Supporting Information

Development of small molecule chimeras that recruit AhR E3 ligase to target proteins

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MATERIALS AND METHODS

Chemical synthesis

The synthesis of β-naphthoflavone-ATRA conjugate (β-NF-ATRA) is outlined in Supporting Information Scheme 1. The ATRA derivative **2** was prepared as previously described [1] and compound **5** was synthesized according to a reported method [2]. Compound **5** was brominated [3] and alkylated with a glycol azide linker to generate **7**. Compound **7** was then converted to **8** *via* the Staudinger reaction. Condensation of **8** with **2** using HATU and DIPEA, followed by deprotection of the cyanoethyl group with TBAF, yielded target compound, β-NF-ATRA. As shown in Supporting Information Scheme 2, α-naphthoflavone-ATRA conjugate **5** (α-NF-ATRA) was synthesized *via* a similar procedure using **13** as an intermediate. The synthesis of ITE-ATRA is described in Supporting Information Scheme 3. Hydrolysis of the methyl ester group of ITE afforded the corresponding carboxylic acid, which was condensed with mono-Boc protected glycol diamine **20** to afford compound **18**. The Boc protecting group of **18** was removed with 4M HCl/dioxane, and the corresponding amine hydrochloride salt was condensed with **2**. Deprotection of the cyanoethyl group of **2** afforded ITE-ATRA. The synthetic route to β-naphthoflavone-(+)-JQ1 conjugate (β-NF-JQ1) is outlined in Supporting Information Scheme 4. The protecting group of (+)-JQ1 was removed with formic acid, and the resulting carboxylic acid (**21**) was condensed with **7** to afford β-NF-JQ1.

All chemicals were obtained from commercial suppliers and were used as received, without further purification. *N*-(*tert*-butoxycarbonyl)-2,2'-(ethylenedioxy)diethylamine (**20**), ITE and (+)-JQ1 were purchased from Sigma-Aldrich. Compounds **10** [4] was synthesized as previously described. TLC analysis, which was used to monitor the progress of reactions, was conducted using Merck silica gel 60 F254 pre-coated plates, visualizing with a 254/365 nm UV lamp and staining with iodine or ninhydrin. Column chromatography was performed using silica gel (spherical, neutral) purchased from Kanto Chemical. ^{1}H and ^{13}C NMR spectra were recorded on a Varian AS 400 spectrometer or a JEOL ECZ 600R spectrometer, and measurements were carried out using deuterated solvents. Chemical shift values (ppm) were calibrated using residual non-deuterated solvent peaks as an internal reference ($CDCl_3$: 7.26 for ^{1}H NMR, 77.0 for ^{13}C NMR; CD_3OD: 3.30 for ^{1}H NMR, 49.0 for ^{13}C NMR; DMSO-d_6: 2.50 for ^{1}H NMR, 39.5 for ^{13}C NMR). Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint.), double of doublets (dd), doublet of doublet of doublets (ddd), multiplet (m), broad (br.). High-resolution mass spectra (HRMS) were measured using a Shimadzu IT-TOF MS equipped with an electrospray ionization source in positive mode.

Analytical HPLC traces were recorded on a LC-20AD instrument (Shimadzu) equipped with a SUPELCO Discovery BIO Wide Pore C18-10 column (4.6 x 250 mm, 10 μm particle size) using a gradient of 60–100% acetonitrile in water containing 0.1% TFA over 30 min (flow rate: 1.0 mL/min, column temperature: 35.0 °C). The purity of all compounds used in assay was > 95% as determined by HPLC.

((2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-((*E*)-2,6,6-trimethyl-3-(((12-oxo-1-(4-(1-oxo-1*H*-benzo[*f*]chromen-3-yl)phenyl)-2,5,8-trioxa-11-azatridecan-13-yl)oxy)imino)cyclohex-1-en-1-yl)nona-2,4,6,8-tetraenoic acid) (β-NF-ATRA)

To a mixture of retinoic acid (ATRA) (1.13 g, 3.80 mmol) and 1,3-hydroxypropionitrile (0.81 g, 11.4 mmol) in CH_2Cl_2 (20 mL) was added DMAP (0.71 g, 5.80 mmol), followed by EDC-HCl (1.15 g, 6.00 mmol), and the reaction mixture was stirred at room temperature. After 18 h, the reaction mixture was diluted with EtOAc and washed with 0.2 M HCl and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The product was used in next step without further purification. To a solution of the crude product (1.39 g, as 3.93 mmol) in CH_2Cl_2 (100 mL) was added MnO_2 (25.0 g, 287.6 mmol) and the reaction mixture was stirred at room temperature. After 22 h, the reaction mixture was diluted with EtOAc, filtered to remove MnO_2 and the filtrate was concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (hexanes/EtOAc = 3:1) to give **1** as a yellowish solid (0.53 g, 13% for 2 steps). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.03 (dd, *J* = 15.6, 15.0 Hz, 1H), 6.37 (d, *J* = 15.0 Hz, 1H), 6.26 (d, *J* = 12.0 Hz, 1H), 6.36 – 6.34 (m, 2H), 5.84 (s, 1H), 4.33 (t, *J* = 6.6 Hz, 2H), 2.74 (t, *J* = 6.6 Hz, 2H), 2.52 (t, *J* = 6.0 Hz, 2H), 2.37 (s, 3H), 2.04 (s, 3H), 1.87 (t, *J* = 6.0 Hz, 2H), 1.86 (s, 3H), 1.19 (s, 6H).

To a solution of compound **1** (735 mg, 2.00 mmol) in pyridine (5 mL) was added carboxymethoxylamine hemihydrochloride (262 mg, 2.40 mmol) and the reaction mixture was stirred at room temperature. After 16 h, the reaction mixture was diluted with EtOAc and washed with saturated aqueous citric acid and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated under reduced pressure and dried under vacuum to afford **2** as a yellowish foam (837 mg, 95%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.03 (dd, *J* = 15.3, 15.0 Hz, 1H), 6.34 (d, *J* = 15.0 Hz, 1H), 6.31 (d, *J* = 15.3 Hz, 1H), 6.32 – 6.02 (m, 2H), 5.83 (s, 1H), 4.67 (s, 2H), 4.33 (t, *J* = 6.0 Hz, 2H), 2.74 (t, *J* = 6.0 Hz, 2H), 2.70 (t, *J* = 6.9 Hz, 2H), 2.37 (s, 3H), 2.03 (s, 3H), 1.88 (s, 3H), 1.63 (t, *J* = 6.6 Hz, 2H), 1.10 (s, 6H).

2'-Hydroxyl-1'-acetonaphthone (**3**) (1.86 g, 10.0 mmol) was dissolved in pyridine (5 mL). *p*-Toluoyl chloride (1.59 mL, 12.0 mmol) was added in a slow, dropwise manner. After being

stirred at room temperature for 1 h, the reaction mixture was poured into ice-cold water (50 mL). The resulting precipitate was collected by filtration, washed with water and dried under high vacuum to afford an off-white solid (3.14 g, quant.). This product was used in the next step without further purification. The crude product (1.52 g, as 5.0 mmol) was suspended in pyridine (10 mL). Powdered KOH (3.30 g, 50.0 mmol) was added and the reaction mixture was stirred at 60 °C for 30 min. After cooling to 0 °C, 10% aqueous AcOH (ca. 50 mL) was added dropwise to the reaction mixture under stirring. EtOAc (100 mL) was added to the resulting mixture and the organic layer was separated. The organic layer was washed with 2M HCl, brine, dried over Na_2SO_4 and concentrated under reduced pressure to give a yellowish-orange solid (**4**), which was used in next step without further purification. The crude product (as 5.0 mmol) was suspended in AcOH (15 mL) and treated with 2 drops of conc. H_2SO_4. After being stirred at 110 °C for 6 h, the reaction mixture was concentrated under vacuum to dryness. EtOH (15 mL) was added to the residue and the resulting precipitate was collected by filtration, washed with a small amount of EtOH and dried under vacuum to afford **5** as a yellowish powder (985 mg, 69% for 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.09 (d, *J* = 9.2 Hz, 1H), 8.11 (d, *J* = 9.2 Hz, 1H), 7.92 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.86 (d, *J* = 8.0 Hz, 2H), 7.77 (ddd, *J* = 10.0, 7.2, 1.6 Hz, 1H), 7.64 – 7.61 (m, 1H), 7.63 (d, *J* = 8.8 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 2H), 6.96 (s, 1H), 2.45 (s, 3H).

To a solution of **5** (286 mg, 1.0 mmol) in CCl_4 (10 mL) was added *N*-bromosuccinimide (NBS) (163 mg, 0.92 mmol), followed by 2,2'-azobis(isobutyronitrile) (AIBN, 64 mg, 0.39 mmol). The reaction mixture was stirred at 80 °C for 12 h. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and filtered to remove the precipitate. The filtrate was concentrated under reduced pressure and dried under vacuum to afford **6** as a yellow powder (300 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.08 (d, *J* = 8.4 Hz, 1H), 8.14 (d, *J* = 9.2 Hz, 1H), 7.96 (d, *J* = 8.4 Hz, 2H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.78 (ddd, *J* = 10.0, 7.2, 1.6 Hz, 1H), 7.66 – 7.62 (m, 1H), 7.64 (d, *J* = 8.8 Hz, 1H), 7.58 (d, *J* = 8.4 Hz, 2H), 7.00 (s, 1H), 4.56 (s, 2H).

To a stirred solution of **10** (150 mg, 0.86 mmol) in THF (10 mL) was slowly added NaH (60% oil suspension, 44 mg, 1.12 mmol) at room temperature under an argon atmosphere. After being stirred for 1 h, **6** (109 mg, 0.30 mmol) was added, and the reaction mixture was stirred at room temperature for a further 24 h. After quenching by the addition of MeOH, the reaction mixture was diluted with EtOAc, and washed with brine. The organic layer was dried over Na_2SO_4, concentrated under reduced pressure and the residue was purified by flash silica gel column chromatography (hexanes/EtOAc = 1:2) to afford **7** as a yellow oil (59 mg, 43%). ^{1}H

NMR (600 MHz, $CDCl_3$) δ 10.09 (d, *J* = 7.6 Hz, 1H), 8.12 (d, *J* = 8.8 Hz, 1H), 7.95 (d, *J* = 8.4 Hz, 2H), 7.92 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.77 (ddd, *J* = 10.0, 7.2, 1.6 Hz, 1H), 7.65 – 7.61 (m, 1H), 7.64 (d, *J* = 9.2 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 2H), 6.98 (s, 1H), 4.67 (s, 2H), 3.76 – 3.68 (m, 10H), 3.40 (t, *J* = 5.2 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 180.4, 161.7, 157.8, 142.7, 136.2, 130.8, 130.3, 130.3, 129.6, 128.4, 128.2, 127.4, 127.0, 126.5, 117.6, 116.9, 109.7, 72.6, 70.8, 70.2, 70.0, 69.1, 67.1, 50.8. ESI-HRMS calcd for $C_{26}H_{26}N_3O_5$ $[M+H]^+$: 460.1867, found: 460.1862.

To a solution of compound **7** (109 mg, 0.24 mmol) in THF (10 mL) was added H_2O (1 mL), followed by triphenylphosphine (PPh_3) (87 mg, 0.33 mmol), and the reaction mixture was stirred at room temperature. After 19 h, the reaction mixture was concentrated under vacuum and the residue was purified by flash silica gel column chromatography (CH_2Cl_2/MeOH/Et_3N = 100:10:1) to afford **8** as a pale-yellow solid (80 mg, 78%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.99 (d, *J* = 9.0 Hz, 1H), 8.02 (d, *J* = 9.0 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.83 (d, *J* = 7.8 Hz, 1H), 7.71 (dd, *J* = 7.5 Hz, 1H), 7.57 (dd, *J* = 7.5 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 2H), 6.92 (s, 1H), 4.63 (s, 2H), 3.78 (t, *J* = 5.2 Hz, 2H), 3.69 – 3.65 (m, 8H), 3.15 (br. t, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 179.9, 164.1, 154.5, 144.2, 137.3, 131.4, 130.7, 129.2, 129.1, 128.6, 127.3, 126.7, 124.8, 123.1, 120.8, 120.6, 108.4, 73.3, 72.9, 71.6, 71.6, 71.3, 71.1, 42.0. ESI-HRMS calcd for $C_{26}H_{28}NO_5$ $[M+H]^+$: 434.1962, found: 434.1953.

