Supplementary Information

Discovery of a potent catalytic p300/CBP inhibitor that targets lineage-specific tumors

Loren M. Lasko[1*], Clarissa G. Jakob[1*], Rohinton P. Edalji[1], Wei Qiu[1], Debra Montgomery[1], Enrico L. Digiammarino[1], T . Matt Hansen[1], Roberto M. Risi[1], Robin Frey[1], Vlasios Manaves[1], Bailin Shaw[1], Mikkel Algire[1], Paul Hessler[1], Lloyd T. Lam[1], Tamar Uziel[1], Emily Faivre[1], Debra Ferguson[1], Fritz G. Buchanan[1], Ruth L. Martin[1], Maricel Torrent[1], Gary G. Chiang[1,2], Kannan Karukurichi[3], J. William Langston[4], Brian T. Weinert[5], Chunaram Choudhary [5], Peter de Vries[6], John H. Van Drie[7], David McElligott[8], Ed Kesicki[3], Ronen Marmorstein[9], Chaohong Sun[1], Philip A. Cole[10], Saul H. Rosenberg[1], Michael R. Michaelides[1], Albert Lai[1,11], and Kenneth D. Bromberg[1,11]

*These authors contributed equally to this study

[1]Discovery, Global Pharmaceutical Research and Development, AbbVie, 1 North Waukegan Road, North Chicago, Illinois 60064

[2]eFFECTOR Therapeutics, 11180 Roselle St., Suite A, San Diego, CA 92121

[3]Petra Pharma Corporation, 430 E. 29th St., Suite 435, New York, NY 10016

[4]Faraday Pharmaceuticals, 1616 Eastlake Ave E, Suite 560 Seattle, WA 98102

[5] Department of Proteomics, the Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3B, DK-2200 Copenhagen, Denmark

[6]Cascadian Therapeutics, Inc., 2601 Fourth Avenue, Suite 500, Seattle, WA 98121

[7]Van Drie Research, 109 Millpond, Andover, MA 01845

[8]Accelerator Corporation, 430 East 29th St., New York, NY 10106

[9]Perelman School of Medicine, University of Pennsylvania, 421 Curie Blvd., Philadelphia, PA 19104

[10]Johns Hopkins University, 725 N. Wolfe St., Baltimore, MD 21205

[11]Corresponding Authors: Kenneth D. Bromberg (kenneth.bromberg@abbvie.com); Albert Lai (Albert.Lai@abbvie.com)

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Key words: EP300, CBP, H3K27Ac, histone acetyltransferase, prostate cancer, androgen receptor

