Supporting Information

Synthesis of CDDO-Amino Acid-Nitric Oxide Donor Trihybrids as Potential Antitumor Agents against Both Drug-sensitive and Drug-resistant Colon Cancer

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Table of concents:

1. Expressing profile of PepT1 in colon cancer and noncancer cells

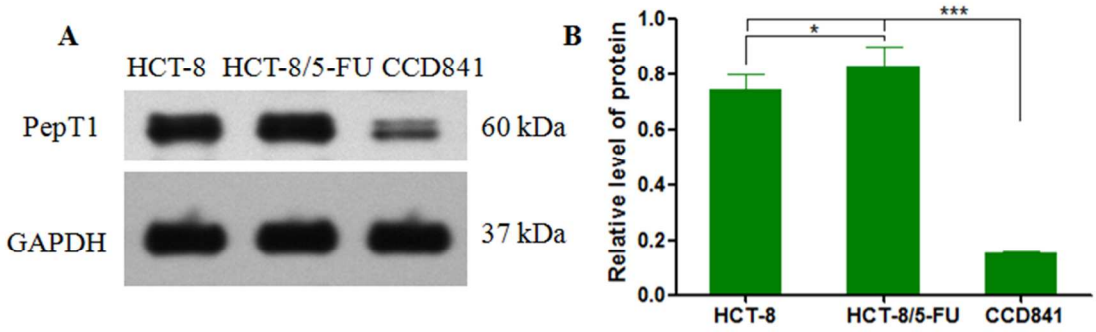


Figure S1. Expression profiles of PepT1 protein in HCT-8, HCT-8/5-FU, and CCD841 cell lines. Cell lysates of HCT-8, HCT-8/5-FU, and CCD841 cells were analyzed by Western blot. The expression of the house keeping protein GAPDH was used as a control of equal protein loading. Data are representative images and expressed as the means ± SD

of each group of cells from three separate experiments. A. Western blot analysis of the relative levels of PepT1 expression. B. Quantitative analysis. *P < 0.05 vs. the HCT-8/5-FU group, **P < 0.001 vs. the CCD841 group.

2. Procedure for the preparation of compound 5

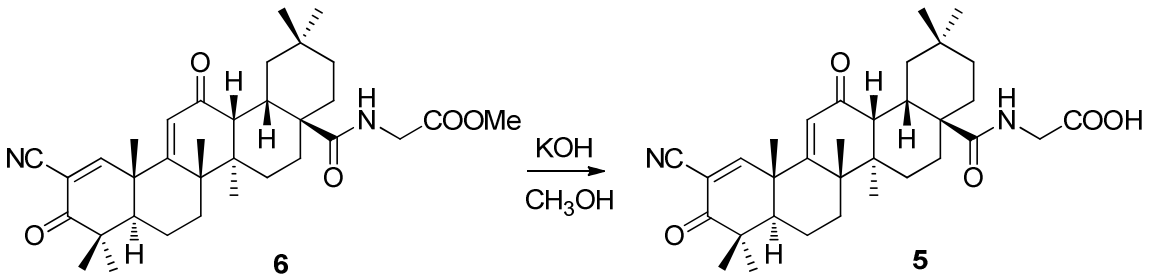


Figure S2. Synthetic Route for Compound **5**.

