 (d,  $J=6.5$ Hz, 3H, CH<sub>3</sub>), 0.77 (d,  $J=6.5$ Hz, 3H, CH<sub>3</sub>), 1.48-1.63 (m, 3H, CH<sub>2</sub>CH), 3.60 (s, 3H, NCH<sub>3</sub>), 4.30- 4.34 (m, 1H, CHCOO), 5.03-5.08 (m, 2H, OCH<sub>2</sub>Ph), 6.25 (d,  $J=7.5$ Hz, 1H, C5-H in pyridinone), 7.28- 7.40 (m, 5H, Ph), 7.63 (d,  $J=7.5$ Hz, 1H, C6-H in pyridinone), 9.18 (d,  $J=7.5$ Hz, 1H, NH). ESI-MS:  $m/z$  373 ([M+H]<sup>+</sup>).

**7g.** Yield: 93%, <sup>1</sup>HNMR (DMSO, 500MHz)  $\delta$  0.67 (d,  $J=10.0$ Hz, 6H, CH<sub>3</sub>), 1.12- 1.22 (m, 3H, CH<sub>2</sub>CH<sub>3</sub>), 1.45- 1.68 (m, 3H, CH<sub>2</sub>CH), 3.87 (q,  $J=7.5$ Hz, 2H, NCH<sub>2</sub>), 4.04- 4.12 (m, 1H, CHCOO), 4.80- 5.24 (m, 2H, OCH<sub>2</sub>Ph), 6.19 (d,  $J=7.5$ Hz, 1H, C5-H in pyridinone), 7.26-7.38 (m, 5H, Ph), 7.56 (d,  $J=7.5$ Hz, 1H, C6-H in pyridinone), 9.07 (d,  $J=7.5$ Hz, 1H,

NH). ESI-MS: *m/z* 387 ([M+H]<sup>+</sup>).

**7h.** Yield: 93%, <sup>1</sup>H NMR (DMSO, 500MHz) δ 0.74 (d, *J*= 6.5Hz, 3H, CH<sub>3</sub>), 0.78(d, *J*= 6.5Hz, 3H, CH<sub>3</sub>), 0.90 (t, *J*= 7.5Hz, 3H, CH<sub>3</sub>), 1.25-1.50 (m, 2H, CH<sub>2</sub>), 1.55- 1.73 (m, 5H, CH<sub>2</sub>CH), 3.98- 4.06 (m, 1H, CHCOO), 4.20- 4.29 (m, 2H, NCH<sub>2</sub>), 4.97- 5.06 (m, 2H, OCH<sub>2</sub>Ph), 6.20 (d, *J*=5.0 Hz, 1H, C5-H in pyridinone), 7.23- 7.36 (m, 5H, Ar), 7.53(d, *J*=5.0Hz, 1H, C6-H in pyridinone), 8.95 (d, *J*=7.5Hz, 1H, NH). ESI-MS: *m/z* 415 ([M+H]<sup>+</sup>).

**7i.** Yield: 91%, <sup>1</sup>H NMR (DMSO, 500MHz) δ 0.73 (d, *J*= 6.5Hz, 3H, CH<sub>3</sub>), 0.77 (d, *J*= 6.5Hz, 3H, CH<sub>3</sub>), 0.84 (t, *J*=7.0Hz, 3H, CH<sub>3</sub>), 1.16-1.35 (m, 6H, CH<sub>2</sub>), 1.37-1.55.(m, 2H, CH<sub>2</sub>), 1.50- 1.79 (m, 3H, CH<sub>2</sub>CH), 3.99- 4.07(m, 1H, CHCOO), 4.12- 4.25 (m, 2H, NCH<sub>2</sub>), 4.99- 5.07 (m, 2H, OCH<sub>2</sub>Ph), 6.21(d, *J*= 6.5Hz, 1H, C5-H in pyridinone), 7.22- 7.35 (m, 5H, Ph), 7.52 (d, *J*=6.5Hz, 1H, C6-H in pyridinone), 9.10 (d, *J*=7.5Hz, 1H, NH). ESI-MS: *m/z* 443 ([M+H]<sup>+</sup>).

