1. Biological Results

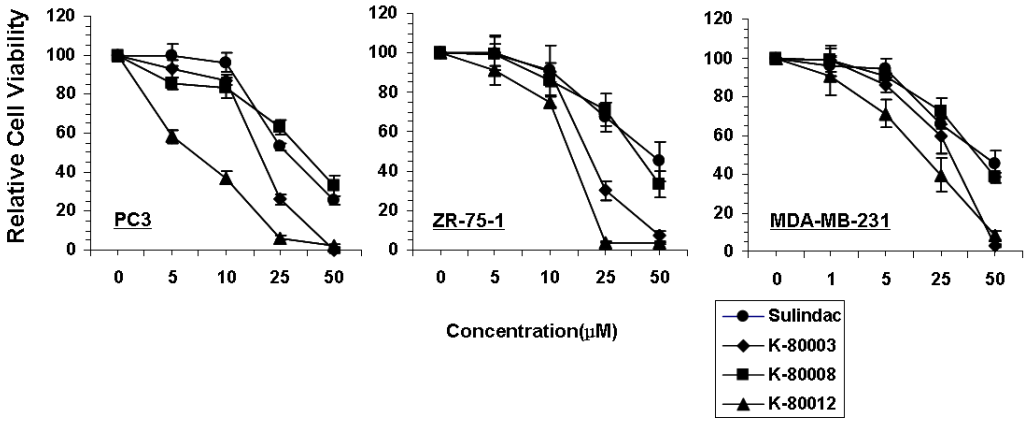


Figure S1 (related to Figure 3). Sulindac and analogs induce apoptosis of cancer cells.

PC3 prostate cancer cell, ZR-75-1 breast cancer, and MDA-MB-231 breast cancer cell lines were treated with the indicated concentration of Sulindac or analogs for 48 h. Cell viability was determined by the MTT assay.

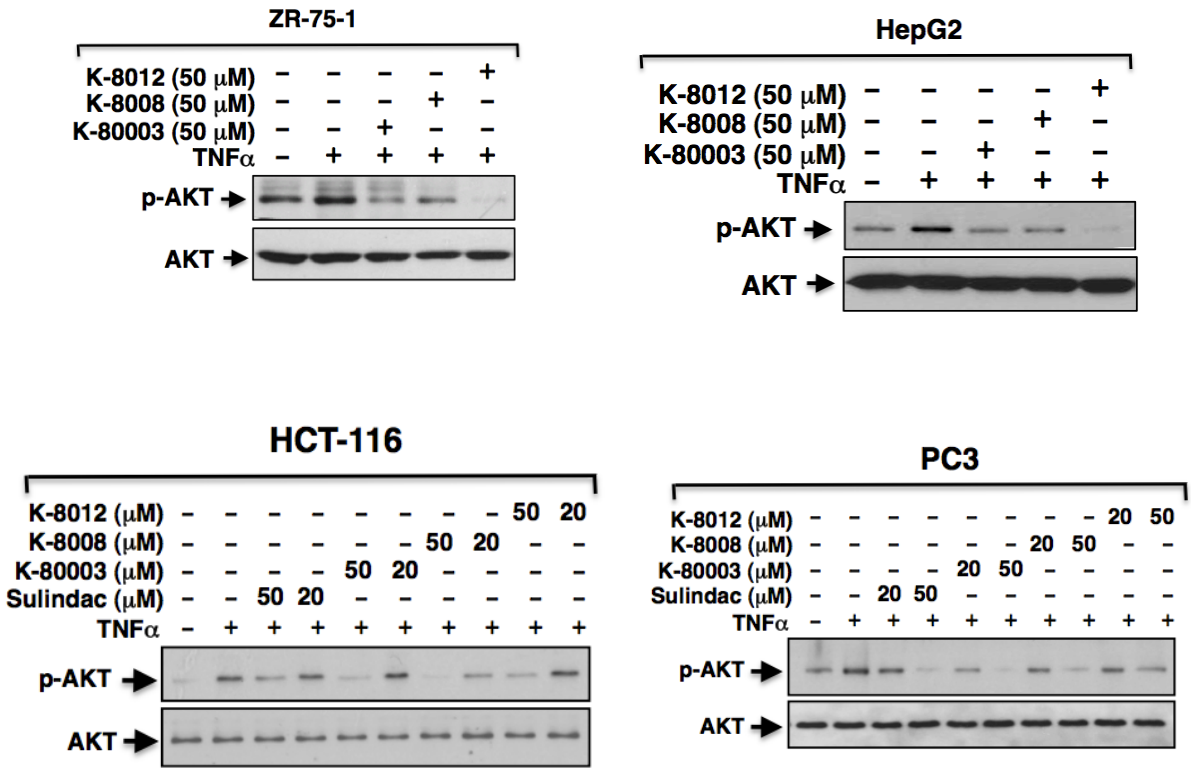


Figure S2 (related to Figure 3). Sulindac and analogs inhibit TNFα-induced AKT activation.

The indicated cell lines were pretreated with indicated compounds for 1 h before exposed to TNFα (10 ng/mL) for 30 min. AKT activation and total AKT expression were analyzed by immunoblotting.