To a mixture of compound **2** (20 mg, 0.046 mmol) and **8** (22 mg, 0.05 mmol) in DMF (0.4 mL) was added HATU (35 mg, 0.09 mmol), followed by DIPEA (32 μL, 0.18 mmol), and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with EtOAc and washed with 1M HCl, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, concentrated under reduced pressure and the residue was purified by flash silica gel column chromatography ($CHCl_3$/MeOH = 100:0 to 98:2) to afford **9** as a yellowish amorphous solid (27 mg, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.08 (d, *J* = 8.4 Hz, 1H), 8.12 (d, *J* = 8.8 Hz, 1H), 7.94 (d, *J* = 6.8 Hz, 2H), 7.92 (d, *J* = 8.4 Hz, 1H), 7.78 – 7.75 (m, 1H), 7.64 – 7.61 (m, 2H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.01 (dd, *J* = 15.4, 11.6 Hz, 1H), 6.97 (s, 1H), 6.70 (t, *J* = 5.2 Hz, 1H), 6.30 (d, *J* = 15.4 Hz, 2H), 6.23 – 6.19 (m, 2H), 5.79 (s, 1H), 4.65 (s, 2H), 4.59 (s, 2H), 4.30 (t, *J* = 6.4 Hz, 2H), 3.71 – 3.68 (m, 4H), 3.67 – 3.64 (m, 4H), 3.63 – 3.60 (m, 2H), 3.56 – 3.53 (m, 2H), 2.72 (t, *J* = 7.2 Hz, 2H), 2.67 (t, *J* = 7.2 Hz, 2H), 2.34 (d, *J* = 1.2 Hz, 3H), 2.01 (s, 3H), 1.87 (s, 3H), 1.61 (t, *J* = 7.2 Hz, 2H), 1.09 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 180.2, 170.1, 166.1, 160.6, 158.6, 157.3, 154.3, 150.0, 141.9, 139.1, 139.0, 135.8, 135.4, 131.3, 131.3, 130.6, 130.4, 129.2, 128.1, 127.9, 127.1, 127.0, 126.6, 126.1, 124.9, 117.5 (2C overlapped), 117.2, 117.0, 110.3, 73.1, 72.6, 70.6, 70.6, 70.3, 69.9, 69.8, 58.0, 38.6, 35.9,

34.8, 27.6, 20.2, 18.1, 14.8, 13.9, 12.8. ESI-HRMS calcd for $C_{51}H_{58}N_3O_9$ $[M+H]^+$: 856.4168, found: 856.4144.

To a solution of compound **9** (26 mg, 0.03 mmol) in THF (0.5 mL) was added TBAF (1M in THF solution, 90 μL, 0.09 mmol) at room temperature. After being stirred for 1.5 h, the reaction mixture was diluted with $CHCl_3$ containing 0.1% AcOH, and then concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography ($CHCl_3$/MeOH = 100:0 to 98:2) to afford β-NF-ATRA as a yellow solid (86 mg, 51%, containing *ca.* 10% of the 13-*cis* isomer). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.00 (d, *J* = 8.4 Hz, 1H), 8.11 (d, *J* = 9.2 Hz, 1H), 7.94 (d, *J* = 7.6 Hz, 2H), 7.92 – 7.91 (m, 1H), 7.76 (ddd, *J* = 8.8, 7.2, 1.6 Hz, 1H), 7.62 (d, *J* = 9.2 Hz, 1H), 7.64 – 7.61 (m, 1H), 7.51 (d, *J* = 7.6 Hz, 2H), 7.01 – 7.00 (m, 2H), 6.74 (t, *J* = 5.4 Hz, 1H), 6.34 – 6.28 (m, 2H), 6.22 – 6.19 (m, 2H), 5.80 (s, 1H), 4.65 (s, 2H), 4.60 (s, 2H), 3.70 – 3.66 (m, 8H), 3.61 – 3.60 (m, 2H), 3.56 – 3.54 (m, 2H), 2.67 (t, *J* = 6.8 Hz, 2H), 2.34 (s, 3H), 2.01 (s, 3H), 1.87 (s, 3H), 1.61 (t, *J* = 6.8 Hz, 2H), 1.09 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 180.4, 171.3, 170.3, 160.7, 158.7, 157.4, 154.4, 150.0, 142.0, 139.2, 138.9, 136.2, 135.5, 131.4, 131.1, 130.6, 130.6, 130.4, 129.2, 128.1, 128.0, 127.1, 126.9, 126.6, 126.1, 124.9, 118.5, 117.5, 117.2, 110.2, 73.0, 72.6, 70.6, 70.6, 70.3, 69.9, 69.8, 38.7, 35.9, 34.8, 27.6, 20.2, 14.9, 13.9, 12.8. ESI-HRMS calcd for $C_{48}H_{55}N_2O_9$ $[M+H]^+$: 803.3902, found: 803.3928. Purity (HPLC) = 96%.

((2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-((*E*)-2,6,6-trimethyl-3-(((12-oxo-1-(4-(4-oxo-4*H*-benzo[*h*]chromen-2-yl)phenyl)-2,5,8-trioxa-11-azatridecan-13-yl)oxy)imino)cyclohex-1-en-1-yl)nona-2,4,6,8-tetraenoic acid) (α-NF-ATRA) 1'-Hydroxyl-2'-acetonaphthone (**11**) (1.86 g, 10.0 mmol) was dissolved in pyridine (5 mL). *p*-Toluoyl chloride (1.59 mL, 12.0 mmol) was added in a slow, dropwise manner. After being stirred at room temperature for 1 h, the reaction mixture was poured into ice-cold water (50 mL). The resulting precipitate was collected by filtration, washed with water and dried under high vacuum to afford a white powder (3.10 g, quant.), which was used in next step without further purification. The crude product (1.52 g, as 5.0 mmol) was suspended in pyridine (10 mL). Powered KOH (3.30 g, 50.0 mmol) was added, and the reaction mixture was stirred at 60 ℃ for 30 min. After cooling to 0 °C, 10% aqueous AcOH (ca. 50 mL) was added dropwise to the reaction mixture under stirring. The resulting precipitate was collected by filtration, washed with water and dried under vacuum to afford a yellowish powder (**12**) (1.28 g), which was used in next step without further purification. **12** (1.22 g, as 4.0 mmol) was suspended in AcOH (20 mL), and treated with 2 drops of conc. H_2SO_4. After being stirred at 110 ℃ for 3 h, the reaction mixture was concentrated under vacuum to dryness.

EtOH (15 mL) was added to the residue, and the resulting precipitate was collected by filtration, washed with a small amount of EtOH and dried under vacuum to afford **13** as a beige floc (960 mg, 84% for 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 – 8.61 (m, 1H), 8.18 (d, *J* = 8.6 Hz, 1H), 7.96 – 7.92 (m, 1H), 7.93 (d, *J* = 8.0 Hz, 2H), 7.79 (d, *J* = 8.6 Hz, 1H), 7.73 – 7.71 (m, 2H), 7.38 (d, *J* = 8.0 Hz, 2H), 6.94 (s, 1H), 2.47 (s, 3H).

To a solution of compound **13** (283 mg, 1.0 mmol) in CCl_4 (5 mL) at 60 °C was added NBS (214 mg, 1.20 mmol), followed by AIBN (33 mg, 0.20 mmol), and the reaction mixture was stirred at 60 °C for 12 h. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 (10 mL), and washed with 15% aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to dryness. The obtained solid was suspended in hexanes/Et_2O (1:1, v/v), collected by filtration and dried under vacuum to afford **14** as a light yellowish solid (283 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 – 8.54 (m, 1H), 8.15 (d, *J* = 8.8 Hz, 1H), 8.02 – 7.97 (m, 2H), 7.93 – 7.92 (m, 1H), 7.79 – 7.76 (m, 2H), 7.72 – 7.70 (m, 2H), 7.58 (d, *J* = 8.8 Hz, 1H), 6.99 (s, 1H), 4.56 (s, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.1, 161.9, 153.5, 141.3, 136.0, 131.9, 129.8, 129.3, 128.3, 127.2, 126.6, 125.4, 124.0, 122.3, 120.7, 120.2, 109.0, 32.2. ESI-HRMS calcd for $C_{20}H_{14}BrO_2$ $[M+H]^+$: 365.0172, found: 365.0196.

To a stirred solution of compound **10** (516 mg, 2.95 mmol) in THF (90 mL) was slowly added NaH (60% oil suspension, 1.05 g, 26.2 mmol) at room temperature under an argon atmosphere. After being stirred for 1 h, compound **14** (885 mg, 2.43 mmol) was added and the reaction mixture was stirred for a further 2 h at room temperature. After quenching by the addition of MeOH, the reaction mixture was filtered through Celite. The filtrate was concentrated under reduced pressure and the residue was purified by flash silica gel column chromatography (hexanes/EtOAc = 4:1) to afford **15** as a yellow oil (186 mg, 17%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 – 8.63 (m, 1H), 8.19 (d, *J* = 8.8 Hz, 1H), 8.02 (d, *J* = 8.4 Hz, 2H), 7.97 – 7.96 (m, 1H), 7.80 (d, *J* = 8.8 Hz, 1H), 7.74 – 7.72 (m, 2H), 7.57 (d, *J* = 8.4 Hz, 2H), 6.98 (s, 1H), 4.69 (s, 2H), 3.76 – 3.68 (m, 10H), 3.40 (t, *J* = 5.0 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.1, 162.4, 153.4, 142.3, 135.9, 131.0, 129.2, 128.1, 128.0, 127.1, 126.2, 125.2, 124.0, 122.2, 120.6, 120.1, 108.5, 72.5, 70.7 (1C overlapped), 70.0, 69.9, 69.8, 50.6. ESI-HRMS calcd for $C_{26}H_{26}N_3O_5$ $[M+H]^+$: 460.1867, found: 460.1860.

To a solution of compound **15** (121 mg, 0.26 mmol) in THF (12 mL) was added H_2O (3 mL), followed by PPh_3 (138 mg, 0.52 mmol). The reaction mixture was stirred at 60 °C for 13 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure, and the residue was purified by flash silica gel column chromatography

(CH_2Cl_2/MeOH = 17 : 3) to afford **16** as a yellow solid (93 mg, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 – 8.52 (m, 1H), 8.12 (d, *J* = 8.8 Hz, 1H), 7.97 (d, *J* = 8.2 Hz, 2H), 7.86 – 7.84 (m, 1H), 7.67 (d, *J* = 8.8 Hz, 1H), 7.65 – 7.63 (m, 2H), 7.53 (d, *J* = 8.4 Hz, 2H), 6.93 (s, 1H), 4.67 (s, 2H), 3.71 – 3.68 (m, 8H), 3.63 (t, *J* = 5.2 Hz, 2H), 3.40 (t, *J* = 5.2 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.2, 162.5, 153.3, 142.2, 136.0, 131.1, 129.2, 128.2, 128.1, 127.1, 126.3, 125.3, 124.1, 122.3, 120.7, 120.2, 108.6, 72.8, 72.5, 70.6 (1C overlapped), 70.2, 69.8, 41.5. ESI-HRMS calcd for $C_{26}H_{28}NO_5$ $[M+H]^+$: 434.1962, found: 434.1937.