**7j.** Yield: 94%, <sup>1</sup>H NMR (DMSO, 500MHz) 0.72 (d, *J*= 6.5Hz, 3H, CH<sub>3</sub>), 0.75 (d, *J*=6.5Hz, 3H, CH<sub>3</sub>), 1.14-1.62 (m, 3H, CH<sub>2</sub>CH), 3.24 (s, 3H, OCH<sub>3</sub>), 3.57-3.64 (m, 2H, OCH<sub>2</sub>), 4.00-4.06 (m, 1H, CHCOO), 4.18- 4.29 (m, 2H, NCH<sub>2</sub>), 5.02- 5.08 (m, 2H, OCH<sub>2</sub>Ph), 6.22 (d, *J*= 7.0Hz, 1H, C5-H in pyridinone), 7.27- 7.40 (m, 5H, Ar), 7.55 (d, *J*= 7.0Hz, 1H, C6-H in pyridinone), 8.95 (d, *J*= 8.0Hz, 1H, CONH). ESI-MS: *m/z* 417 ([M+H]<sup>+</sup>).

General procedure for the preparation of compounds **8**:

To a solution of **7** (2mmol) and hydrochloride salt of ALA methyl ester (2mmol) in DMF (10mL) was added HCTU (2.2mmol), followed by the addition of Et<sub>3</sub>N (6mmol). The reaction solution was stirred at room temperature overnight. After removal of the solvent, the residue was dissolved in dichloromethane (40mL). The resulting solution was washed with 5% NaHCO<sub>3</sub> and brine successively, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After removal of the solvent, residue was purified by column chromatography (MeOH/EtOAc, 1:20 to 1:2) to

obtain the product **8**.

**8a.** Yield: 77%,  $^1\text{H}$ NMR(DMSO-d<sub>6</sub>, 400MHz)  $\delta$  2.46 (t,  $J= 6.4\text{Hz}$ , 2H, CH<sub>2</sub>COO), 2.66(t,  $J= 6.4\text{Hz}$ , 2H, COCH<sub>2</sub>), 2.80 and 3.09 (m, 2H, CH<sub>2</sub>Ph), 3.13 (s, 3H, NCH<sub>3</sub>), 3.57 (s, 3H, COOCH<sub>3</sub>), 3.92 (m, 2H, NHCH<sub>2</sub>CO), 4.82 and 4.99 (m, 2H, OCH<sub>2</sub>Ph), 4.86 (m, 1H, CH), 6.17 (d,  $J= 7.2\text{Hz}$ , 1H, C5-H in pyridinone), 7.12-7.31 (m, 10H, Ph), 7.52 (d,  $J= 7.2\text{Hz}$ , 1H, C6-H in pyridinone), 8.45 (t,  $J= 5.6\text{Hz}$ , 1H, NH), 9.25 (d,  $J= 8.0\text{Hz}$ , 1H, NH). ESI-MS: *m/z* 534 ([M+H]<sup>+</sup>).

**8b.** Yield: 81%,  $^1\text{H}$ NMR(DMSO-d<sub>6</sub>, 400MHz)  $\delta$  1.02 (t,  $J= 7.2\text{Hz}$ , 3H, CH<sub>3</sub>), 2.45 (t,  $J= 6.4\text{Hz}$ , 2H, CH<sub>2</sub>COO), 2.65 (t,  $J= 6.4\text{Hz}$ , 2H, COCH<sub>2</sub>), 2.79 and 3.12 (m, 2H, CH<sub>2</sub>Ph), 3.30 and 3.55 (m, 2H, NHCH<sub>2</sub>CO), 3.57 (s, 3H, COOCH<sub>3</sub>), 3.88 (d,  $J= 5.6\text{Hz}$ , 2H, NCH<sub>2</sub>), 4.82 and 4.99 (m, 2H, OCH<sub>2</sub>Ph), 4.89 (m, 1H, CH), 6.21(d,  $J= 7.6\text{Hz}$ , 1H, C5-H in pyridinone), 7.13-7.31 (m, 10H, Ph), 7.59 (d,  $J= 7.6\text{Hz}$ , 1H, C6-H in pyridinone), 8.42 (t,  $J= 5.6\text{Hz}$ , 1H, NH), 9.29 (d,  $J= 8.0\text{Hz}$ , 1H, NH). ESI-MS: *m/z* 548 ([M+H]<sup>+</sup>).