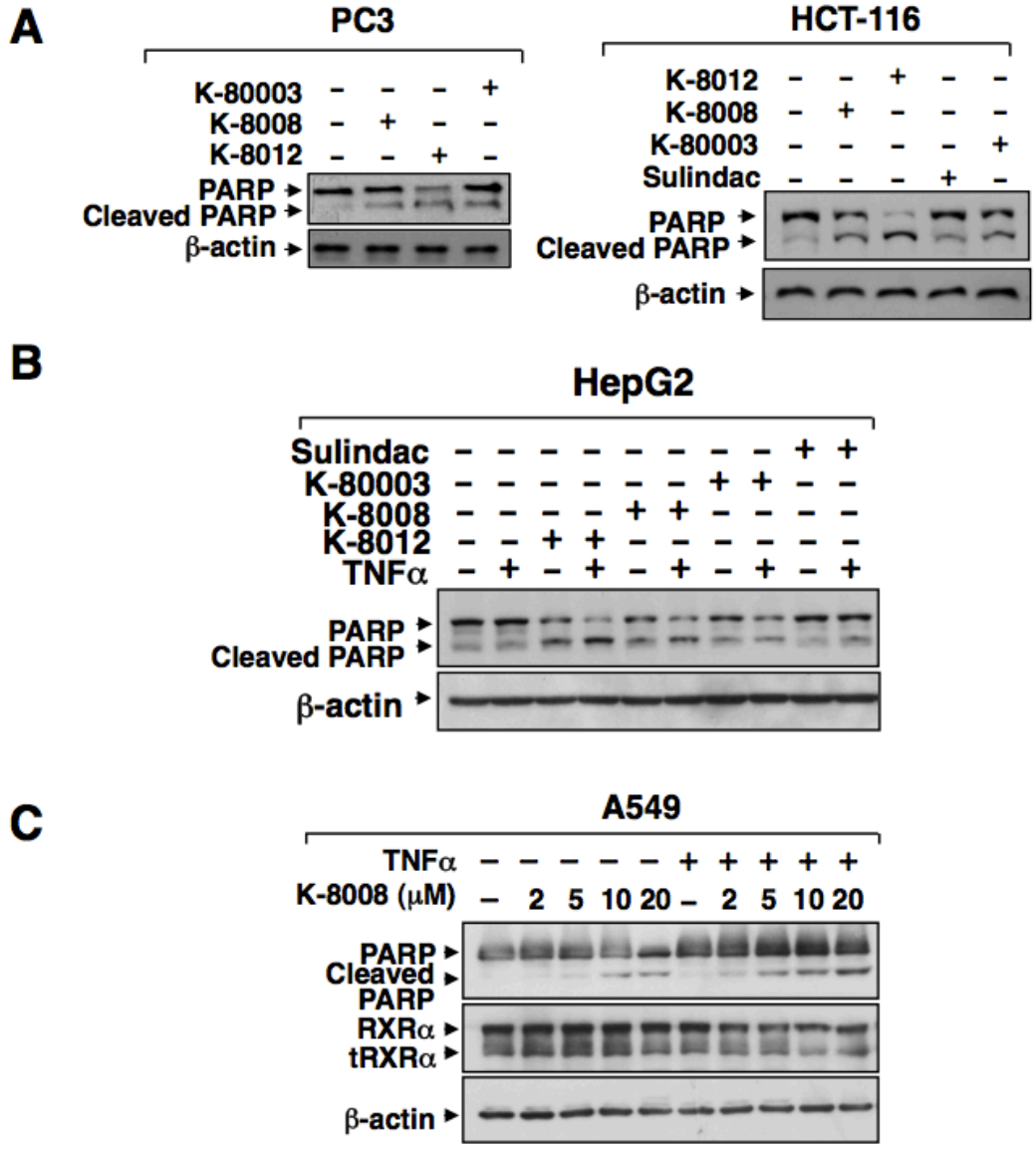


Figure S3 (related to Figure 3). Induction of apoptosis by Sulindac and its analogs.

(A) The apoptotic effects of Sulindac and its analogs in different cell lines. PC3 human prostate cancer cells and HCT-116 human colon cancer cells were treated with 40 μM indicated compound for 6 h. PARP cleavage was analyzed by immunoblotting. (B) Synergistic induction of apoptosis by compound and TNFα combination. HepG2 human liver cancer cells grown in medium with 1% FBS were treated with TNFα (10 ng/mL) and/or indicated compound (40 μM) for 4 h and analyzed by immunoblotting. (C) Dose dependent effect of K-8008 on apoptosis induction. A549 lung cancer cells cultured in medium with 1% FBS were treated with TNFα (10 ng/mL) in the presence or absence of the indicated concentration of compound K-8008 for 4 h and analyzed by immunoblotting.