To a mixture of compound **2** (40 mg, 0.09 mmol) and **16** (43 mg, 0.10 mmol) in DMF (0.8 mL) was added HATU (69 mg, 0.18 mmol), followed by DIPEA (63 μL, 0.36 mmol), and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with EtOAc, washed with 1M HCl, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, concentrated under reduced pressure and the residue was purified by flash silica gel column chromatography ($CHCl_3$/MeOH = 100:0 to 98:2) to afford **17** as a yellowish amorphous solid (67 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 – 8.61 (m, 1H), 8.19 (d, *J* = 8.8 Hz, 1H), 8.02 (d, *J* = 8.4 Hz, 2H), 7.99 – 7.95 (m, 1H), 7.81 (d, *J* = 8.8 Hz, 1H), 7.76 – 7.71 (m, 2H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.04 – 6.94 (m, 1H), 6.97 (s, 1H), 6.68 (br. t, 1H), 6.32 – 6.19 (m, 2H), 6.23 – 6.19 (m, 2H), 5.79 (s, 1H), 4.68 (s, 2H), 4.59 (s, 2H), 4.31 (t, *J* = 6.4 Hz, 2H), 3.72 – 3.51 (m, 12H), 2.73 (t, *J* = 6.4 Hz, 2H), 2.67 (t, *J* = 7.2 Hz, 2H), 2.34 (s, 3H), 2.01 (s, 3H), 1.87 (s, 3H), 1.61 (t, *J* = 7.2 Hz, 2H), 1.09 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.0, 170.0, 166.0, 162.3, 158.5, 154.2, 153.4, 149.9, 142.1, 139.0, 139.0, 135.9, 135.8, 131.2, 131.2, 131.1, 129.2, 128.1, 128.0, 127.1, 126.9, 126.2, 125.2, 124.8, 124.0, 122.2, 120.6, 120.1, 117.5, 117.0, 108.5, 73.0, 72.4, 70.5, 70.5, 70.2, 69.8, 69.8, 57.9, 38.6, 35.9, 34.7, 27.5, 20.1, 18.0, 14.7, 13.8, 12.7. ESI-HRMS calcd for $C_{51}H_{58}N_3O_9$ $[M+H]^+$: 856.4168, found: 856.4123.

To a solution of compound **17** (31 mg, 0.036 mmol) in THF (0.6 mL) was added TBAF (1M in THF solution, 109 μL, 0.109 mmol) at room temperature. After being stirred for 1.5 h, the reaction mixture was diluted with $CHCl_3$ containing 0.1% AcOH, and then concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography ($CHCl_3$/MeOH = 100:0 to 98:2) to afford α-NF-ATRA as a yellow solid (24 mg, 83%, containing *ca.* 10% of the 13-*cis* isomer). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 – 8.62 (m, 1H), 8.19 (d, *J* = 8.8 Hz, 1H), 8.06 – 8.01 (m, 1H), 7.97 – 7.95 (m, 2H), 7.81 (d, *J* = 8.8 Hz, 1H), 7.75 – 7.72 (m, 2H), 7.55 (d, *J* = 8.4 Hz, 2H), 7.03 – 6.94 (m, 1H), 6.98 (s, 1H), 6.72 – 6.67 (m, 1H), 6.34 – 6.27 (m, 2H), 6.24 – 6.18 (m, 2H), 5.80 (s, 1H), 4.67 (s, 2H), 4.59 (s, 2H), 3.72 – 3.68 (m, 4H), 3.67 – 3.64 (m, 4H), 3.62 – 3.58 (m, 2H), 3.56 – 3.52 (m, 2H), 2.67 (t, *J* = 6.4 Hz, 2H), 2.34 (s, 3H), 2.01 (s, 3H), 1.87 (s, 3H), 1.61 (t, *J* = 7.2 Hz, 2H), 1.09 (s, 6H); ^{13}C NMR

(151 MHz, $CDCl_3$) δ 178.3, 171.2, 170.3, 162.5, 158.7, 154.3, 153.5, 150.0, 142.2, 139.2, 138.9, 136.2, 136.0, 131.4, 131.1, 131.0, 129.3, 128.2, 128.1, 127.2, 126.8, 126.3, 125.4, 124.9, 124.1, 122.3, 120.7, 120.2, 118.6, 108.6, 73.0, 72.6, 70.6, 70.6, 70.3, 69.9, 69.8, 38.7, 35.9, 34.8, 27.6, 20.2, 14.8, 13.9, 12.8. ESI-HRMS calcd for $C_{48}H_{55}N_2O_9$ $[M+H]^+$: 803.3902, found: 803.3932. Purity (HPLC) = 96%.

((2*E*,4*E*,6*E*,8*E*)-9-((*E*)-3-(((1-(2-(1*H*-indole-3-carbonyl)thiazol-4-yl)-1,12-dioxo-5,8-dioxa-2,11-diazatridecan-13-yl)oxy)imino)-2,6,6-trimethylcyclohex-1-en-1-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid) (ITE-ATRA) To a solution of 2-(1′ H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) (48 mg, 0.17 mmol) in THF (1.6 mL) was added 4M aqueous KOH (105 μL, 0.42 mmol). After being stirred at room temperature for 16 h, the reaction mixture was neutralized with 2M HCl and concentrated under vacuum to dryness. The obtained product was used in the next reaction without further purification.

To a mixture of the above crude compound (as 0.17 mmol) and **20** (62 mg, 0.25 mmol) in DMF (0.24 mL) was added HATU (127 mg, 0.33 mmol), followed by DIPEA (175 μL, 1.0 mmol). After being stirred at room temperature for 16 h, the reaction mixture was diluted with EtOAc and washed with 1M HCl, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, concentrated under reduced pressure and the residue was purified by flash silica gel column chromatography ($CHCl_3$/MeOH = 100:0 to 99:1 to 49:1) to afford **18** as a yellow oil (48 mg, 57% for 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.73 (br. s, 1H), 8.97 (s, 1H), 8.50 (d, J = 7.8 Hz, 1H), 8.39 (s, 1H), 7.73 (s, 1H), 7.53 (d, J = 7.2 Hz, 1H), 7.35 – 7.30 (m, 2H), 5.54 (s, 1H), 3.77 – 3.74 (m, 2H), 3.70 – 3.68 (m, 4H), 3.65 – 3.58 (m, 4H), 3.35 (d, J = 4.8 Hz, 2H), 1.48 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.1, 170.4, 161.0, 156.8, 151.0, 136.8, 136.3, 128.3, 126.8, 123.9, 123.0, 122.4, 113.4, 112.0, 80.2, 70.6, 70.0, 69.8 (1C overlapped), 40.2, 39.3, 28.4. ESI-HRMS calcd for $C_{24}H_{31}N_4O_6S^+$ $[M+H]^+$: 503.1959, found: 503.2000.

Compound **18** (25 mg, 0.05 mmol) was treated with 4M HCl/1,4-dioxane (1.2 mL) and stirred at room temperature for 2 h. The volatiles were removed by evaporation under reduced pressure to afford the crude product as an HCl salt. This product was used in next reaction without further purification.

To a mixture of the above compound (as 0.05 mmol) and compound **2** (18.5 mg, 0.04 mmol) in DMF (120 μL) was added HATU (32 mg, 0.08 mmol), followed by DIPEA (44 μL, 0.25 mmol). After being stirred at room temperature for 4 h, the reaction mixture was diluted with EtOAc and washed with 1M HCl, saturated aqueous $NaHCO_3$ and brine. The organic layer was

dried over Na_2SO_4, concentrated under reduced pressure and the residue was purified by flash silica gel column chromatography ($CHCl_3$/MeOH = 99:1 to 49:1) to afford **19** as a yellow foam (19 mg, 55% for 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.21 (s, 1H), 9.00 (d, *J* = 3.6 Hz, 1H), 8.51 (d, *J* = 7.8 Hz, 1H), 8.38 (s, 1H), 7.74 (t, *J* = 5.4 Hz, 1H), 7.56 (d, *J* = 6.6 Hz, 1H), 7.36 – 7.32 (m, 2H), 7.02 (dd, *J* = 15.3, 15.3 Hz, 1H), 6.80 (t, *J* = 5.4 Hz, 1H), 6.35 (d, *J* = 15.3 Hz, 1H), 6.28 (d, *J* = 15.3 Hz, 1H), 6.22 – 6.17 (m, 2H), 5.82 (s, 1H), 4.68 (s, 2H), 4.33 (t, *J* = 6.0 Hz, 2H), 3.75 – 3.63 (m, 10H), 3.61 – 3.58 (m, 2H), 2.74 (t, *J* = 6.0 Hz, 2H), 2.65 (t, *J* = 6.6 Hz, 2H), 2.37 (s, 3H), 2.02 (s, 3H), 1.86 (s, 3H), 1.59 (t, *J* = 6.6 Hz, 2H), 1.08 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.2, 171.6, 170.5, 166.1, 160.9, 159.3, 154.3, 151.0, 150.7, 139.3, 139.0, 136.7, 136.6, 136.0, 131.5, 131.3, 128.3, 126.9, 126.8, 124.6, 123.9, 123.1, 122.4, 117.7, 117.0, 113.4, 112.2, 72.9, 70.9, 70.3, 70.3, 69.7, 58.0, 39.2, 39.0, 35.9, 34.9, 27.5, 20.2, 18.1, 14.8, 14.0, 12.8. ESI-HRMS calcd for $C_{44}H_{53}N_6O_8S$ $[M+H]^+$: 825.3640, found: 825.3645.

To a solution of compound **19** (19 mg, 0.02 mmol) in THF (0.39 mL) was added TBAF (1M in THF solution, 69 μL, 0.069 mmol) at room temperature. After being stirred for 1.5 h, the reaction mixture was diluted with $CHCl_3$ containing 0.1% AcOH and then concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography ($CHCl_3$/MeOH = 99:1 to 97:3) to afford ITE-ATRA as a yellow solid (9.0 mg, 51%, containing *ca.* 20% of the 13-*cis* isomer). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.10 (s, 1H), 8.53 – 8.52 (m, 1H), 8.41 (s, 1H), 7.83 (t, *J* = 6.0 Hz, 1H), 7.41 – 7.37 (m, 3H), 7.00 (dd, *J* = 15.0, 15.0 Hz, 1H), 6.70 (t, *J* = 6.0 Hz, 1H), 6.34 (d, *J* = 15.0 Hz, 1H), 6.28 (d, *J* = 16.2 Hz, 1H), 6.21 – 6.17 (m, 2H), 5.82 (s, 1H), 4.56 (s, 2H), 3.71 – 3.64 (m, 8H), 3.60 – 3.58 (m, 2H), 3.50 – 3.48 (m, 2H), 2.64 (t, *J* = 6.6 Hz, 2H), 2.35 (s, 3H), 2.01 (s, 3H), 1.85 (s, 3H), 1.59 (t, *J* = 6.6 Hz, 2H), 1.07 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.4, 170.9, 170.5, 169.5, 161.2, 158.8, 154.6, 151.3, 150.3, 139.2, 139.0, 138.8, 136.2, 135.7, 131.4, 131.2, 128.7, 126.9, 124.8, 124.4, 123.8, 123.2, 118.3, 117.6, 113.6, 108.9, 73.0, 70.3, 70.1, 69.8, 69.7, 39.0, 38.7, 35.9, 34.8, 27.6, 20.2, 14.9, 14.0, 12.8. ESI-HRMS calcd for $C_{41}H_{50}N_5O_8S$ $[M+H]^+$: 772.3375, found: 772.3380. Purity (HPLC) = 95.4%.

((*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(2-(2-(2-((4-(1-oxo-1*H*-benzo[*f*]chromen-3-yl)benzyl)oxy)ethoxy)ethoxy)ethyl) acetamide) (β-NF-JQ1) (+)-JQ1 (3.3 mg, 7.24 μmol) was added to formic acid (100 μL), and the mixture was stirred at room temperature. After 22 h, the reaction mixture was concentrated under vacuum to give a pale-yellow solid (3.0 mg, quant.). This product (**21**) was used in next reaction without further purification. ^{1}H NMR (400 MHz, CD_3OD) δ 7.46 (d, *J* = 8.8 Hz, 2H),

7.42 (d, *J* = 8.8 Hz, 2H), 4.61 (t, *J* = 7.1 Hz, 1H), 3.51 (d, *J* = 7.1 Hz, 2H), 2.72 (s, 3H), 2.46 (s, 3H), 1.71 (s, 3H).

