Upconversion Nanotheranostic Agent Activated by Hypoxia Combined with NIR Irradiation for Selective Hypoxia Imaging and Tumour Therapy

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Supplementary Information
1. The characterization of FDU-CA\(_{2}\)-NO\(_2\) and UCNP-CA\(_{2}\)-FDU/NO\(_2\)

2-(4-Nitrobenzyloxy)-4-(prop-2-ynyloxy)benzaldehyde (2): A yellow solid, Mp: 163.3-164.8 °C; \(^1H\)-NMR (600 MHz, DMSO-\(d_6\)): \(\delta\) 10.31 (s, 1 H), 8.28 (d, 2 H, \(J = 8.4\) Hz), 7.80 (d, 1 H, \(J = 2.4\) Hz), 7.73 (d, 1 H, \(J = 8.4\) Hz), 6.86 (d, 1 H, \(J = 2.4\) Hz), 6.76 (d, 1 H, \(J = 8.4\) Hz), 5.46 (s, 2 H), 4.94 (d, 2 H, \(J = 2.4\) Hz), 3.64 (s, 1 H). \(^{13}C\) NMR (DMSO-\(d_6\), 150 MHz, ppm): \(\delta\) 187.42, 163.66, 161.66, 147.10, 144.15, 130.18, 128.19, 123.68, 118.99, 107.70, 100.66, 78.90, 78.38, 68.67, 56.04. HRMS (ESI) \(m/z\) calcd for C\(_{17}\)H\(_{13}\)NO\(_5\) [M+H]\(^+\) 312.08665, found 312.08673.

(E)-\(\text{tert}\)-Butyl-3-(2-(4-nitrobenzyloxy)-4-(prop-2-ynyloxy)phenyl) acrylate (4): A white solid, Mp: 145.7-148.3 °C; \(^1H\)-NMR (DMSO-\(d_6\), 600 MHz, ppm): \(\delta\) 8.29 (d, 2 H, \(J = 9.0\) Hz), 7.83 (d, 1 H, \(J = 15.6\) Hz), 7.74 (d, 2 H, \(J = 9.0\) Hz), 7.72 (d, 1 H, \(J = 2.4\) Hz), 6.76 (d, 1 H, \(J = 2.4\) Hz), 6.67 (dd, 1 H, \(J = 9.0, 2.4\) Hz), 6.42 (d, 1 H, \(J = 15.6\) Hz), 5.39 (s, 2 H), 4.86 (d, 2 H, \(J = 2.4\) Hz), 3.60 (s, 1 H), 1.47 (s, 9 H). \(^{13}C\) NMR (DMSO-\(d_6\), 150 MHz, ppm): \(\delta\) 166.00, 160.20, 157.53, 147.11, 144.40, 137.70, 129.67, 128.22, 123.67, 117.71, 116.37, 107.27, 100.73, 79.46, 78.74, 78.51, 68.64, 55.73, 27.81. HRMS (ESI) \(m/z\) calcd for C\(_{23}\)H\(_{23}\)NO\(_6\) [M+H]\(^+\) 410.15981, found 410.16003.

(E)-3-(2-(4-Nitrobenzyloxy)-4-(prop-2-ynyloxy)phenyl) acrylic acid (5): A white solid, Mp: 197.6-199.5 °C. \(^1H\)-NMR (DMSO-\(d_6\), 600 MHz, ppm): \(\delta\) 8.28 (d, 2 H, \(J = 8.4\) Hz), 7.73 (d, 2 H, \(J = 8.4\) Hz), 7.50 (d, 1 H, \(J = 16.0\) Hz), 7.47 (d, 1 H, \(J = 2.4\) Hz), 6.69 (d, 1 H, \(J = 16.0\) Hz), 6.60 (dd, 1 H, \(J = 8.8, 2.4\) Hz), 6.30 (d, 1 H, \(J = 15.6\) Hz), 5.34 (s, 2 H), 4.79 (d, 2 H, \(J = 2.4\) Hz), 3.56 (s, 1 H). \(^{13}C\) NMR (DMSO-\(d_6\), 150 MHz, ppm): \(\delta\) 167.99, 160.14, 157.54, 147.18, 144.35, 138.22, 128.39, 123.73, 117.22, 116.48, 107.29, 100.76, 78.76, 78.51, 68.64, 55.74. HRMS (ESI) \(m/z\) calcd for C\(_{19}\)H\(_{15}\)NO\(_6\) [M+H]\(^+\) 354.09721, found 354.09723.

FDU-CA\(_{2}\)-NO\(_2\): A white solid. Mp: 143.4-146.3 °C. \(^1H\) NMR (DMSO-\(d_6\), 600 MHz, ppm): \(\delta\) 11.88 (s, 1 H), 8.28 (d, 2 H, \(J = 8.7\) Hz), 7.92 (d, 1 H, \(J = 16.0\) Hz), 7.87 (d, 1 H, \(J = 6.8\) Hz), 7.75 (d, 1 H, \(J = 8.8\) Hz), 7.72 (d, 2 H, \(J = 8.7\) Hz), 6.77 (d, 1 H, \(J = 2.0\) Hz), 6.69 (dd, 1 H, \(J = 8.8, 2.0\) Hz), 6.60 (d, 1 H, \(J = 16.0\) Hz), 6.14 (t, 1 H, \(J = 6.4\) Hz), 5.51 (d, 1 H, \(J = 4.4\) Hz), 5.40 (d, 2 H, \(J = 3.2\) Hz), 4.87 (d, 2 H, \(J = 2.0\) Hz), 4.82 (d, 2 H, \(J = 8.4\) Hz), 3.57 (s, 1 H).
Hz), 4.40-4.28 (m, 3 H), 4.01 (s, 1 H), 3.61 (s, 1 H), 2.24-2.09 (m, 2 H). 13C NMR (DMSO-d6, 150 MHz, ppm):
δ 166.47, 160.54, 157.03, 157.03, 156.77, 148.86, 147.09, 144.29, 141.12, 139.43, 138.83, 130.21, 128.26,
124.53, 124.19, 123.71, 116.12, 115.53, 107.34, 100.73, 84.55, 83.97, 78.71, 78.59, 70.00, 68.65, 63.67,
55.77. HRMS (ESI) m/z calcd for C29H24N3F10 [M+H]+ 582.15185, found 582.15216.