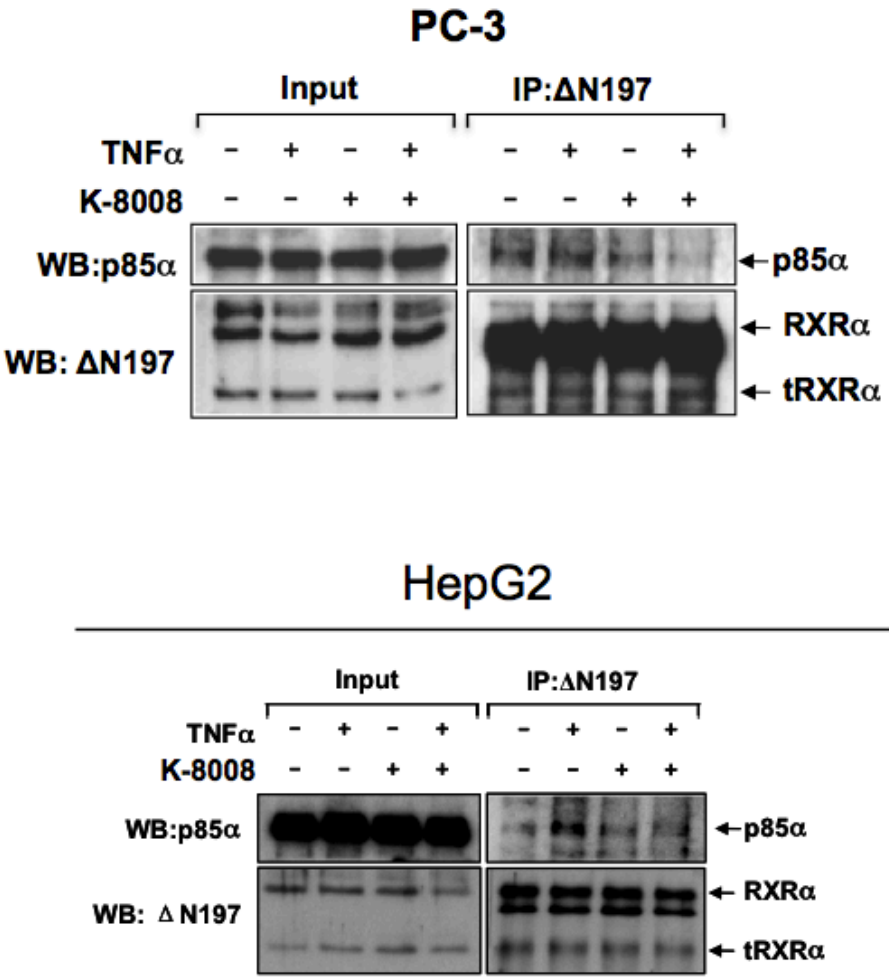
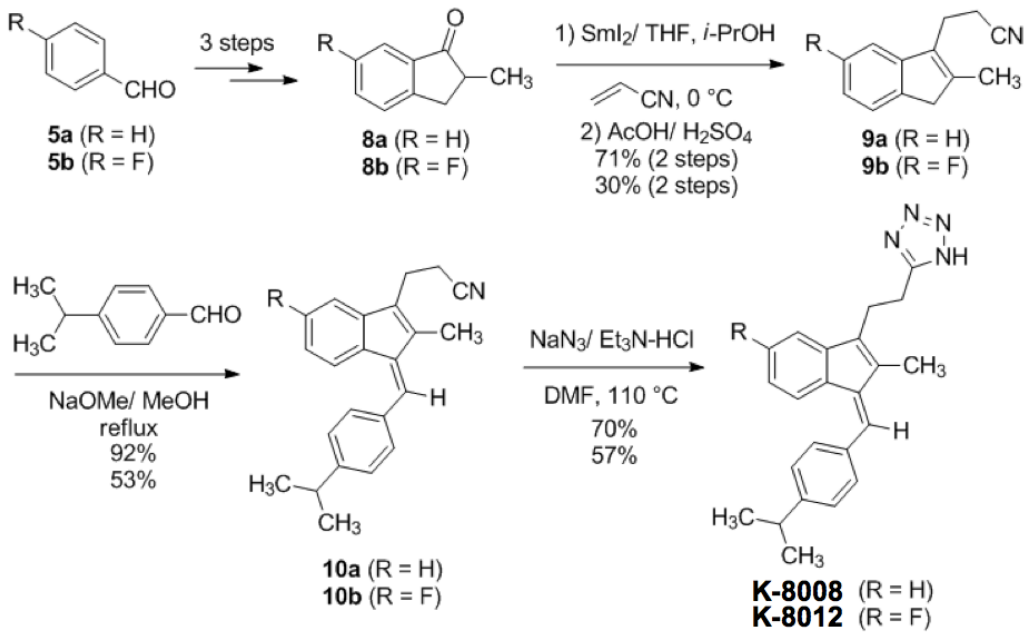


Figure S4 (related to Figure 3). K-8008 inhibits TNFα induced tRXRα-p85α interaction.

Co-immunoprecipitation assays were carried out in PC3 cells to determine tRXRα interaction with p85α. Cells treated with TNFα and/or K-8008 (40 μM) for 1 h were analyzed for tRXRα and p85α interaction by immunoprecipitation assay using ΔN197 anti-RXRα antibody. The co-immunoprecipitates were then subjected to immunoblotting analysis for tRXRα expression and its co-precipitated p85α by ΔN197 anti-RXRα and anti-p85α antibodies, respectively.