To a mixture of compound **8** (1.3 mg, 3.2 μmol) and compound **21** (2.1 mg, 4.8 μmol) in CH_2Cl_2 (3 mL) was added HATU (3.4 mg, 8.9 μmol), followed by DIPEA (1.3 mg, 9.8 μmol). After being stirred at room temperature for 4 h, the reaction mixture was concentrated under reduced pressure, and the residue was dissolved in EtOAc and washed with 0.1% HCl, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, concentrated under reduced pressure and the residue was purified by flash silica gel column chromatography (CH_2Cl_2/MeOH = 9:1) to afford β-NF-JQ1 as a colorless oil (2.5 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.00 (d, *J* = 8.4 Hz, 1H), 8.04 (d, *J* = 9.2 Hz, 1H), 7.86 – 7.84 (m, 1H), 7.85 (d, *J* = 8.2 Hz, 2H), 7.69 (ddd, *J* = 8.4, 6.8, 1.2 Hz, 1H), 7.57 – 7.53 (m, 1H), 7.56 (d, *J* = 9.2 Hz, 1H), 7.45 (d, *J* = 8.2 Hz, 2H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 2H), 6.86 (s, 1H), 6.77 (br. s, 1H), 4.61 (s, 2H), 4.58 (t, *J* = 6.8 Hz, 1H), 3.07 – 3.62 (m, 8H), 3.59 – 3.54 (m, 2H), 3.50 – 3.43 (m, 3H), 3.33 – 3.18 (m, 1H), 2.59 (s, 3H), 2.30 (s, 3H), 1.58 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 180.2, 170.5, 163.8, 160.7, 157.3, 155.6, 149.9, 142.0, 136.7, 136.6, 135.4, 132.1, 130.8, 130.7, 130.6, 130.5, 130.5, 130.4, 129.8, 128.6, 128.1, 128.0, 127.1, 126.6, 126.1, 117.6, 117.2, 110.3, 77.2, 72.6, 70.6, 70.4, 69.8, 69.8, 54.3, 39.4, 39.1, 29.7, 14.4, 13.1, 11.8. ESI-HRMS calcd for $C_{45}H_{43}ClN_5O_6S$ $[M+H]^+$: 816.2617, found: 816.2579. Purity (HPLC) = 96%.

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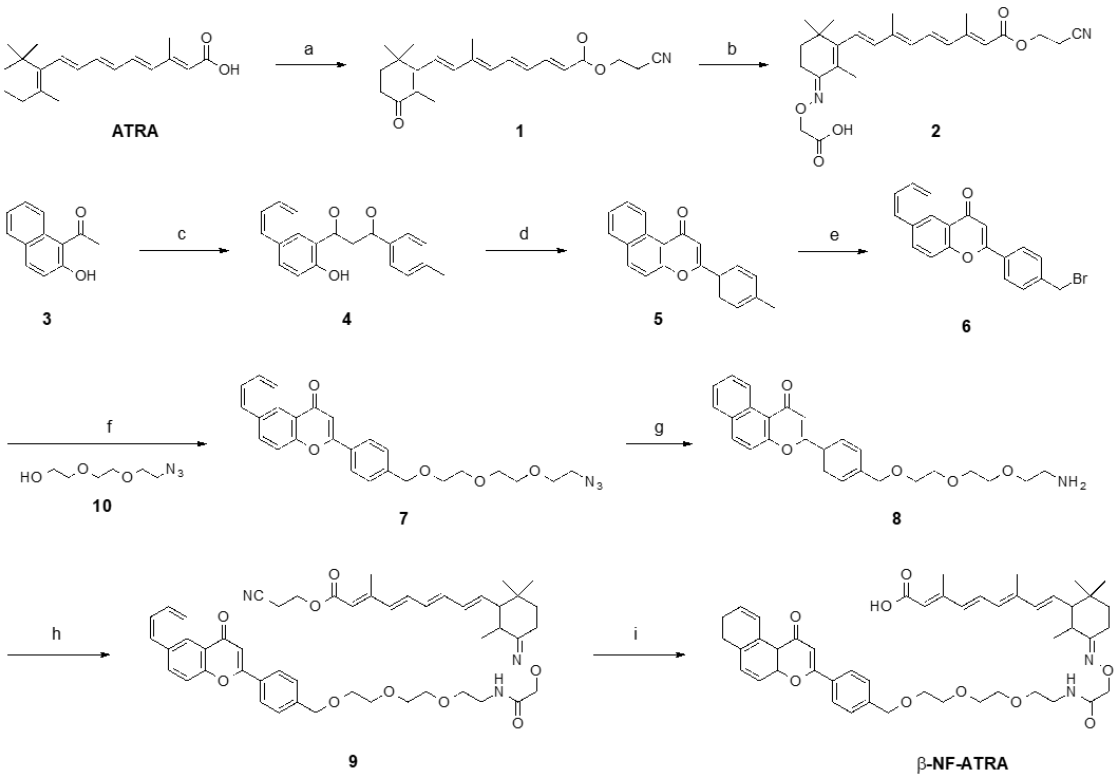
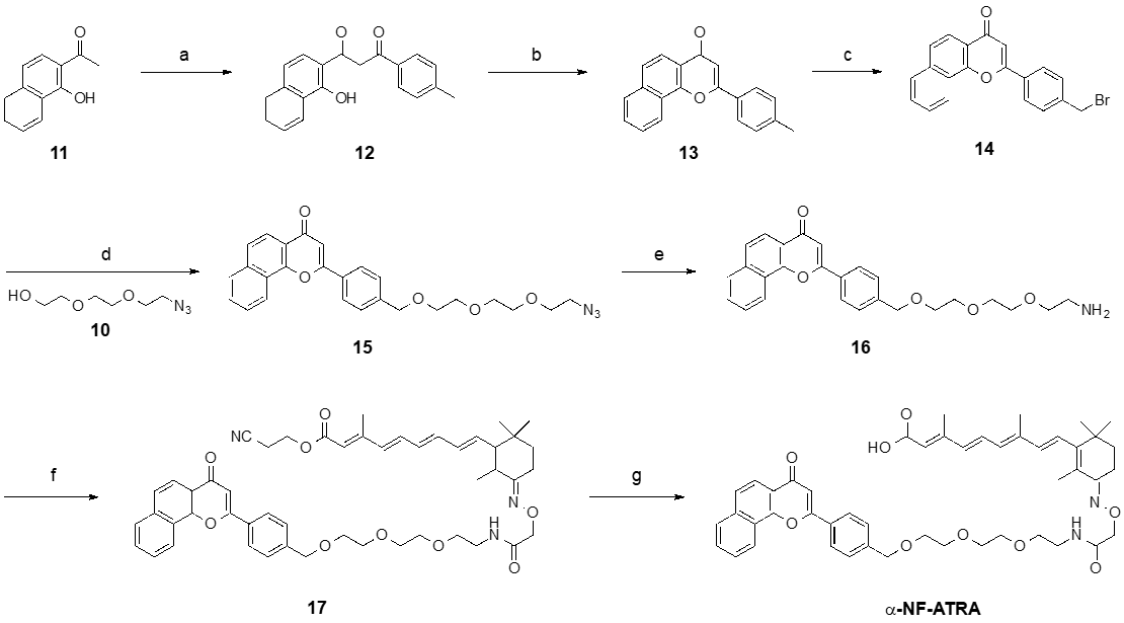
Chemical Schemes

Scheme 1. Synthesis of β-NF-ATRA



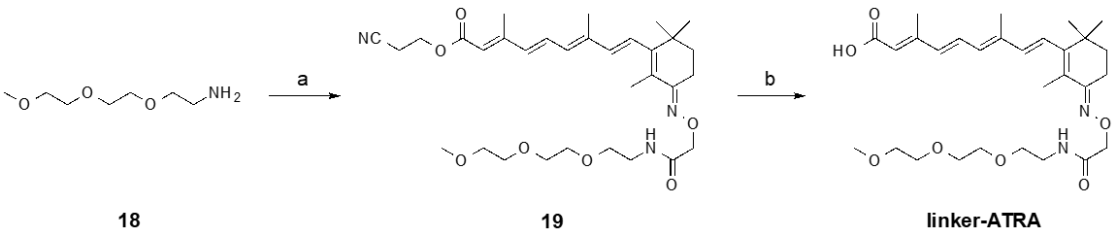
(a) 1) 3-Hydroxypropionitrile, EDC-HCl, DMAP, CH_2Cl_2; 2) Mn_2O, CH_2Cl_2; (b) (Aminooxy)acetic acid hemihydrochloride, pyridine; (c) 1) *p*-Toluoyl chloride, pyridine; 2) KOH, pyridine; (d) H_2SO_4 (cat.), AcOH; (e) NBS, AIBN, CCl_4; (f) **10**, NaH, THF; (g) PPh_3, THF, H_2O; (h) **2**, HATU, DIPEA, DMF; (i) TBAF, THF.

Scheme 2. Synthesis of α-NF-ATRA



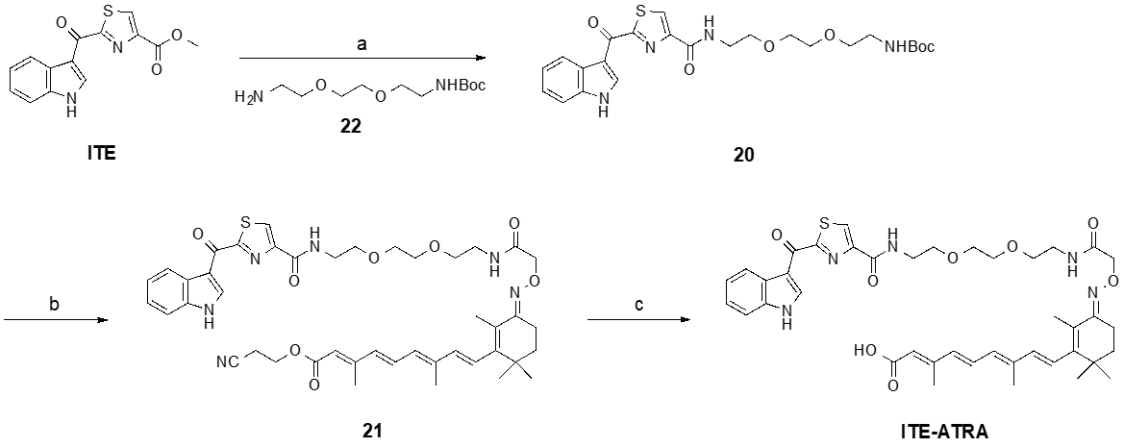
(a) 1) *p*-Toluoyl chloride, pyridine; 2) KOH, pyridine; (b) H_2SO_4 (cat.), AcOH; (c) NBS, AIBN, CCl_4; (d) **10**, NaH, THF; (e) PPh_3, THF, H_2O; (f) **2**, HATU, DIPEA, DMF; (g) TBAF, THF.

Scheme 3. Synthesis of linker-ATRA



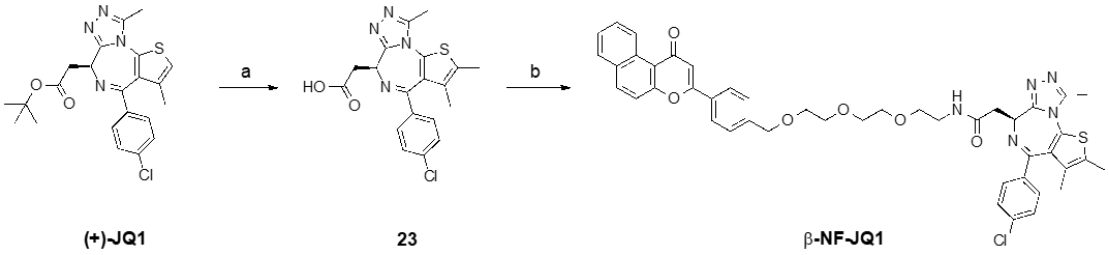
(a) **2**, HATU, DIPEA, DMF, (b) TBAF, THF

Scheme 4. Synthesis of ITE-ATRA



(a) 1) 4M KOH (aq.), THF; 2) HATU, DIPEA, DMF; (b) 1) 4M HCl/1,4-dioxane; 2) **2**, HATU, DIPEA, DMF; (c) TBAF, THF.

Scheme 5. Synthesis of β-NF-JQ1



a) HCOOH; (b) **8**, HATU, DIPEA, DMF.