**8c.** Yield: 80%,  $^1\text{H}$ NMR (DMSO-d<sub>6</sub>, 400MHz)  $\delta$  0.77 (t,  $J= 7.2\text{Hz}$ , 3H, CH<sub>3</sub>), 1.01-1.08(m, 2H, CH<sub>2</sub>), 1.40-1.48(m, 2H, CH<sub>2</sub>), 2.45 (t,  $J= 6.4\text{Hz}$ , 2H, CH<sub>2</sub>COO), 2.64 (t,  $J= 6.4\text{Hz}$ , 2H, COCH<sub>2</sub>), 2.80 and 3.09 (m, 2H, CH<sub>2</sub>Ph), 3.23 and 3.63 (m, 2H, NHCH<sub>2</sub>CO), 3.56 (s, 3H, COOCH<sub>3</sub>), 3.86 (d,  $J= 4.8\text{Hz}$ , 2H, NCH<sub>2</sub>), 4.79-4.88 and 5.00 (m, 3H, OCH<sub>2</sub>Ph and CH), 6.20 (d,  $J= 7.6\text{Hz}$ , 1H, C5-H in pyridinone), 7.14-7.31 (m, 10H, Ph), 7.59 (d,  $J= 7.6\text{Hz}$ , 1H, C6-H in pyridinone), 8.43 (t,  $J= 5.6\text{Hz}$ , 1H, NH), 9.28 (d,  $J= 8.0\text{Hz}$ , 1H, NH). ESI-MS: *m/z* 576 ([M+H]<sup>+</sup>).

**8d.** Yield: 77%,  $^1\text{H}$ NMR (CDCl<sub>3</sub>, 400MHz)  $\delta$  0.88 (t,  $J= 6.8\text{Hz}$ , 3H, CH<sub>3</sub>), 1.01-1.09 (m, 2H, CH<sub>2</sub>), 1.13-1.19 (m, 2H, CH<sub>2</sub>), 1.23-1.30 (m, 2H, CH<sub>2</sub>), 1.47-1.57 (m, 2H, CH<sub>2</sub>), 2.48 (m, 2H, CH<sub>2</sub>COO), 2.59 (m, 2H, COCH<sub>2</sub>), 2.93 and 3.14 (m, 2H, CH<sub>2</sub>Ph), 3.05 and 3.85 (m, 2H, NHCH<sub>2</sub>CO), 3.42 (m, 2H, NCH<sub>2</sub>), 3.64 (s, 3H, COOCH<sub>3</sub>), 4.83 and 5.26 (m, 2H, OCH<sub>2</sub>Ph), 4.86-4.92 (m, 1H, CH), 6.27 (d,  $J= 7.6\text{Hz}$ , 1H, C5-H in pyridinone), 6.89 (t,  $J= 5.2\text{Hz}$ , 1H,

NH), 7.13 (d,  $J=7.6$ Hz, 1H, C6-H in pyridinone), 7.19-7.32 (m, 10H, Ph), 8.62 (br, 1H, NH).

ESI-MS:  $m/z$  604 ([M+H]<sup>+</sup>).