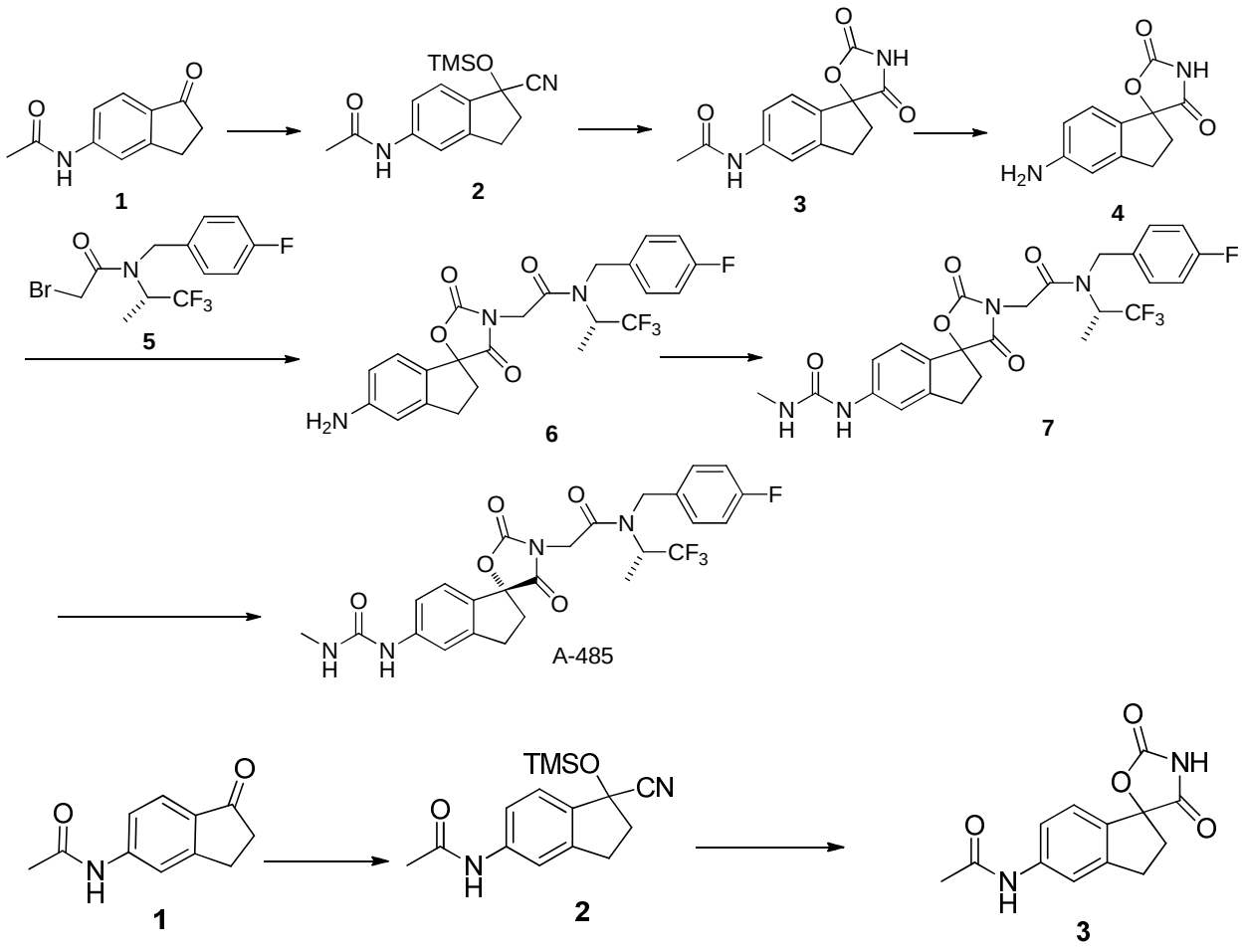
Running title: Discovery of p300/CBP inhibitor A-485

Supplemental Text

A-485 was primarily metabolized by CYP3A4 *in vitro* indicating that careful consideration should be taken when considering combinations with other agents that induce, activate, or inhibit CYP3A4. A-485 also exhibited modest inhibition of CYP2C8 (IC_{50}=0.99 µM) and CYP2C9 (IC_{50}=1.6 µM). There was no activity toward hERG (>30 µM).

Tool Compound Synthesis

Intermediates were characterized by LC-MS to confirm the structures and purity and carried to the next step without further purification unless otherwise noted. The schema for synthesis of A-485 is described below followed by synthesis of indicated intermediates.



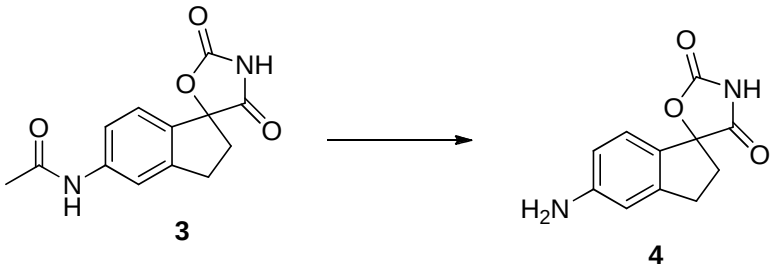
N-(1-cyano-1-(trimethylsilyloxy)-2,3-dihydro-1H-inden-5-yl)acetamide (2):

To a stirring solution of **1** (commercially available) (31 g, 164 mmol) in toluene and MeCN (400 mL/400 mL) was added ZnI_2 (5.2g, 16.4 mmol) and TMSCN (33 g, 328 mmol). The mixture was heated at reflux for 1 hour, then treated with an additional 33g of TMSCN and 4 g of zinc iodide and refluxed for an additional 2 h. The reaction was cooled and the solvent from reaction mixture was removed under reduced pressure. The residue was purified by silica gel column chromatography to afford compound **2** (14 g, 30%).