Figure S1

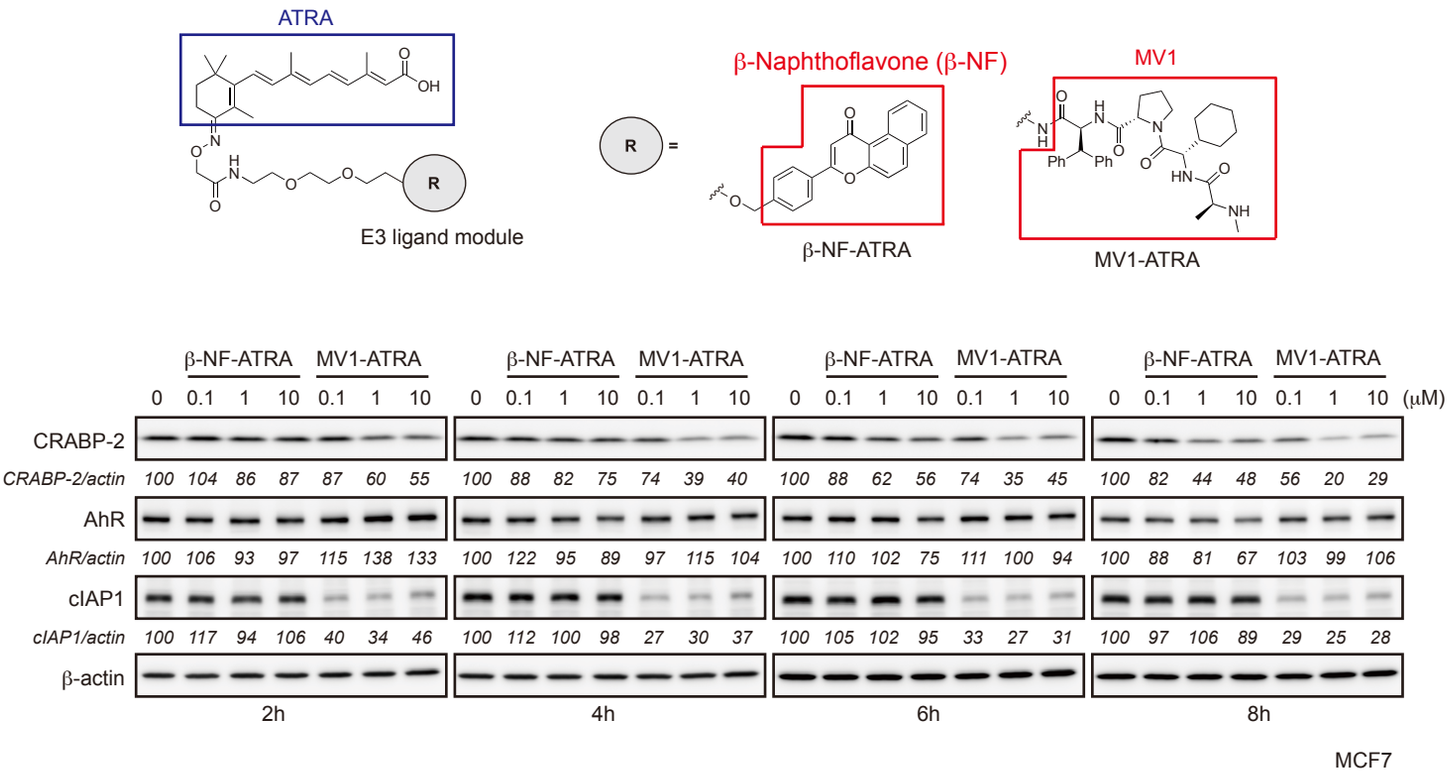


Fig. S1 Comparison of the CRABP-2 degradation rates by β-NF-ATRA and MV1-ATRA. (A) Chemical structures of MV1-ATRA. MV1-ATRA contains an IAP antagonist MV1 as an E3 ligand. (B) MCF-7 cells were treated with β-NF-ATRA and MV1-ATRA for the indicated periods. Whole-cell lysates were analyzed by western blotting.

Figure S2

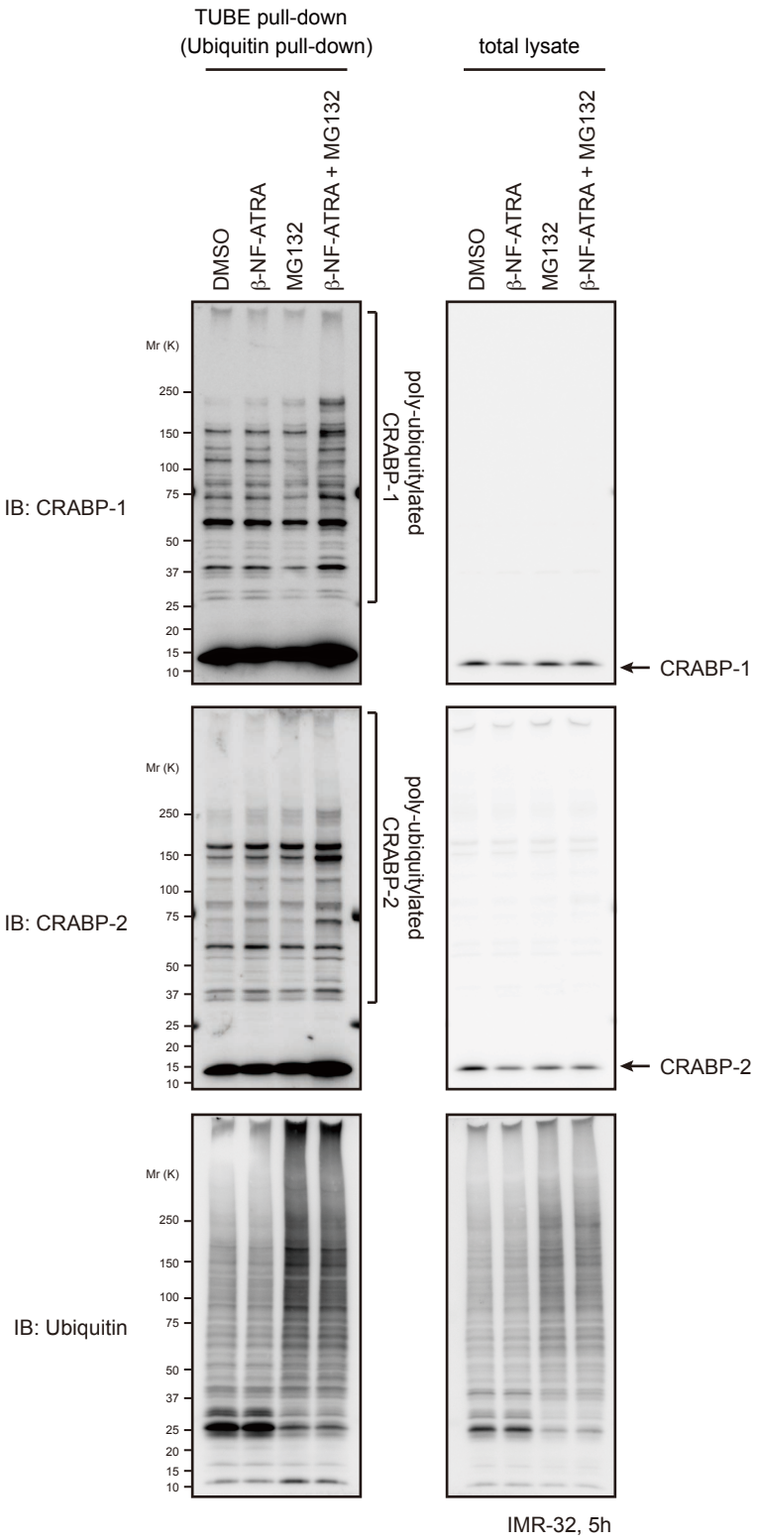


Fig. S2 ubiquitylation of CRABP-1 and CRABP-2 by β-NF-ATRA. IMR-32 cells were treated with10 μM β-NF-ATRA in the presence or absence of 10 μM MG132 for 5 h. Whole-cell lysates and lysates pulled down with TUBEs were analyzed by Western blotting with the indicated antibodies.

Figure S3

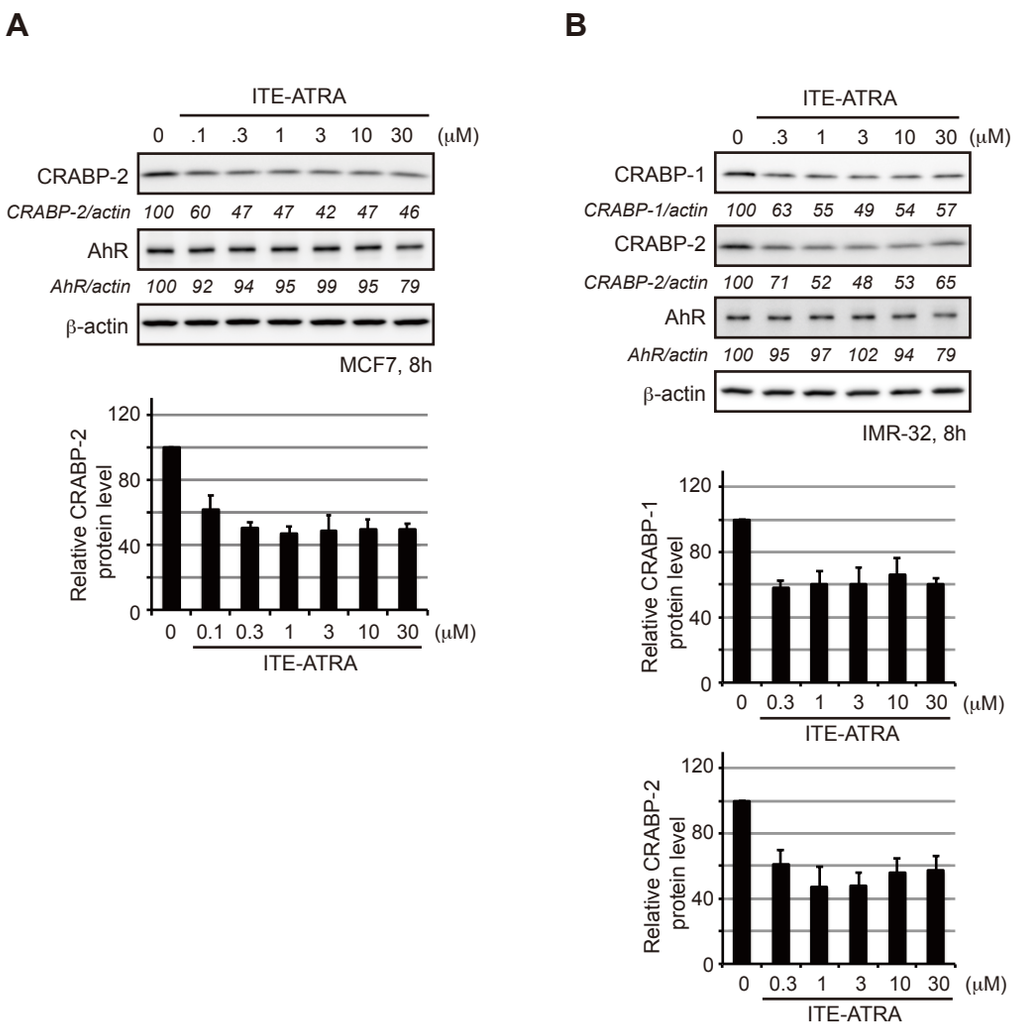


Fig. S3 The effects of increasing concentrations of ITE-ATRA on the CRABP-1 and CRABP-2 degradation rates. (A, B) MCF-7 cells (A) and IMR-32 cells (B) were treated with ITE-ATRA for 8 h. Whole-cell lysates were analyzed by western blotting. Data in the bar graph are the mean ± SD of three independent experiments.