**8e.** Yield: 75%, <sup>1</sup>HNMR (DMSO-d<sub>6</sub>, 400MHz) δ 2.46 (t,  $J=6.8$ Hz, 2H, CH<sub>2</sub>COO), 2.66 (t,  $J=6.8$ Hz, 2H, COCH<sub>2</sub>), 2.79 and 3.11 (m, 2H, CH<sub>2</sub>Ph), 3.16 (s, 3H, OCH<sub>3</sub>), 3.22-3.31(m, 2H, OCH<sub>2</sub>), 3.47 and 3.81 (m, 2H, NHCH<sub>2</sub>CO), 3.57 (s, 3H, COOCH<sub>3</sub>), 3.89 (m, 2H, NCH<sub>2</sub>), 4.81 and 4.98 (m, 2H, OCH<sub>2</sub>Ph), 4.84 (m, 1H, CHCH<sub>2</sub>Ph), 6.17 (d,  $J=7.2$ Hz, 1H, C5-H in pyridinone), 7.13-7.31(m, 10H, Ph), 7.45 (d,  $J=7.2$ Hz, 1H, C6-H in pyridinone), 8.45 (t,  $J=5.6$ Hz, 1H, NH), 9.30 (d,  $J=8.0$ Hz, 1H, NH). ESI-MS:  $m/z$  578 ([M+H]<sup>+</sup>).

**8f.** Yield: 79%, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 500MHz) δ 0.85 (t,  $J=6.5$ Hz, 6H, CH<sub>3</sub>), 1.69-1.78 (m, 3H, CH<sub>2</sub>CH), 2.53-2.59 (m, 2H, CH<sub>2</sub>COO), 2.64 (m, 2H, COCH<sub>2</sub>), 3.64 (s, 3H, NCH<sub>3</sub>), 3.68 (s, 3H, OCH<sub>3</sub>), 3.75 and 3.93 (m, 2H, NHCH<sub>2</sub>CO), 4.65 (m, 1H, CH), 4.87-5.15 (m, 2H, OCH<sub>2</sub>Ph), 6.30 (d,  $J=7.0$ Hz, 1H, C5-H in pyridinone), 7.08 (d,  $J=7.0$ Hz, 1H, C6-H in pyridinone), 7.28-7.33(m, 5H, Ph), 7.52 (t,  $J= 5.0$ Hz, 1H, NH), 8.98 (d,  $J=8.5$ Hz, 1H, NH). ESI-MS:  $m/z$  500 ([M+H]<sup>+</sup>).

**8g.** Yield: 78%, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 500MHz) δ 0.88 (d,  $J=6.0$ Hz, 6H, CH<sub>3</sub>), 1.46 (t,  $J=7.5$ Hz, 3H, CH<sub>3</sub>), 1.72-1.81(m, 3H, CH<sub>2</sub>CH), 2.50-2.59 (m, 2H, CH<sub>2</sub>COO), 2.62 (m, 2H, COCH<sub>2</sub>), 3.55 (m, 1H, from CH<sub>2</sub> in NHCH<sub>2</sub>CO), 3.64 (s, 3H, COOCH<sub>3</sub>), 3.94- 3.98 (m, 3H, NCH<sub>2</sub> and buried 1H from CH<sub>2</sub> in NHCH<sub>2</sub>CO), 4.65 (m, 1H, CH), 5.03 (s, 2H, OCH<sub>2</sub>Ph), 6.38 (d,  $J=7.5$ Hz, 1H, C5-H in pyridinone), 7.18 (d,  $J=7.5$ Hz, 1H, C6-H in pyridinone), 7.29-7.35 (m, 6H, Ph and NH), 9.06 (d,  $J=8.5$ Hz, 1H, NH). ESI-MS:  $m/z$  514 ([M+H]<sup>+</sup>).

**8h.** Yield: 78%, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 500MHz) δ 0.84 (m, 6H, CH<sub>3</sub>), 0.88 (t,  $J=7.5$ Hz, 3H, CH<sub>3</sub>), 1.25-1.31 (m, 2H, CH<sub>2</sub>), 1.67-1.81(m, 5H, CH<sub>2</sub> and CH<sub>2</sub>CH), 2.47 (m, 2H, CH<sub>2</sub>COO), 2.53 (m, 2H, COCH<sub>2</sub>), 3.42 (m, 1H, from CH<sub>2</sub> in NHCH<sub>2</sub>CO), 3.59 (s, 3H, COOCH<sub>3</sub>), 3.74-3.87(m, 3H, NCH<sub>2</sub> and buried 1H from CH<sub>2</sub> in NHCH<sub>2</sub>CO), 4.60 (m, 1H, CH), 4.91-5.04 (m, 2H, OCH<sub>2</sub>Ph), 6.32 (d,  $J=7.5$ Hz, 1H, C5-H in pyridinone), 7.12(d,  $J= 7.5$ Hz, 1H, C6-H in

pyridinone), 7.23-7.28 (m, 6H, Ph and NH), 9.03 (d,  $J=8.0\text{Hz}$ , 1H, NH). ESI-MS:  $m/z$  542 ([M+H]<sup>+</sup>).