***N*-(2',4'-dioxo-2,3-dihydrospiro[indene-1,5'-ozazolidine]-5-yl)acetamide (3):**

Compound **2** (11.5 g, 39.8 mmol) was dissolved in EtOH (160 mL), cooled with ice bath to 0 degrees and HCl gas was bubbled in for 2 h.(Exothermic reaction). The solvent was removed under reduced pressure. The residue was partitioned between Ethyl acetate and aq. NaHCO3, the organic extract was dried, concentrated and triturated with 1 to 1 mixture of petroleum ether and Ethyl Acetate. The crude product ethyl 5-acetamido-1-hydroxy-2,3-dihydro-1H-indene-1-carbimidate (7.6 g) was used directly for subsequent step.

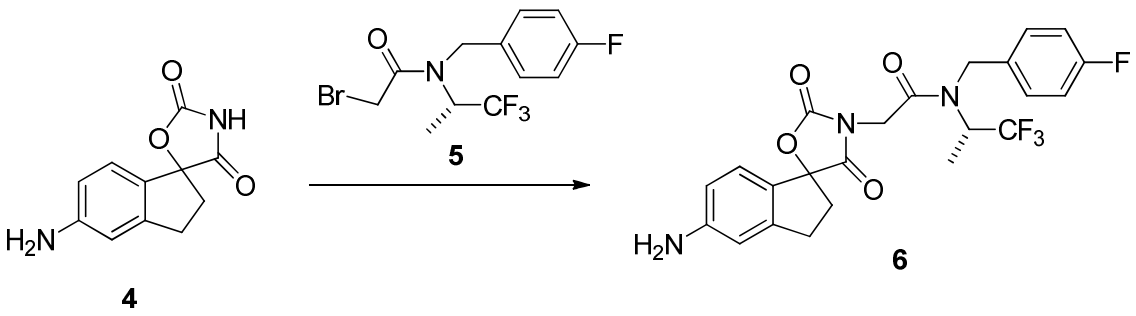
A solution of crude product (7.6 g) in THF (150 mL) and triethylamine (6g g, 58 mmol) was cooled to 5 ^{o}C and carefully treated with a solution of triphosgene (4.3 g, 14.5 mmol) in 20 ml THF, so as to maintain temperature below 10 ^{o}C. The mixture was stirred for 1 h, treated with 6N HCl until pH2, and the resultant mixture was stirred at 5 ^{o}C for an additional 30 minutes. About half the volume of the solvent from the reaction mixture was removed under reduced pressure and the remaining mixture was partitioned between water and Ethyl acetate. The organic extract was dried, concentrated, and the residue was purified by silica gel column chromatography to afford *N*-(2',4'-dioxo-2,3-dihydrospiro[indene-1,5'-ozazolidine]-5-yl)acetamide (**3**) (5.89 g, 57%) as a yellow solid. LC-MS: *m/z* = 261[M+H]$^{+}$