#. NMR SPECTRA OF COMPOUNDS

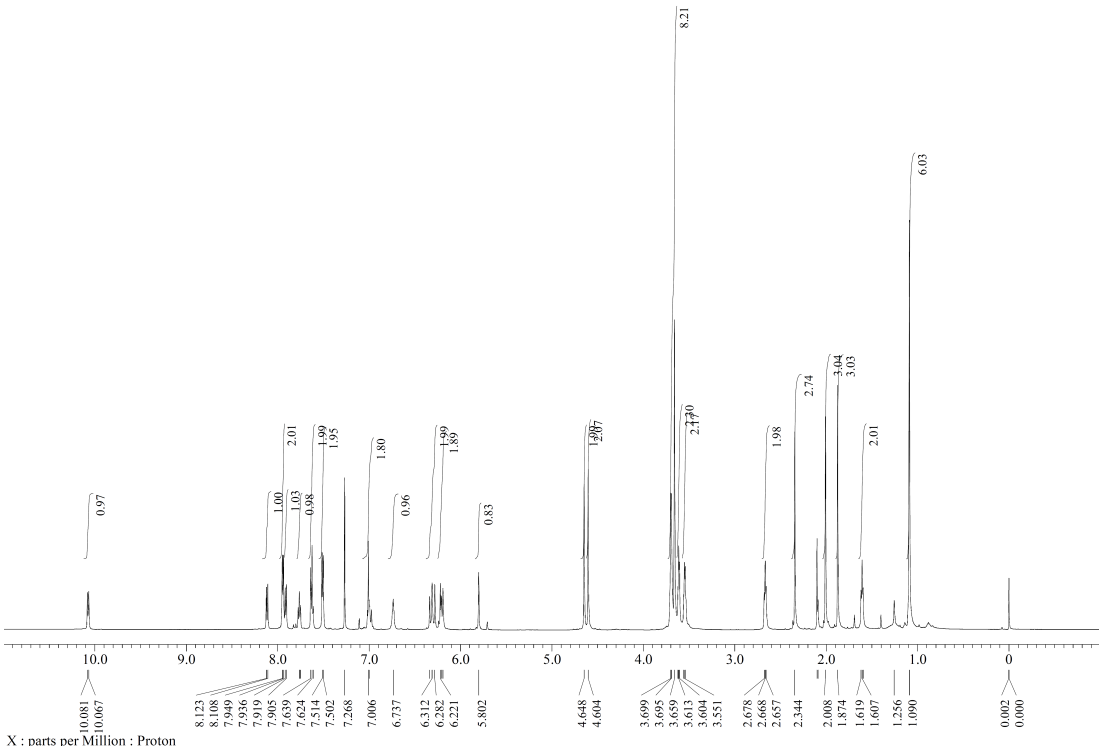


Fig. S4 ^{1}H NMR of **β-NF-ATRA**

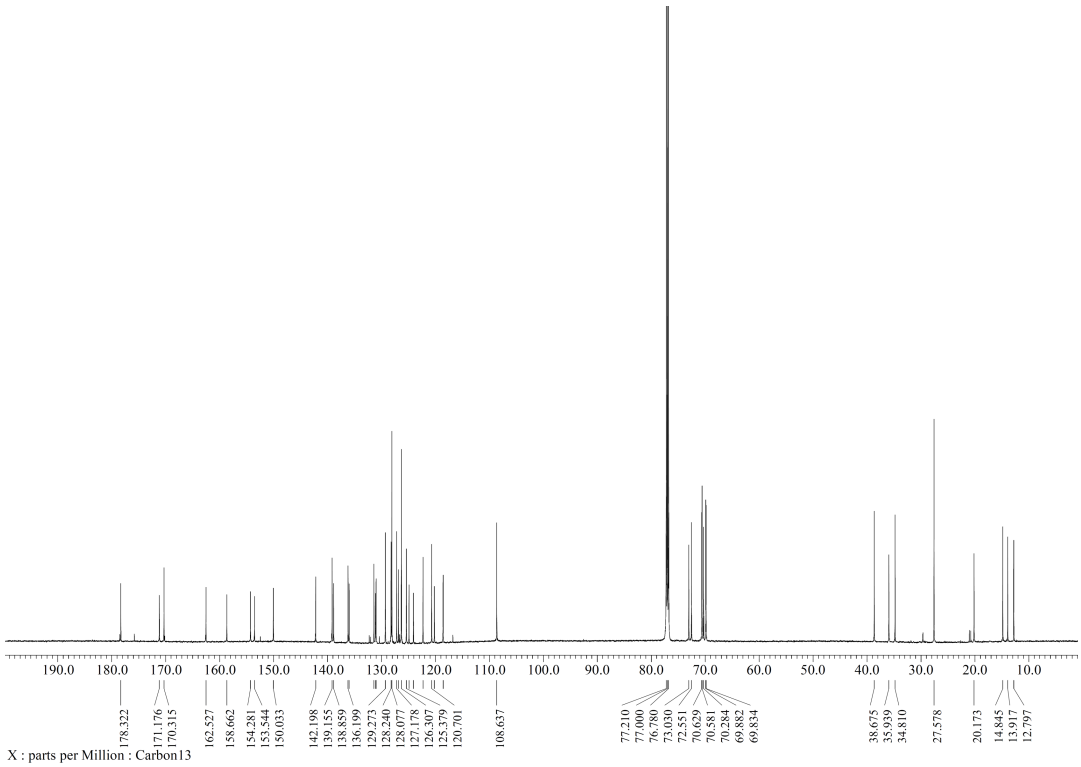


Fig. S5 ^{13}C NMR of **β-NF-ATRA**

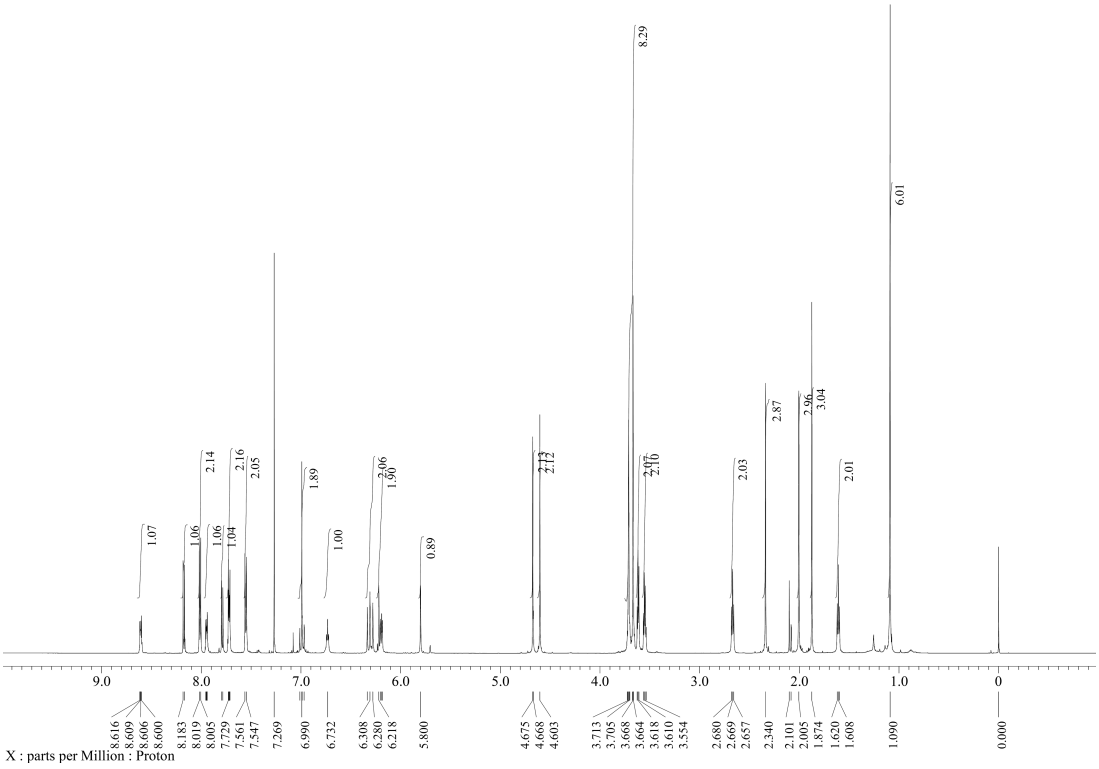


Fig. S6 ^{1}H NMR of **α-NF-ATRA**

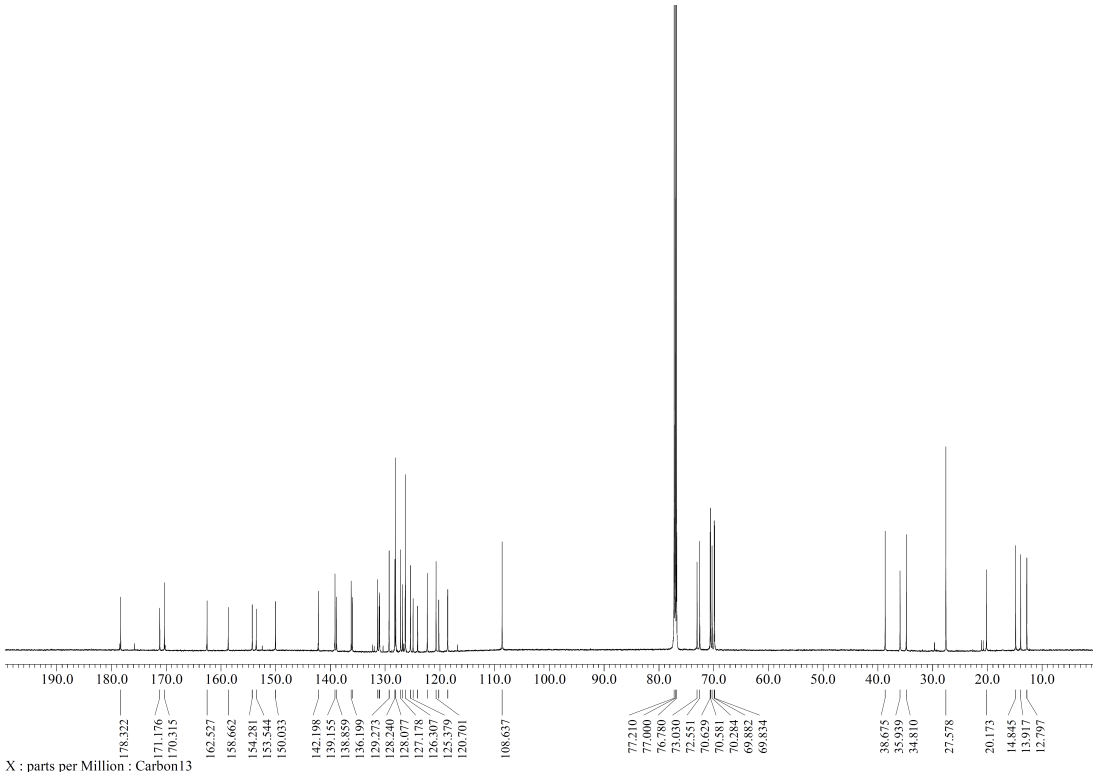


Fig. S7 ^{13}C NMR of **α-NF-ATRA**

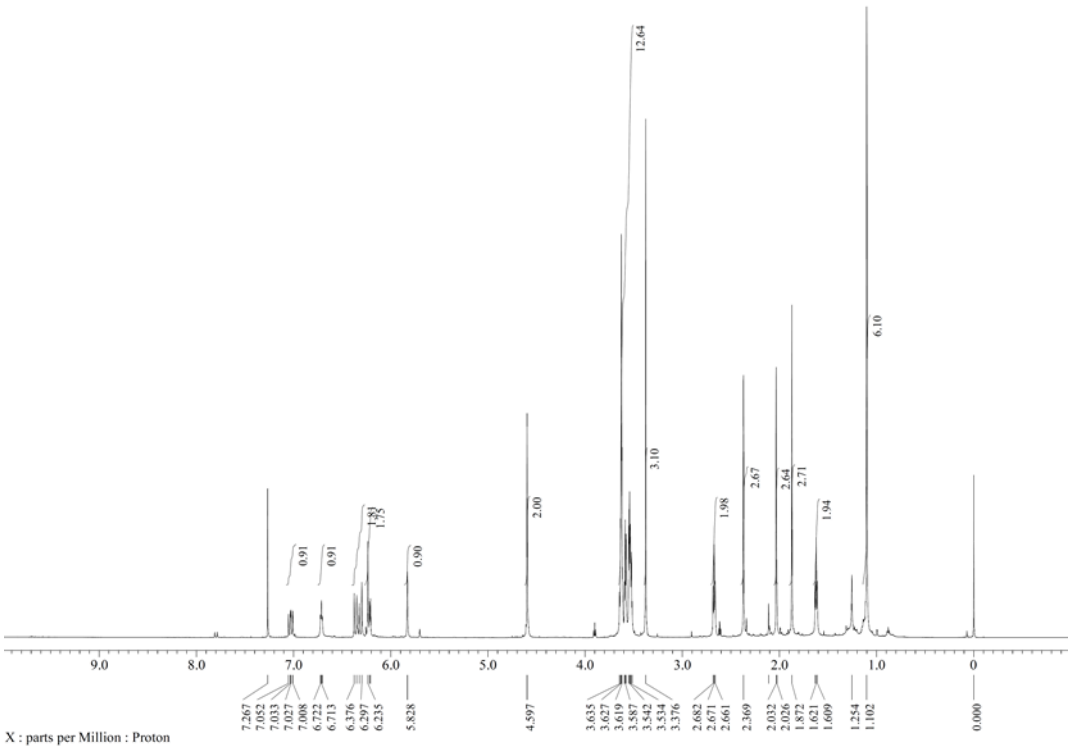


Fig. S8 ^{1}H NMR of **linker-ATRA**

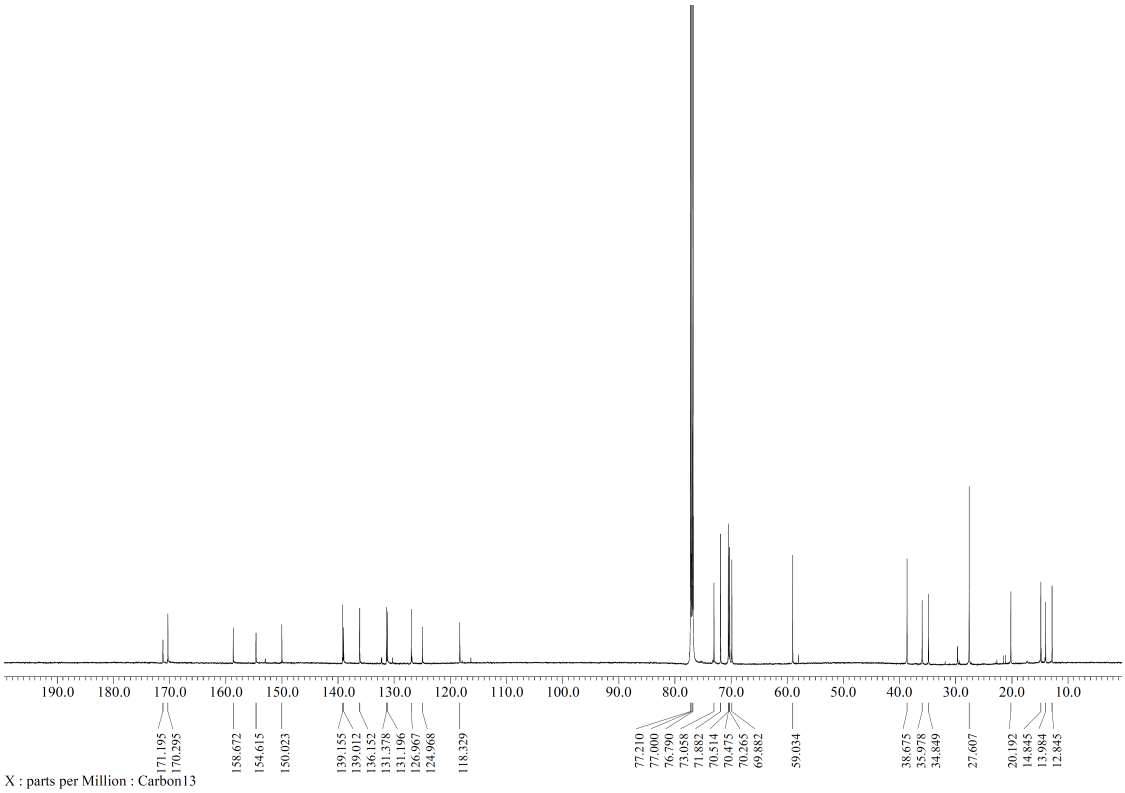


Fig. S9 ^{13}C NMR of **linker-ATRA**