Compound **6** was prepared according to the method described previously.[1] The crude product was used without further purification. Thus, a mixture of **6** (0.2 g, 0.36 mmol), KOH (0.08 g, 2.4 mmol) in methenol was stirred at room temperature until the starting material was totally consumed as indicated by TLC. The mixture was then poured into CH_2Cl_2 (50 mL), and washed sequentially with 1 N HCl (3 × 25 mL) and saturated NaCl solution, and the organic fraction was dried over sodium sulfate. After removal of the solvent, the crude product was purified by column chromatography (PE : AcOEt = 2:1-1:1 v/v) to afford the title product **5** in 82 % yield as a white solid: mp 165-167 °C; ^{1}H NMR (300 M Hz, $CDCl_3$, 25 °C, TMS): δ 8.10 (s, 1H, C_1-H), 6.66 (br s, 1H, CONH), 6.02 (s, 1H, C_{11}-H), 4.04 (m, 2H, NH$\underline{CH_2}$CO), 3.08 (d, J = 4.2 Hz, 1H, C_{13}-H), 2.96-2.92 (m, 1H, C_{18}-H), 1.49 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 1.17 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.91 (s, 3H, CH_3) ppm; ^{13}C NMR (75 M Hz, $CDCl_3$, 25 °C, TMS): δ 199.2, 196.1, 178.0, 172.7, 169.2, 165.5, 123.4, 117.9, 114.0, 49.1, 47.1, 46.1, 45.5, 44.5, 42.1, 41.6, 35.6, 34.0, 33.5, 32.7, 31.3, 31.2, 30.1, 29.2, 28.8, 27.1, 26.5, 26.1, 24.4, 22.6, 21.3, 21.1, 17.7; ESI-MS: 549 $[M+H]^+$.

3. **HPLC assessment of compound purity**.

All tested compounds (**4a-i**) with a purity of > 95% were used for subsequent biological assays. We provided the spectra of HPLC assays as below.

Column: Inertex C18 (150 mm × 4.6 mm × 5 μm);

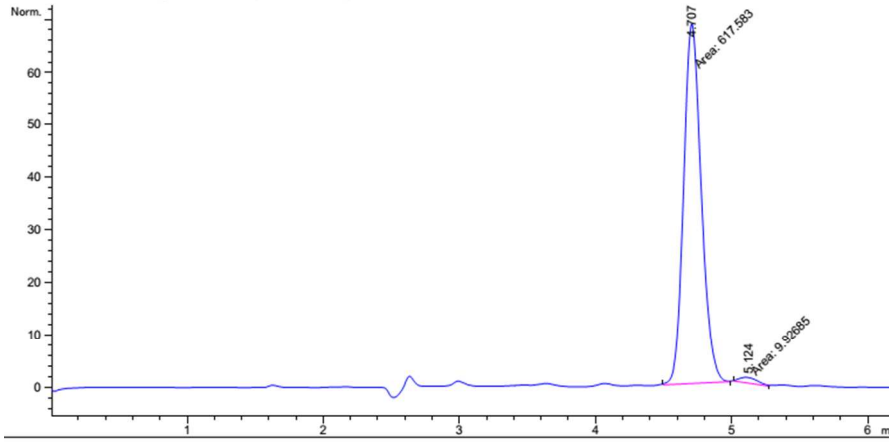
Mobile phase: Methanol-Water (80:20 to 95:5, v/v);

Wavelength: 244 nm;

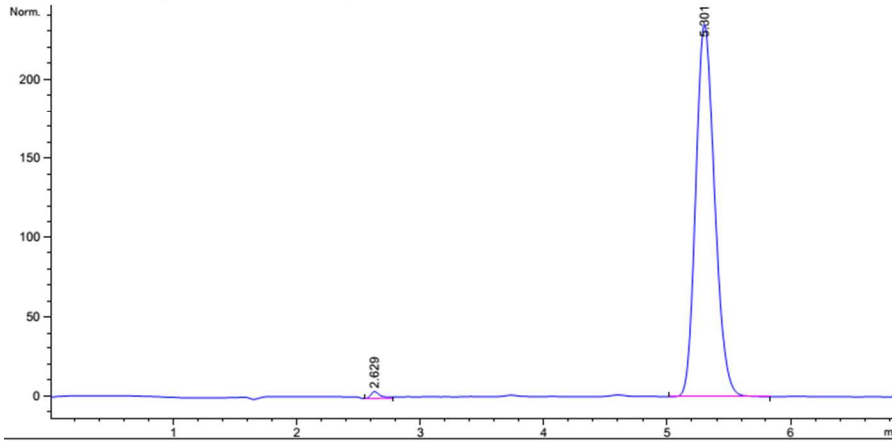
Rate: 0.8 mL/min;

Temperature: 25 °C;