3 **4**

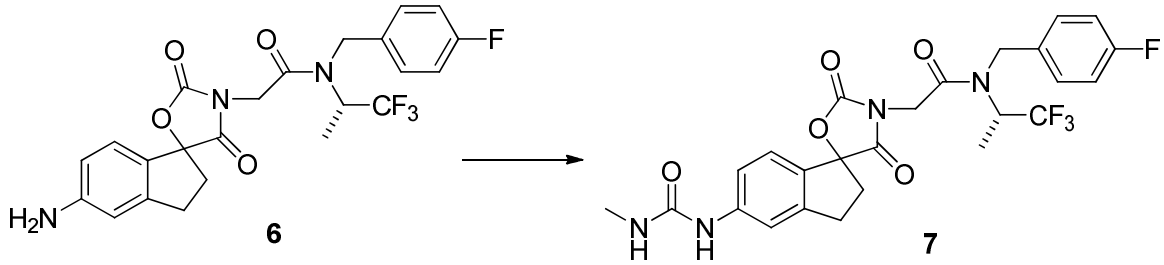
5-amino-2,3-dihydrospiro[indene-1,5'-ozazolidine]-2',4'-dione (4):

To a stirring suspension of intermediate **3** (29.5 g, 113 mmol) in CH_3OH (300 mL) was added concentrated HCl aqueous solution (75 mL). The resulting mixture was heated to reflux for 3 h. The precipitate was filtered and dried to afford **4** (16 g, 65%) as a yellow solid. LC-MS: *m/z* = 219.1 [M+H]$^{+}$



2-(5-amino-2',4'-dioxo-2,3-dihydrospiro[indene-1,5'-ozazolidine]-3'-yl)-*N*-(4-fluorobenzyl)-*N*-((*S*)-1,1,1-trifluoropropan-2-yl)acetamide (6):

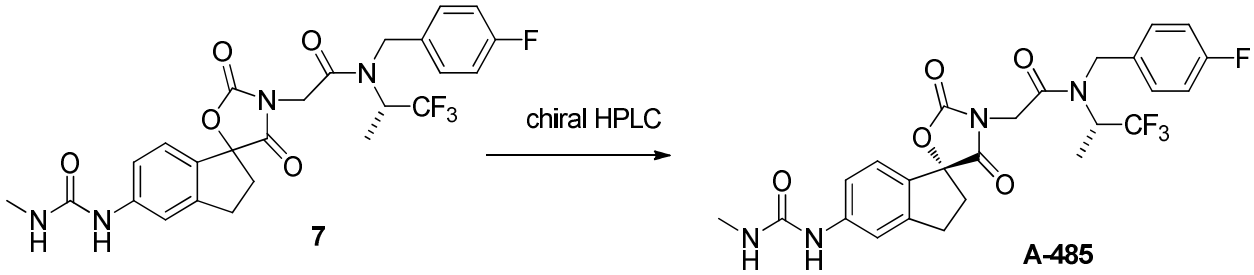
To a solution of **4** (27.7 g, 127 mmol) and **5** (43.5 g, 127 mmol) in DMF (200 mL) was added K_2CO_3 (52.6 g, 381 mmol). The reaction mixture was then stirred at RT for 2 h, quenched with water, extracted with EtOAc, washed with water and brine, dried, concentrated under reduced pressure, and purified by silica gel column chromatography (methanol: CH_2Cl_2= 1:20) to afford **6** (43.9 g, 72%) as a white solid. LC-MS: *m/z* = 480.0 $[M+H]^+$



N-(4-fluorobenzyl)-2-(5-(3-methylureido)-2',4'-dioxo-2,3-dihydrospiro[indene-1,5'-ozazolidine]-3'-yl)-N-((S)-1,1,1-trifluoropropan-2-yl)acetamide (7):

To a stirring solution of intermediate 6 (8.6 g, 18 mmol) in THF (100 mL) was added Et3N (7.5 mL, 54 mmol) followed by triphosgene (2.7 g, 9 mmol) in one portion at room temperature (exothermic reaction). The reaction mixture was stirred at room temperature for 30 min, and then methyl amine (2M in THF, 45mL, 90 mmol) was added and stirred at room temperature for an additional 0.5 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water and EtOAc. Combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain crude product, which was purified by silica gel column chromatography eluting with 50% EtOAc/Petroleum Ether to afford compound **7** (7.96 g, 83%) as a yellow solid. LC-MS: *m/z* = 537.1 $[M+H]^+$ at RT 4.74 (98.44% purity). ^{1}H NMR (301 MHz, DMSO-d_6) δ 8.72 (s, 1H), 7.55 (s, 1H), 7.41 – 7.32