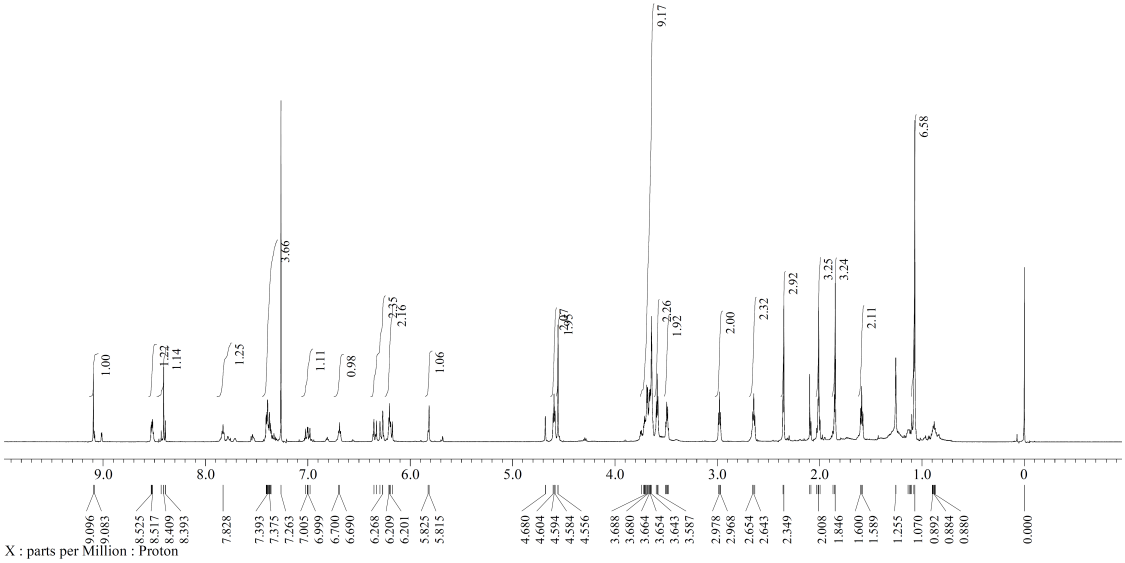


Fig. S10 ^{1}H NMR of **ITE-ATRA**

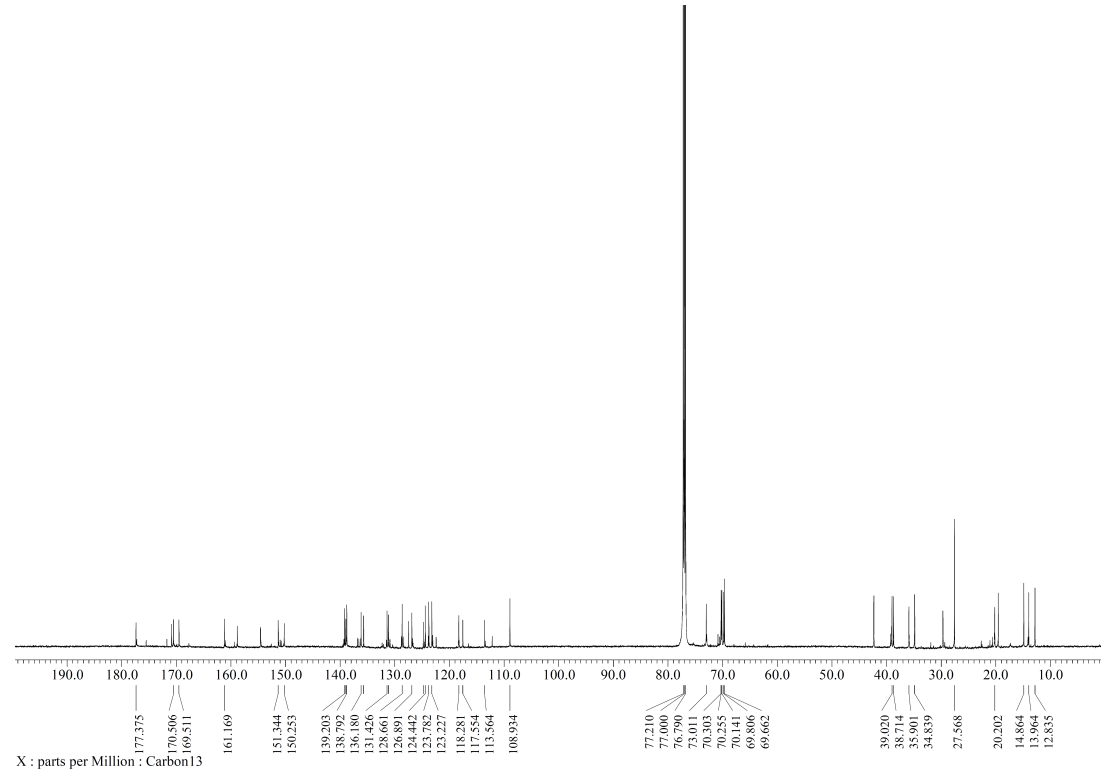


Fig. S11 ^{13}C NMR of **ITE-ATRA**

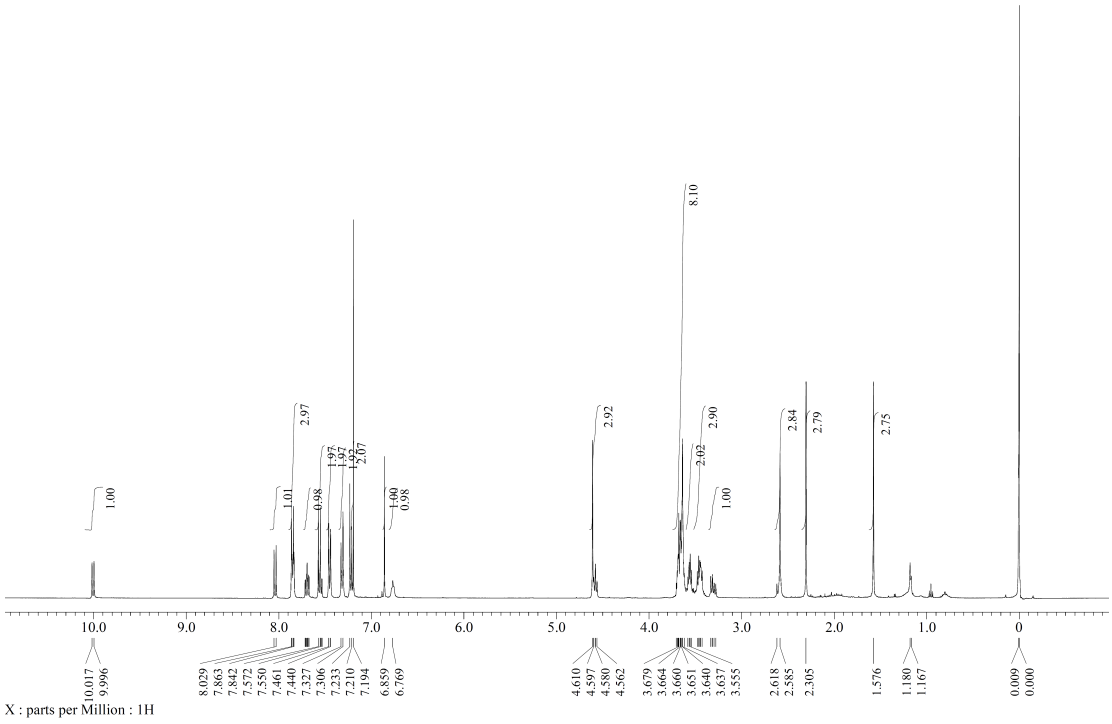


Fig. S12 ^{1}H NMR of **β-NF-JQ1**

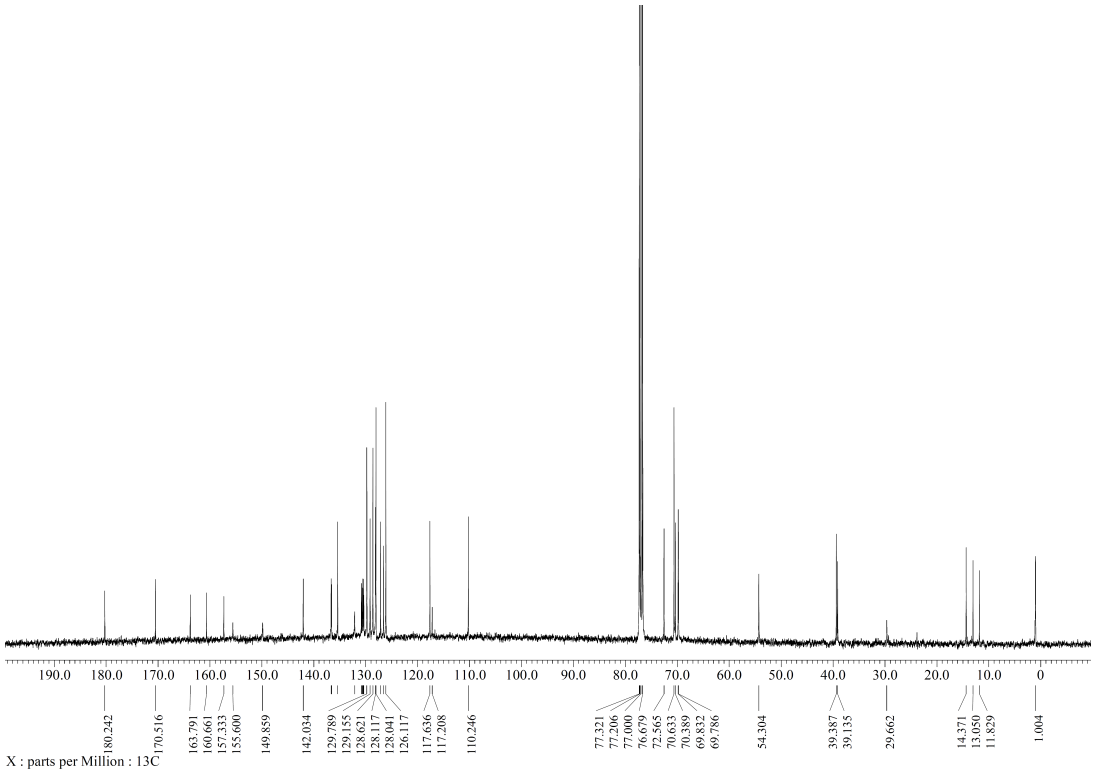
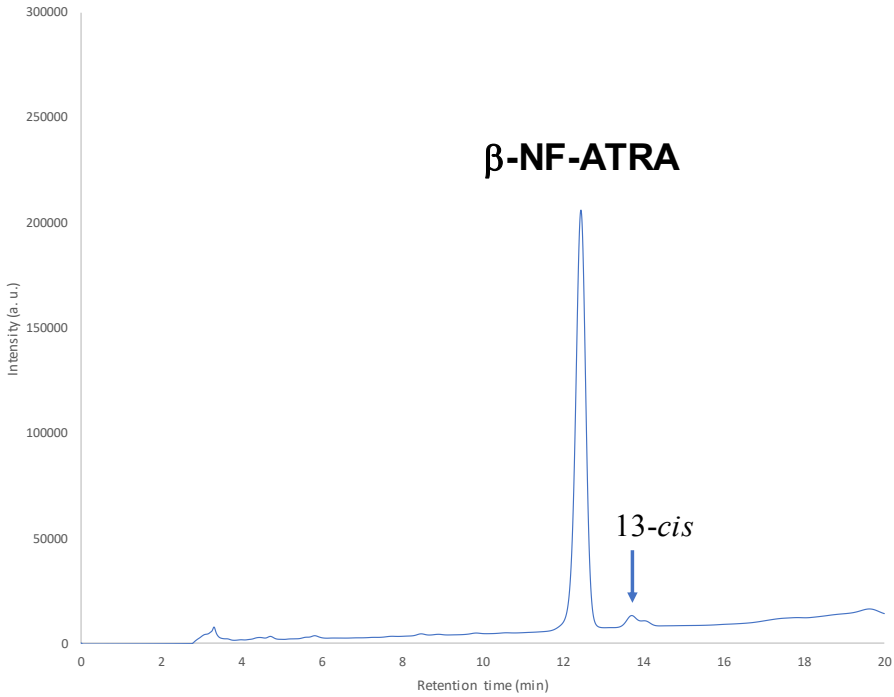


Fig. S13 ^{13}C NMR of **β-NF-JQ1**

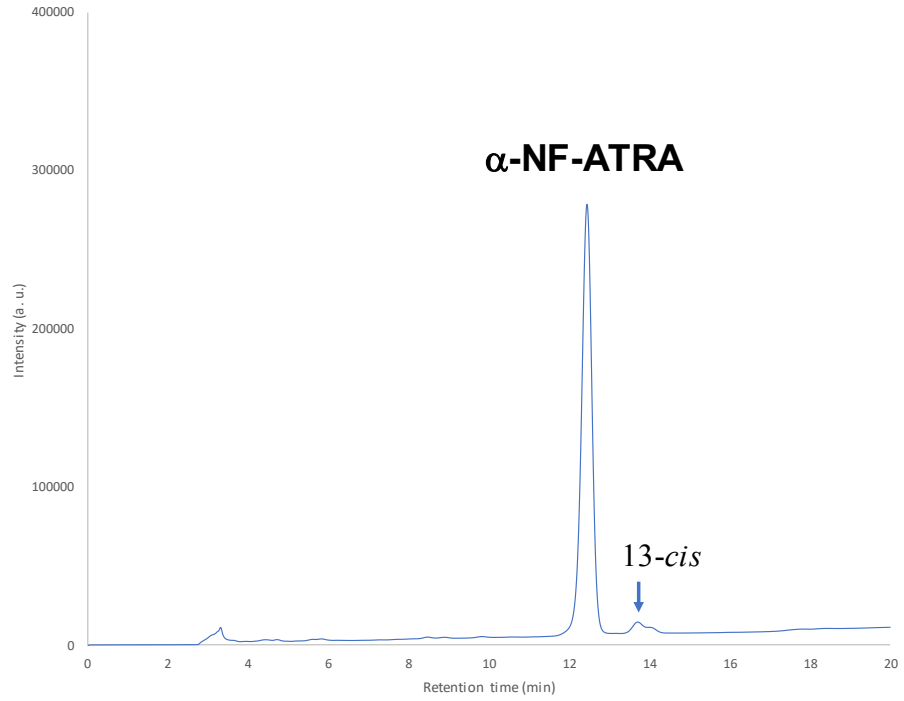
#. HPLC TRACES OF COMPOUNDS

HPLC conditions; column : SUPELCO Discovery BIO Wide Pore C18-10 (4.6 x 250 mm, 10 μm), Mobile