2. Synthetic Procedures (related to Figure 1)

Compound Synthesis:



3-(2-Methyl-1 *H*-inden-3-yl)propanenitrile (9a)

A solution of the 2-methylinden-1-one **8a** (500.0 mg, 3.42 mmol), and *iso*-propanol (1.3 mL, 17.1 mmol), and acrylonitrile (2.26 mL, 34.2 mmol) in anhydrous THF (10.0 mL) was purged with argon for 20 min and cooled to 0 °C. Then, A SmI_2 (10.3 mmol) solution in THF (103 mL) was added through transfer needle. After another 10 min, the reaction was quenched with a saturated aqueous $NaHCO_3$ (10 mL). The resulting mixture was extracted with Et_2O (20 mL × 3). The combined organic layers were washed with a saturated aqueous $Na_2S_2O_4$, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. To the residue was added HOAc / H_2SO_4 (10 / 1, 5.0 mL). Then, after stirring overnight at room temperature, the mixture was extracted with EtOAc (15 mL × 3). The combined extracts were washed successively with water, saturated $NaHCO_3$, and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : PE = 1 : 50) to afford compound **9a** (443 mg, 71%) as a white solid. M.p. 53-54 °C (ethyl acetate / PE); IR (film) : ν_{max} 3430, 3015, 2909, 2244, 1631, 1607, 1467, 1394, 1028 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.14 (s, 3H, C=CCH_3), 2.57 (t, J = 7.4 Hz, 2H, CH_2CH$_2$CN), 2.89 (t, J = 7.4 Hz, 2H, CH$_2$CH_2CN), 3.33 (s, 2H, ArCH_2C=C), 7.12-7.18 (m, 2H, Ar-*H*), 7.24-7.29 (m, 1H, Ar-*H*), 7.37-7.42 (m, 1H, Ar-*H*) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 16.6, 21.4, 42.8, 117.4, 119.4, 123.6, 124.1, 126.2, 133.1, 141.8, 142.4, 145.0 ppm; MS (ESI) *m/z* 206.1 (M+Na^+); HRMS (ESI) calcd for $C_{13}H_{13}NNa^+$ [M+Na^+]: 206.0940; found: 206.0943.

3-(5-Fluoro-2-methyl-1*H*-inden-3-yl)propanenitrile (9b)

A solution of compound **8b** (300.0 mg, 1.8 mmol), and *iso*-propanol (0.7 mL, 9.0 mmol), and acrylonitrile (1.2 mL, 18.0 mmol) in THF (4 mL) was purged with argon for 20 min and cooled to 0 °C. A SmI_2 (5.4 mmol) solution in THF (54 mL) was added through transfer needle. After 5 min, the reaction was quenched with saturated aqueous Na_2CO_3 (10 mL). The resulting mixture was extracted with Et_2O (15 mL × 3). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. To the residue was added HOAc / H_2SO_4 (10 / 1, 3.0 mL). After stirring for 4 h at room temperature, the mixture was extracted with EtOAc (15 mL × 3). The combined extracts were washed successively with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : PE = 1 : 50) to afford compound **9b** as a white solid (108 mg, 30%). M.p. 91-92 °C (hexane / EtOAc); IR (film): ν_{max} 2915, 2247, 1610, 1592, 1476, 1275, 1190, 1165 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.16 (s, 3H, C=CCH_3), 2.57 (t, J = 7.3 Hz, 2H, CH_2CH$_2$CN), 2.86 (t, J = 7.3 Hz, 2H, CH$_2$CH_2CN), 3.31 (s, 2H, CH_2C=C), 6.79-6.88 (m, 2H, Ar-*H*), 7.27-7.32 (m, 1H, Ar-*H*) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 16.6, 21.3, 41.2, 104.8 (d, $J_{C\text{-}F}$ = 24.0 Hz), 110.5 (d, $J_{C\text{-}F}$ = 23.0 Hz), 119.2, 124.17 (d, $J_{C\text{-}F}$ = 9.0 Hz), 132.8, 137.5, 144.6, 146.9 (d, $J_{C\text{-}F}$ = 9.0 Hz), 162.4 (d, $J_{C\text{-}F}$ = 241.0 Hz) ppm; MS (ESI) *m/z* 224.1 (M+Na^+, 100%); HRMS (ESI) calcd for $C_{13}H_{12}FNNa^+$ [M+Na^+]: 224.0846; found: 224.0848.