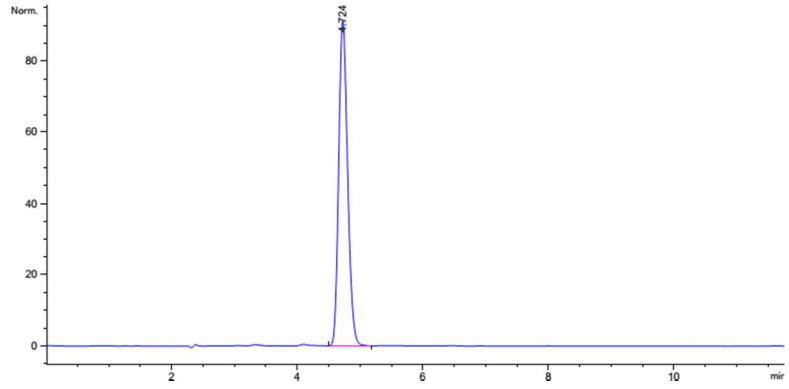
4a, 98.4 %



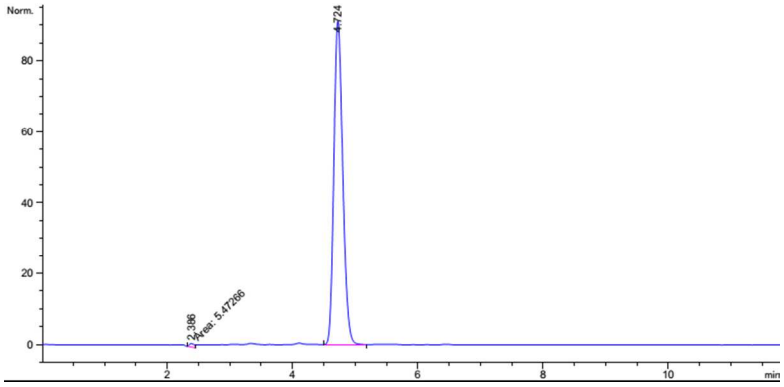
4b, 99.1 %



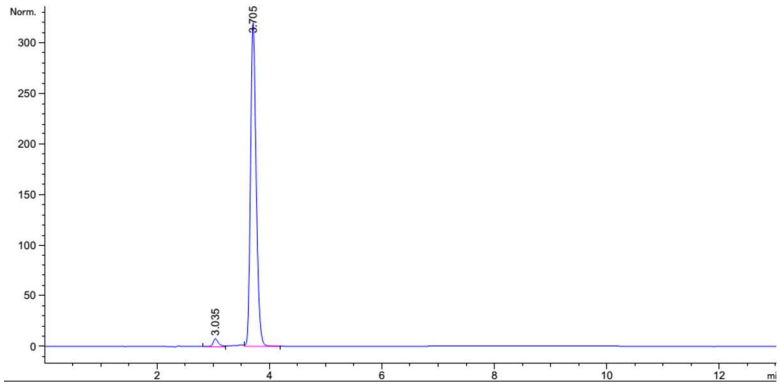
4c, 100 %



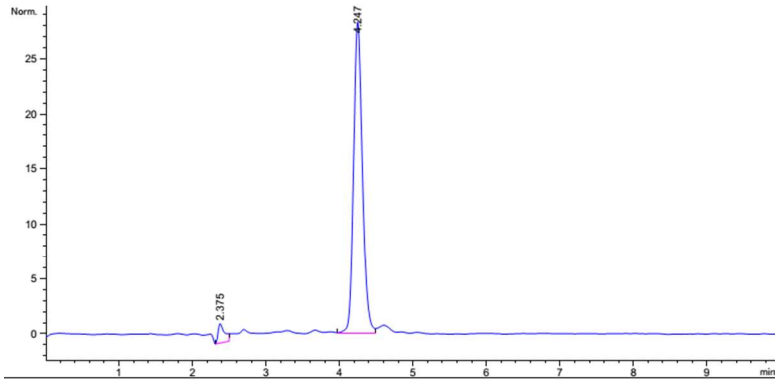
4d, 99.4 %



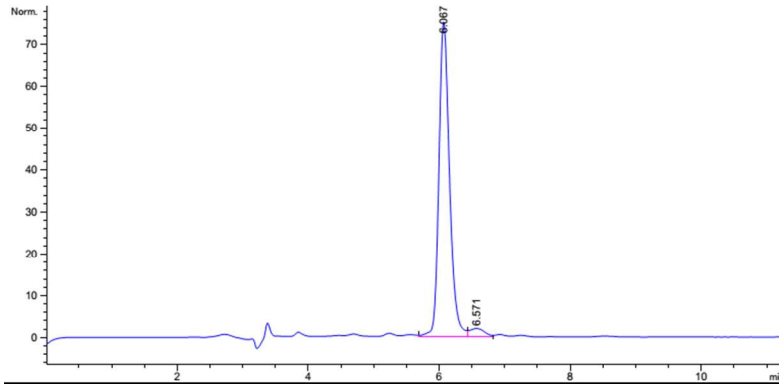
4e, 97.7 %