phase; A : 0.1% TFA in H_2O, B : 0.1% TFA in CH_3CN, Gradient : B60-100% (30 min), flow rate : 1.0 mL/min, column oven : 35.0 °C



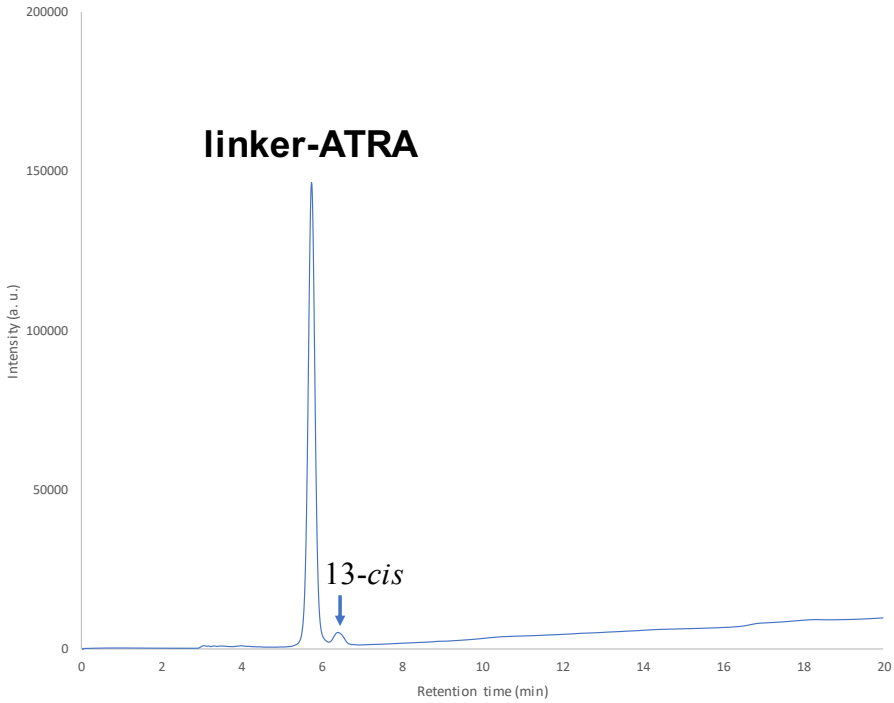
Purity 96%

Fig. S14 HPLC trace of **β-NF-ATRA**



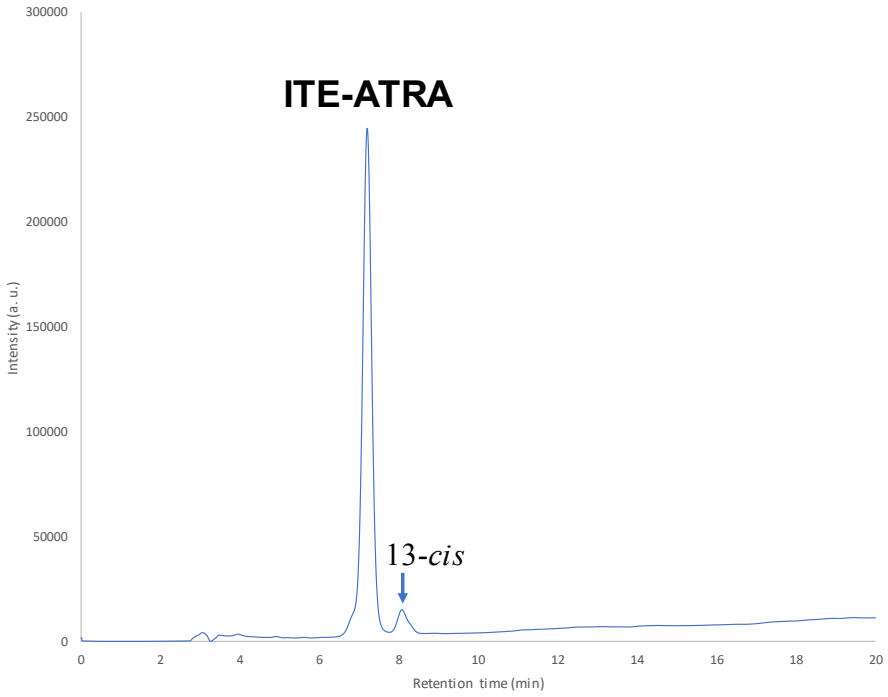
Purity 96%

Fig. S15 HPLC trace of **α-NF-ATRA**



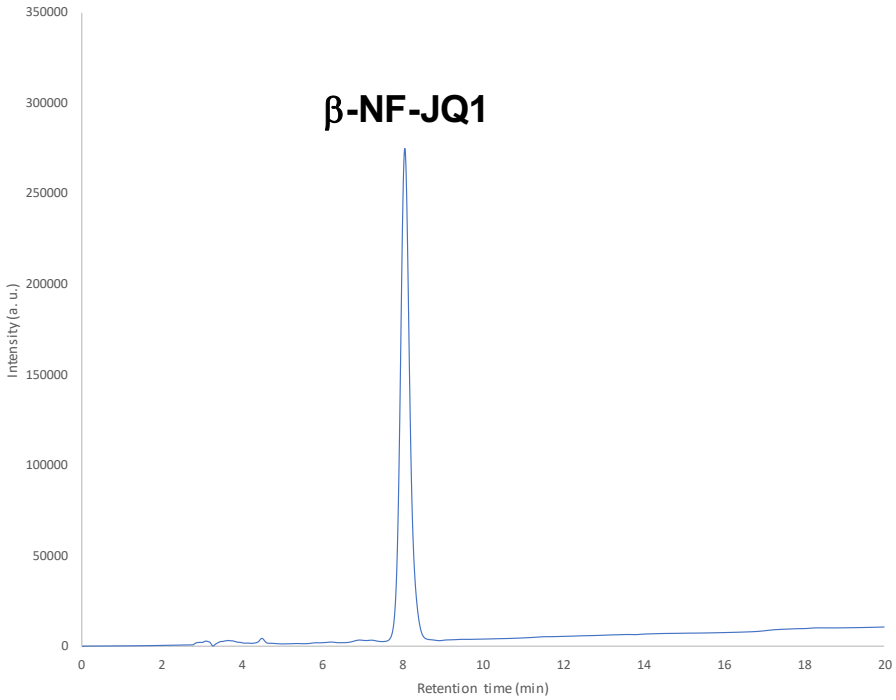
Purity 98%

Fig. S16 HPLC trace of **linker-ATRA**



Purity 95%

Fig. S17 HPLC trace of **ITE-ATRA**



Purity 96%

Fig. S18 HPLC trace of **β-NF-JQ1**