(*Z*)-3-(1-(4-*iso*-Propylbenzylidene)-2-methyl-1*H*-inden-3-yl)propanenitrile (10a)

To a solution of compound **9a** (238 mg, 1.3 mmol) in MeOH (4.0 mL) was added 2.5 N NaOMe (1.6 mL, 4.0 mmol) at room temperature to get an orange mixture. After stirring for 30 min, to the mixture was added 4-isopropylbenzaldehyde (0.3 mL, 2.0 mmol). The resulting mixture was refluxed at 80 °C for 4 h. After concentrated under reduced pressure, the residue was acidified with a 1N HCl solution to pH 4.0~6.0. After stirring for another 0.5 h at room temperature, the mixture was extracted with EtOAc (15 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (ethyl acetate : PE = 1 : 50) to afford indene derivative **10a** as a yellow solid (374 mg, 92%). M.p. 88-90 °C (Et_2O / hexane); IR (film): ν_{max} 3022, 2957, 2241, 1604, 1506, 1461, 1330, 1055 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.23 (d, J = 6.9 Hz, 6H, CH(CH_3)$_2$), 2.14 (s, 3H, C=CCH_3), 2.51 (t, J = 7.4 Hz, 3H, CH_2CH$_2$CN), 2.83-2.93 (m, 1H, CH(CH$_3$)$_2$), 2.88 (t, J = 7.4 Hz, 2H, CH$_2$CH_2CN), 6.80-6.88 (m, 1H, Ar-*H*), 6.97-7.03 (m, 1H, Ar-*H*), 7.07-7.12 (m, 1H, Ar-*H*), 7.12 (s, 1H, vinyl-*H*), 7.18-7.23 (m, 2H, Ar-*H*), 7.36-7.43 (m, 3H, Ar-*H*) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.4, 16.6, 21.7, 23.9 (2C), 34.0, 117.0, 119.3, 123.0, 124.6, 126.5, 127.7, 129.4, 131.1, 134.0, 134.4, 134.7, 136.0, 140.6, 143.1, 149.1 ppm; MS (ESI) *m/z* 336.2 (M+Na^+); HRMS (ESI) calcd for $C_{23}H_{23}NNa^+$ [M+Na^+]: 336.1723; found: 336.1729.

(*Z*)-3-(5-Fluoro-1-(4-isopropylbenzylidene)-2-methyl-1*H*-inden-3-yl)propanenitrile (10b)

To a solution of compound **9b** (261 mg, 1.3 mmol) in MeOH (4.0 mL) was added 2.5 N NaOMe (1.6 mL, 4.0 mmol) at room temperature to get an orange mixture. After stirring for 30 min, to the mixture was added 4-isopropylbenzaldehyde (0.3 mL, 2.0 mmol). The resulting mixture was refluxed at 80 °C for 4 h. After concentrated under reduced pressure, the residue was acidified with a 1N HCl solution to pH 4.0~6.0. After stirring for another 0.5 h at room temperature, the mixture was extracted with EtOAc (15 mL × 3). The combined organic layers were dried over anhydrous Na2SO4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (ethyl acetate : PE = 1 : 50) to afford indene derivative **10b** as a yellow solid (228 mg, 53%). M.p. 108-109 °C (hexane / EtOAc); IR (film): ν_{max} 2957, 2927, 2866, 2247, 1598, 1464, 1199, 1162, 1138, 1055, 1016 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.32 (d, J = 6.9 Hz, 6H, CH(CH_3)$_2$), 2.24 (s, 3H, C=CCH_3), 2.60 (t, J = 7.4 Hz, 2H, CH_2CH$_2$CN), 2.93 (t, J = 7.4 Hz, 2H, CH$_2$CH_2CN), 2.98 (sept, J = 6.9 Hz, 1H, CH(CH$_3$)$_2$), 6.58-6.65 (m, 1H, Ar-*H*), 6.75-6.80 (m, 1H, Ar-*H*), 7.21 (s, 1H, vinyl-*H*), 7.28-7.33 (m, 2H, Ar-*H*), 7.39-7.48 (m, 3H, Ar-*H*) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.5, 16.6, 21.6, 23.9, 34.0, 104.7 (d, J_{C-F} = 23.0 Hz), 110.6 (d, J_{C-F} = 22.0 Hz), 119.1, 124.0 (d, J_{C-F} = 8.0 Hz), 126.5 (2C), 129.4 (2C), 130.2, 131.1, 133.68, 133.78, 138.2, 139.6, 145.47 (d, J_{C-F} = 8.0 Hz), 149.3, 163.0 (d, J_{C-F} = 244.0 Hz) ppm; MS (ESI) *m/z* 354.2 (M+Na^+, 100%); HRMS (ESI) calcd for $C_{23}H_{22}FNNa^+$ [M+Na^+]: 354.1628; found: 354.1625.

(*Z*)-5-(2-(1-(4-*iso*-Propylbenzylidene)-2-methyl-1*H*-inden-3-yl)ethyl)-1*H*-tetrazole (K-8008)