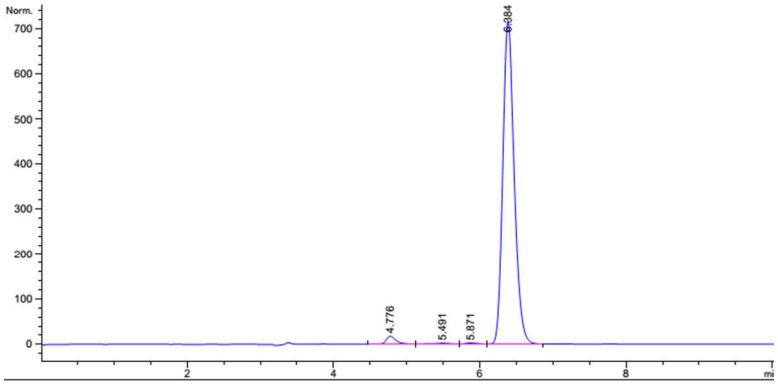
4f, 95.7 %



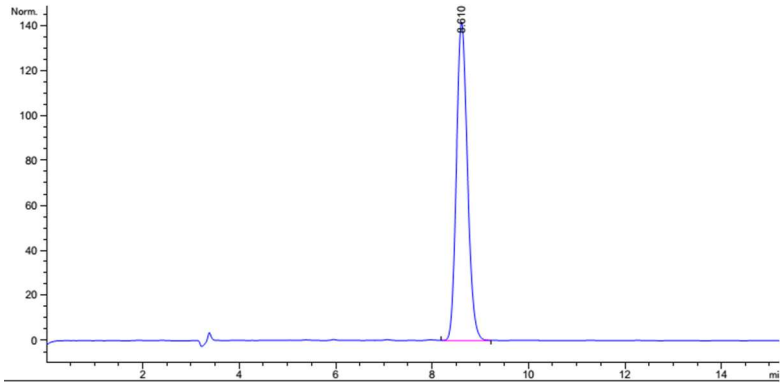
4g, 96.4 %



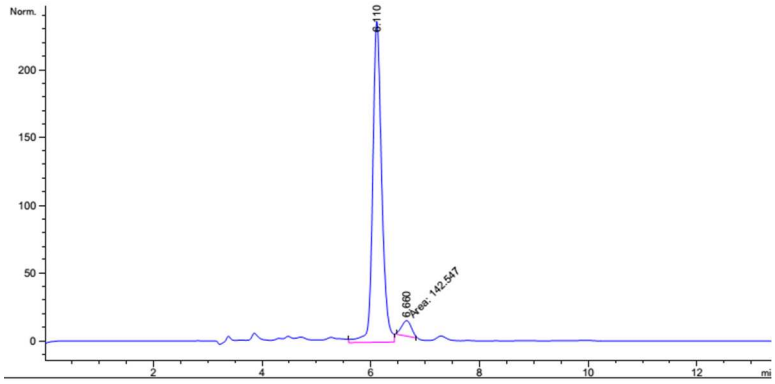
4h, 97.0 %



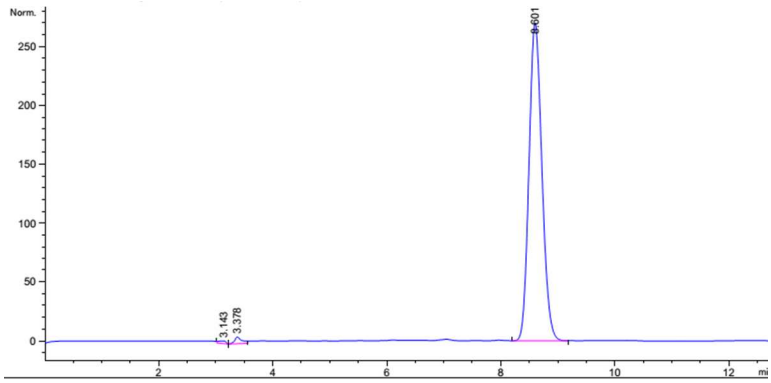
4i, 100 %



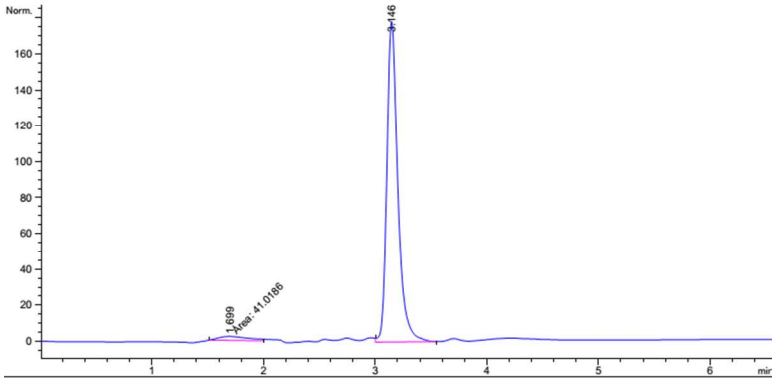
4j, 95.0 %



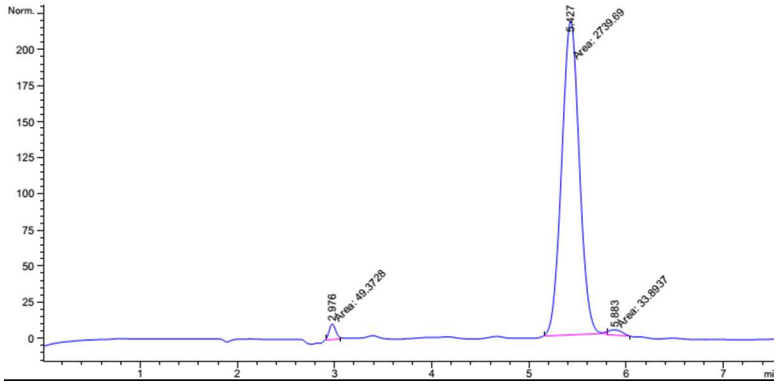