**8i.** Yield: 80%, <sup>1</sup>HNMR ( $\text{CDCl}_3$ , 500MHz)  $\delta$  0.86-0.90 (m, 9H,  $\text{CH}_3$ ), 1.27 (m, 6H,  $\text{CH}_2$ ), 1.68-1.84 (m, 5H,  $\text{CH}_2$  and  $\text{CH}_2\text{CH}$ ), 2.48-2.55 (m, 2H,  $\text{CH}_2\text{COO}$ ), 2.58 (m, 2H,  $\text{COCH}_2$ ), 3.47 (m, 1H, from  $\text{CH}_2$  in  $\text{NHCH}_2\text{CO}$ ), 3.63 (s, 3H,  $\text{COOCH}_3$ ), 3.75-3.92 (m, 3H,  $\text{NCH}_2$  and buried 1H from  $\text{CH}_2$  in  $\text{NHCH}_2\text{CO}$ ), 4.63 (m, 1H, CH), 4.98-5.11 (m, 2H,  $\text{OCH}_2\text{Ph}$ ), 6.36 (d,  $J=7.5\text{Hz}$ , 1H, C5-H in pyridinone), 7.14 (d,  $J=7.5\text{Hz}$ , 1H, C6-H in pyridinone), 7.22 (t,  $J=5.0\text{Hz}$ , 1H, NH), 7.28-7.31 (m, 5H, Ph), 8.76 (d,  $J=8.0\text{Hz}$ , 1H, NH). ESI-MS:  $m/z$  570 ([M+H]<sup>+</sup>).

**8j.** Yield: 76%, <sup>1</sup>HNMR ( $\text{CDCl}_3$ , 500MHz)  $\delta$  0.73 (t,  $J=5.5\text{Hz}$ , 6H,  $\text{CH}_3$ ), 1.58-1.68 (m, 3H,  $\text{CH}_2\text{CH}$ ), 2.50 (m, 2H,  $\text{CH}_2\text{COO}$ ), 2.58 (m, 2H,  $\text{COCH}_2$ ), 3.24 (s, 3H,  $\text{OCH}_3$ ), 3.57 (s, 3H,  $\text{COOCH}_3$ ), 3.63 (m, 2H,  $\text{NHCH}_2\text{CO}$ ), 3.72-3.86 (m, 2H,  $\text{NCH}_2$ ), 3.99 (t,  $J=5.0\text{Hz}$ , 2H,  $\text{OCH}_2$ ), 4.53 (m, 1H,  $\text{NHCHCH}_2$ ), 4.92-5.10 (m, 2H,  $\text{OCH}_2\text{Ph}$ ), 6.22 (d,  $J=7.5\text{Hz}$ , 1H, C5-H in pyridinone), 7.19-7.24 (m, 5H, Ph), 7.52 (d,  $J=7.5\text{Hz}$ , 1H, C6-H in pyridinone), 8.45 (t,  $J=5.0\text{Hz}$ , 1H, NH), 8.80 (d,  $J=8.5\text{Hz}$ , 1H, NH). ESI-MS:  $m/z$  544 ([M+H]<sup>+</sup>).

General procedure for the preparation of compounds **Z01-10**:

To a suspension of **8** (2 mmol) and benzyl chloride (3 mmol) in MeOH (30 mL) was added 5% palladium/charcoal (0.3 g). Hydrogenation was carried out at 30 psi  $\text{H}_2$  for 3–4 h. After filtration to remove the catalyst, the filtrate was concentrated to dryness. The residue was purified by crystallization from methanol–diethyl ether. Conjugates **Z01-10** were obtained as hydrochlorides in 93-97% yield.