A flask (10 mL) was charged with nitrile **10a** (45 mg, 0.14 mmol) and dry DMF (0.8 mL), triethylamine hydrochloride (110 mg, 0.80 mmol) and sodium azide (52 mg, 0.80 mmol) were added to the solution under nitrogen. The mixture was heated for 40 h at 110 °C, then cooled to the room temperature, concentrated in vacuo and diluted with water (10 mL). The aqueous solution was then acidified to pH 2.0 using concentrated HCl, extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (ethyl acetate : PE = 1 : 1) to afford crude **K-8008** (36 mg, 70%). M.p. 173-175 °C (CH_2Cl_2 / hexane); IR (film): ν_{max} 3136, 3022, 2957, 2737, 2616, 1911, 1598, 1564, 1463, 1326, 1254 1049 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.29 (d, J = 6.9 Hz, 6H, CH(CH_3)$_2$), 1.92 (s, 3H, C=CCH_3), 2.90-2.99 (m, 1H, CH(CH$_3$)$_2$), 3.05 (t, J = 7.1 Hz, 2H, CH_2CH$_2$-Tetrazole), 3.27 (t, J = 7.1 Hz, 2H, CH$_2$CH_2-Tetrazole), 6.85-6.92 (m, 1H, Ar-*H*), 7.05-7.14 (m, 3H, vinyl-*H*, Ar-*H*), 7.20-7.28 (m, 2H, Ar-*H*), 7.35-7.49 (m, 3H, Ar-*H*) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.9, 22.6, 23.9 (2C), 29.7, 34.0, 117.4, 122.9, 124.6, 126.5, 127.8, 129.4, 130.9, 133.9, 134.4, 135.6, 135.7, 140.5, 143.4, 149.1, 155.9 ppm; MS (ESI) *m/z* 379.2 (M+Na^+); HRMS (ESI) calcd for $C_{23}H_{24}N_4Na^+$ [M+Na^+] 379.1893; found 379.1894.

(*Z*)-5-(2-(5-Fluoro-1-(4-isopropylbenzylidene)-2-methyl-1*H*-inden-3-yl)ethyl)-1*H*-tetrazole (K-8012)

A flask (10 mL) was charged with the nitrile **10b** (96 mg, 0.29 mmol) and dry DMF (3.0 mL), triethylamine hydrochloride (200 mg, 1.45 mmol) and sodium azide (94.3 mg, 1.45 mmol) were added to the solution under nitrogen. The mixture was heated for 40 h at 110 °C, then cooled to the room temperature, concentrated in vacuo and diluted with water (10 mL).The aqueous solution was then acidified to pH 2.0 using concentrated HCl, extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography to afford compound **K-8012** as a yellow solid (62 mg, 57%). M.p. 201-202 °C (hexane / EtOAc); IR (film): ν_{max} 3143, 2964, 2930, 2866, 2731, 2619, 2454, 1597, 1464, 1266, 1180, 1134, 1101, 1052 cm^{-1}; ^1H NMR (400 MHz, Methanol-d_4) δ 1.29 (d, J = 6.8 Hz, 6H, $CH(CH_3)_2$), 1.96 (s, 3H, C=CCH_3), 2.96 (sept, J = 6.8 Hz, 1H, $CH(CH_3)_2$), 3.03 (t, J = 7.1 Hz, 2H, CH_2CH_2-Tetrazole), 3.19 (t, J = 7.1 Hz, 2H, CH_2CH_2-Tetrazole), 6.50-6.60 (m, 1H, Ar-*H*), 6.86-6.94 (m, 1H, Ar-*H*), 7.17 (s, 1H, vinyl-*H*), 7.24-7.35 (m, 3H, Ar-*H*), 7.37-7.44 (m, 2H, Ar-*H*) ppm; ^{13}C NMR (100 MHz, Methanol-d_4) δ 10.0, 23.5, 24.3, 24.8, 35.3, 106.0 (d, $J_{C\text{-}F}$ = 24.0 Hz), 111.1 (d, $J_{C\text{-}F}$ = 23.0 Hz), 124.8 (d, $J_{C\text{-}F}$ = 9.0 Hz), 127.6 (2C), 130.5 (2C), 131.5, 131.6, 135.4, 136.67 (d, $J_{C\text{-}F}$ = 2.0 Hz), 138.5, 141.2, 147.69 (d, $J_{C\text{-}F}$ = 9.0 Hz), 150.5, 157.3, 164.5 (d, $J_{C\text{-}F}$ = 243.0 Hz) ppm; MS (ESI) *m/z* 397.2 ($M+Na^+$, 100%); HRMS (ESI) calcd for $C_{23}H_{23}FN_4Na^+$ [$M+Na^+$]: 397.1799; found: 397.1804.

3. Crystallographic Studies

Both crystal structures have the space group $P2_1$ and similar unit cell parameters, however, axes b and c have been replaced in them (see Table S1). This difference was not induced by differences in ligands, because K-8008 co-crystals were also obtained in same unit cell as K-8012 co-crystals (data are not presented). More likely, the crystal packing changed due to the presence of different salt additives (Mg Formate in case of the K-8008 co-crystal and Na Acetate in case of the K-8012 co-crystal) during the crystallization. Nevertheless, the change of the unit cell did not affect protein structures significantly. They both crystallized as nocrystallographic tetramers with molecular symmetry P222, and the rms deviation between their 760 Ca atoms (out of 788) is 0.36 Å, which is comparable with the overall error of the structures (0.3 Å). In both structures, the chains A and D as well as chains B and C have a very high degree of pairwise similarity. Thus, in the RXRα LBD/K-8008 structure, the rms deviations between 193 out of 197 Ca atoms of the chains A and D was 0.24 Å (0.22 Å for the chains B and C). The rms deviation between chains A and B (as well as A and C) was 0.6 Å (for the same group of Ca atoms). Several N- and C-terminal residues of both structures are disordered. Thus, the electron density is present for residues 261-457 of all four chains of the RXRα LBD/K-8008 structure. In the RXRα LBD /K-8012-binding structure, however, the 10 additional residues at the N-termini of the chains B and C (residues 231-241) are also ordered. Interestingly, in the crystal

structure of RXRα LBD complexed with an inactive retinoic acid isomer (PDB entry 1G5Y) (Ref2), the entire region 231- 458 of all four chains is ordered, even though its unit cells parameters (a, b, c = 51.0, 99.7 96.3 Å β = 96.7°) are very close to those of the K-8008 co-crystal.