4k, 98.4 %



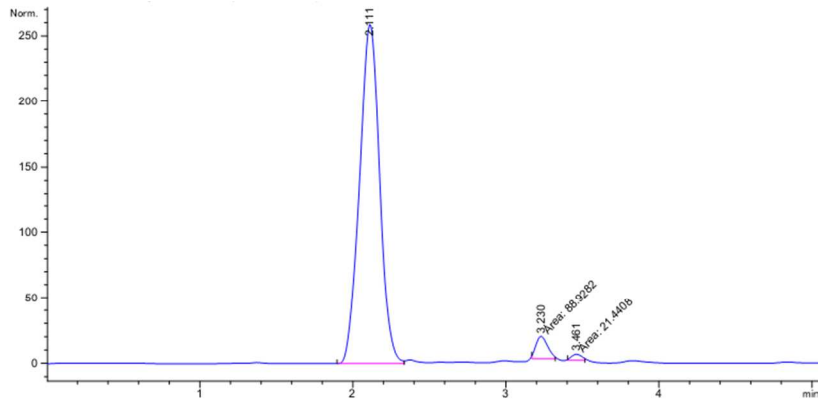
4l, 96.7 %



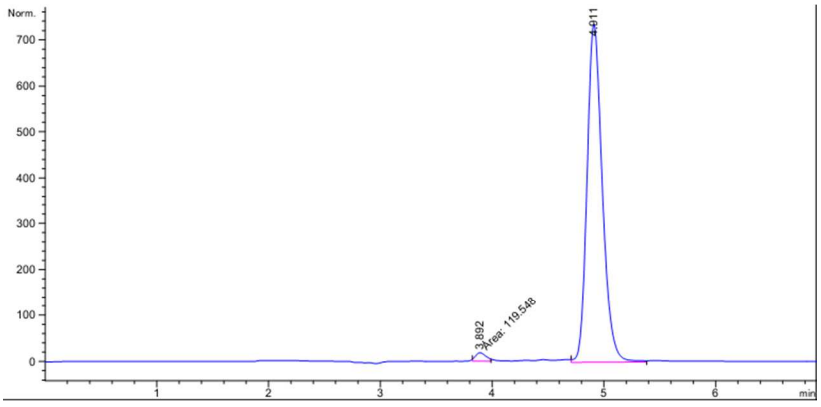
4m, 97.1 %



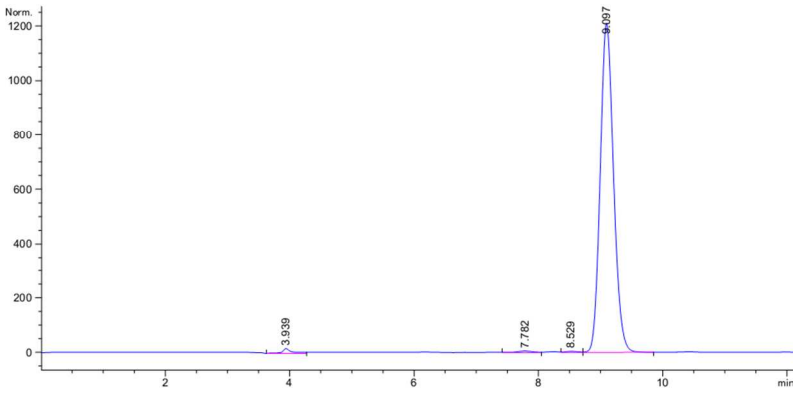
4n, 95.5%



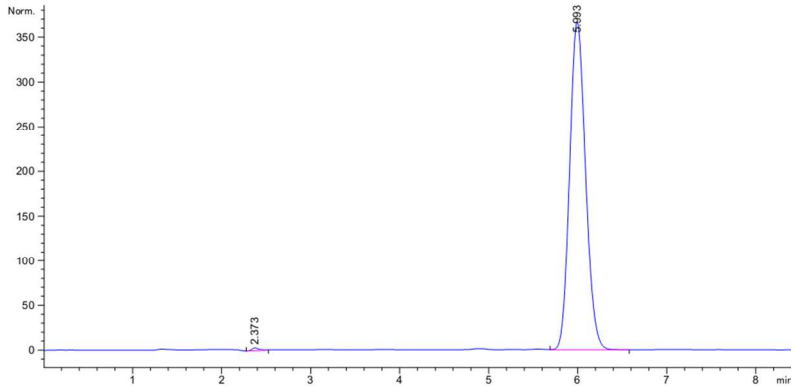
4o, 98.3%



4q, 98.0 %



4p, 99.6%



References

(1) Onyango, E. O.; Fu, L.; Cao, M.; Liby, K. T.; Sporn, M. B.; Gribble, G. W. Synthesis and biological evaluation of amino acid methyl ester conjugates of 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid against the production of nitric oxide (NO). *Bioorg. Med. Chem. Lett.* **2014**, *24*, 532-534.