**7-Propargyloxycoumarin (CM)**: A white solid yield, Mp: 70.3-71.8 ºC. 1H NMR (CDCl3, 600 MHz, ppm): δ 7.64 (d, 1 H, J = 9.6 Hz), 7.41 (d, 1 H, J = 8.4 Hz), 6.94 (s, 1 H), 6.92 (d, 1 H, J = 8.4 Hz),
6.29 (d, 1 H, J = 9.6 Hz), 4.77 (d, 2 H, J = 2.4 Hz), 2.56 (s, 1 H). 13C NMR (CDCl3, 150 MHz, ppm): δ 160.98,
160.55, 155.66, 143.27, 128.84, 113.67, 113.20, 113.05, 102.16, 77.38, 76.57, 56.22. HRMS (ESI) m/z calcd for C12H8O3 [M+H]+ 201.05462, found 201.05461.

2. **The size of UCNP-CAEC-FDU/NO2 and UCNP analyzed by DLS and TEM**

The samples for TEM and DLS were prepared by dispersing UCNP-CAEC-FDU/NO2 (200 μg/mL) in PBS (25 mmol/L, pH 7.4) with intermittent ultrasonic by a needle type ultrasonic instrument. The samples of UCNP were dispersed in cyclohexane (20 μg/mL). The TEM sample was prepared by dropping on the surface of a copper grid and negative staining for 30 s by a droplet of phosphotungstic acid. The DLS of the samples were measured using a Nano-ZS system in disposable cuvettes.

3. **HPLC and HRMS analysis**

![HPLC profiles](image)

**Figure S1.** HPLC profiles of a) FDU; b) FDU-CAEC-NO2; c) CM; d) solution of FDU-CAEC-NO2 incubated with Na2S2O4 and then illuminated by UV light at 365 nm; e) UCNP-CAEC-FDU/NO2 incubated with Na2S2O4 and then illuminated by NIR light at 980 nm
Figure S2. HRMS of the solution of FDU-CA\textsubscript{F}-NO\textsubscript{2} incubated with Na\textsubscript{2}S\textsubscript{2}O\textsubscript{4} and then illuminated by UV-light at 365 nm.
Figure S3. HRMS of the mixture of UCNP-CA$_2$-FDU/NO$_2$ with Na$_2$S$_2$O$_4$ and then illuminated by NIR-light at 980 nm

4. NMR, IR and HRMS spectra

Figure S4. $^1$H NMR of 2-(4-nitrobenzyloxy)-4-(prop-2-ynyloxy)benzaldehyde (2)
**Figure S5.** $^{13}$C NMR of 2-(4-nitrobenzyloxy)-4-(prop-2-ynyloxy)benzaldehyde (2)

**Figure S6.** HRMS of 2-(4-nitrobenzyloxy)-4-(prop-2-ynyloxy)benzaldehyde (2)
Figure S7. IR of 4-nitrobenzylxy-4-diethylaminobenzaldehyde (2)

Figure S8. $^1$H NMR of (E)-tert-butyl-3-(2-(4-nitrobenzylxy)-4-(prop-2-ynyloxy)phenyl)acrylate (4)
Figure S9. $^{13}$C NMR of (E)-tert-butyl-3-(2-(4-nitrobenzyloxy)-4-(prop-2-ynyloxy)phenyl)acrylate (4)

Figure S10. HRMS of (E)-tert-butyl-3-(2-(4-nitrobenzyloxy)-4-(prop-2-ynyloxy)phenyl)acrylate (4)
Figure S11. IR of (E)-tert-butyl-3-(2-(4-nitrobenzyloxy)-4-(prop-2-ynyloxy)phenyl)acrylate (4)

Figure S12. $^1$H NMR of (E)-3-(2-(4-nitrobenzyloxy)-4-(prop-2-ynyloxy)phenyl)acrylic acid (5)
Figure S13. $^{13}$C NMR of (E)-3-(2-(4-nitrobenzyloxy)-4-(prop-2-ynyloxy)phenyl)acrylic acid (5)

Figure S14. HRMS of (E)-3-(2-(4-nitrobenzyloxy)-4-(prop-2-ynyloxy)phenyl)acrylic acid (5)
Figure S15. $^1$H NMR of FDU-CA$_E$-NO$_2$

Figure S16. $^{13}$C NMR of FDU-CA$_E$-NO$_2$
Figure S17. HRMS of FDU-CA\textsubscript{E}-NO\textsubscript{2}

Figure S18. IR of FDU-CA\textsubscript{E}-NO\textsubscript{2}
Figure S19. $^1$H NMR of CM

Figure S20. $^{13}$C NMR of CM
Figure S21. HRMS of CM

Figure S22. IR of CM
Figure S23. IR of UCNPs

Figure S24. IR of UCNP-CA-FDU/NO₂