Table S1 (related to Experimental Procedures: Crystallization and structure solution of the RXR LBD-ligand complexes.). Data collection and refinement statistics of the crystal structures

RXR LBD co-crystalized with:	K-8008	K-8012
Space Group	$P2_1$	$P2_1$
Unit Cell *a*, b, c /Å, β	51.0 99.3 94.0 98.7°	46.6 98.8 110.6 99.0°
Resolution/Å (outer shell)	68-2.0 (2.08-2.03)	37-2.1 (2.17-2.11)
Unique reflections collected	58010	48947
Completeness (%)	97 (92)	86 (50)
Average Redundancy	4.3 (3.5)	3.6 (3.4)
<I/s(I)>	8.6 (1.0)	8.1 (1.6)
CC(1/2)	0.988 (0.86)	0.997 (0.71)
R_{meas}	0.16 (1.4)	0.079 (0.80)
Refinement statistics		
Resolution range (Å)	50-2.0	37-2.2
No reflections work set (R_{FREE} set)	57918 (3719)	44889 (4288)
R_{WORK} (R_{FREE})	0.199 (0.237)	0.199 (0.247)
RMS Deviations		
bond lengths (Å)	0.003	0.003
bond angles (Å)	0.74	0.76
Ramachandran plot (%)		
Favored by MolProbity (%)	97.6	98.2
Outliers by MolProbity (%)	0.0	0.0
Coordinate errors, estimated by Phenix (Å)	0.30	0.29
No. of protein residues observed (present)	788 (976)	810 (976)
No. of ligand residues	2	2
No. of Water molecules	652	280
Temperature factors ($Å^2$)		
overall	21.4	50.4
protein	20.8	50.6
ligands	29.7	53.4
solvent	26.5	45.6
from Wilson B plot	28.1	40.3

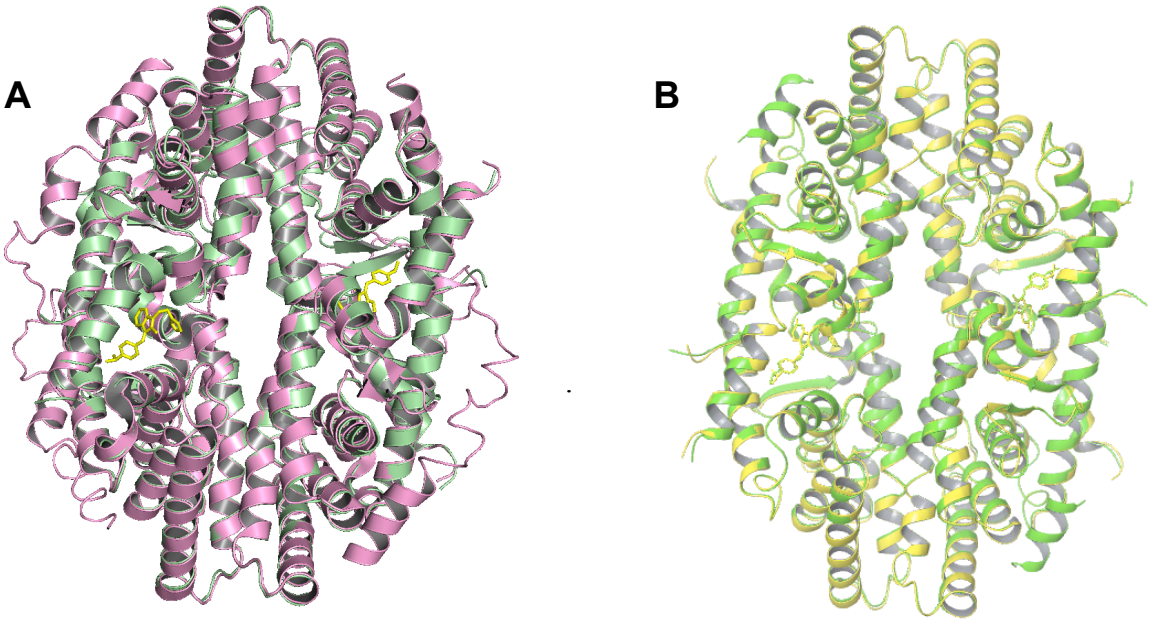


Figure S5 (related to Figure 6). (A) Superposition of the RXRα LBD tetramer in complex with K-8008 (green ribbons) and the apo protein structure (pink ribbons, from PDB 1G1U). Yellow sticks are the bound K-8008 molecules. (B) Superposition of the RXRα LBD tetramer in complex with K-8008 (green ribbons) and the RXRα LBD tetramer in complex with K-8012 (yellow ribbons). Green sticks and yellow sticks are the bound K-8008 and K-8012 molecules respectively.

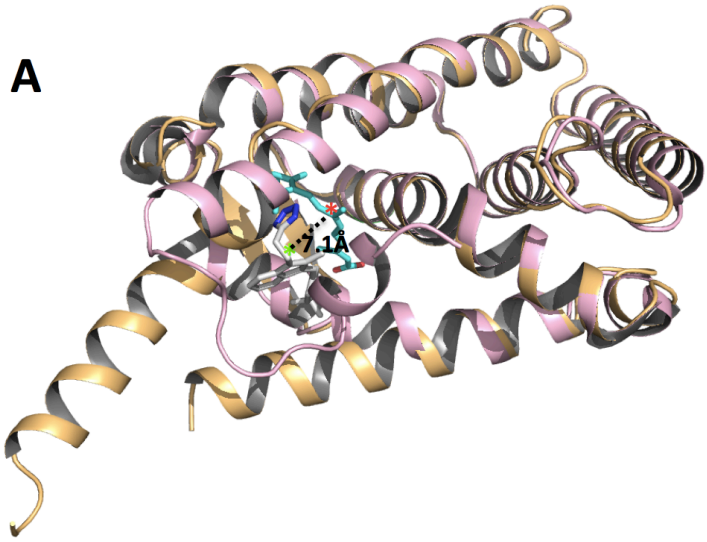


Figure S6 (related to Figure 6 and Figure 7). (A) Distance between the binding region of K-8008 and the binding region of 9-cis-RA. Distance between the centroids of bound 9-cis-RA (red *) and the bound K-8008 (green *) is measured. The 9-cis-RA bound structure is in pink cartoon and the K-8008 in light orange cartoon.

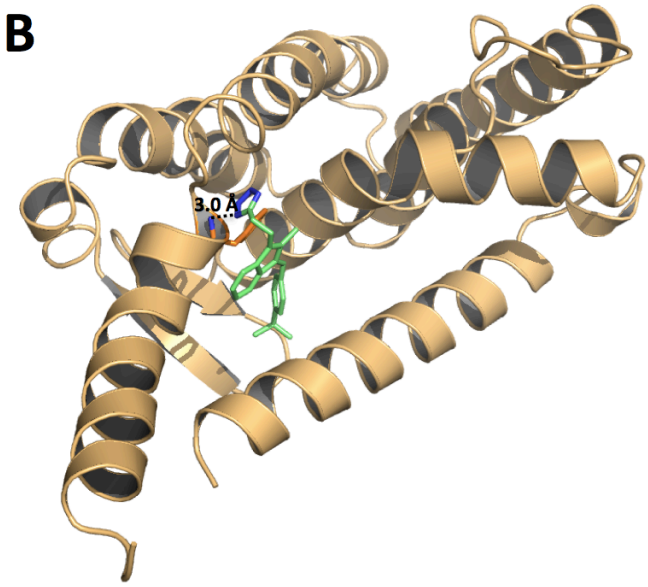


Figure S6 (related to Figure 6 and Figure 7). (B) Distance between closest N of the tetrazol of K-8008 and the backbone N of residue of Phe438 (side chain in orange and N in blue).

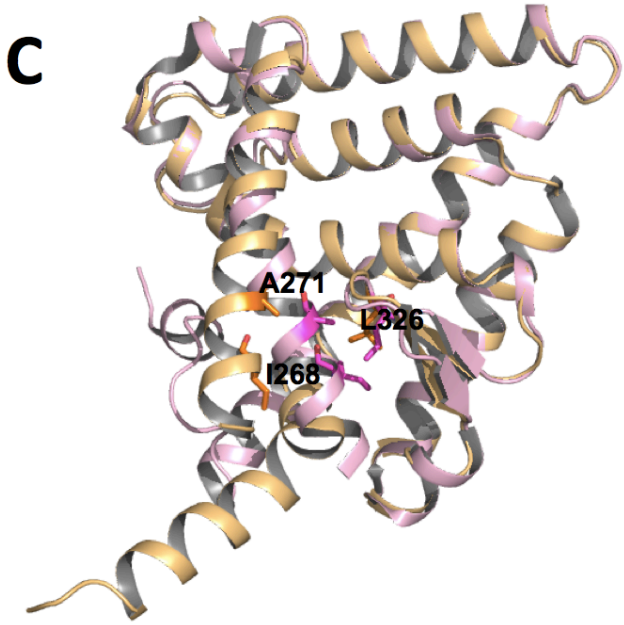


Figure S6 (related to Figure 6 and Figure 7). (C). Locations of residues that contribute both to the K-8008 binding and the 9-cis-RA binding. The 9-cis-RA bound structure is in pink cartoon and side chains are in pink sticks. The K-8008 bound structure is in light orange cartoon and side chains in orange sticks.