(m, 2H), 7.29 – 7.09 (m, 4H), 6.08 (s, 1H), 5.46 – 5.42 (m, 0.5H), 4.99 – 4.88 (s, 1.5H), 4.83 – 4.69 (m, 1H), 4.68 – 4.54 (m, 1H), 4.24 – 4.16 (m, 1H), 3.13 – 2.95 (m, 2H), 2.67 – 2.47 (m, 5H), 1.43 – 1.32 (m, 3H).



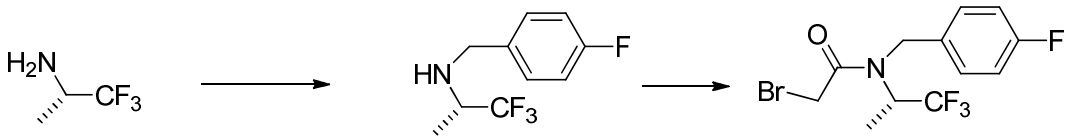
N-(4-fluorobenzyl)-2-((R)-5-(3-methylureido)-2',4'-dioxo-2,3-dihydrospiro[indene-1,5'-ozazolidine]-3'-yl)-N-((S)-1,1,1-trifluoropropan-2-yl)acetamide – A-485:

Compound **7** (2.5 g, 4.7 mmol) was purified by SFC (column: CHIRALPAK AS-H; Manufacturer: DAICEL CHIRAL TECHNOLOGIES (CHINA) CO., LTD; condition: Isopropyl alcohol /CO_2 (3:7)) to give compound **A-485** (1.01 g, 40%) as a white solid.

High temperature NMR resolved the rotamers seen at room temperature: ^{1}H NMR (400 MHz, DMSO-d_6, 120 ^{o}C)
δ 8.29 (s, 1H), 7.51 (t, *J* = 1.2 Hz, 1H), 7.36 (dd, *J* = 8.4, 5.4 Hz, 2H), 7.26 (d, *J* = 1.7 Hz, 2H), 7.15 (t, *J* = 8.7 Hz, 2H), 5.87 (d, *J* = 5.4 Hz, 1H), 5.31 – 5.12 (m, 1H), 4.86 (d, *J* = 17.7 Hz, 1H), 4.74 – 4.54 (m, 2H), 4.40 (d, *J* = 16.8 Hz, 1H), 3.22 – 3.07 (m, 1H), 3.02 (ddd, *J* = 16.3, 8.7, 4.2 Hz, 1H), 2.75 – 2.61 (m, 4H), 2.50 – 2.43 (m, 2H), 1.40 (d, *J* = 7.1 Hz, 3H); MS (ESI) m/e 1072.9 $(2M+H)^+$; ^{13}C NMR (101 MHz, DMSO-d_6, 120 ^{o}C) δ 174.04, 167.67, 163.18, 160.75, 156.23, 154.35, 146.54, 143.73, 133.74, 130.33, 128.68, 127.38, 124.47, 117.96, 115.63, 114.39, 94.60, 46.31, 42.14, 35.60, 35.56, 30.26, 26.50; HRMS (m/z): $[M+H]^+$ calcd for $C_{25}H_{25}F_4N_4O_5$, 537.1756 ; found, 537.1733.
$[\alpha]_D^{20}$ + 46.1^o (*c* 0.74, MeOH); HPLC purity: 100% PA at 254 nm (Condition 1, Phenomenex Luna 5 μm, C18(2), 100 A, 4.6 x 250 mm, room temperature, 1 h gradient (H_2O (0.1% TFA) : Acetonitrile 10-95% gradient). HPLC purity: 99% PA at 254 nm (Condition 2, Waters XBridge 3.5 μm, C18, 4.6 x 150 mm, room temperature, 1 h gradient (H_2O (0.1% H_3PO_4) : Acetonitrile 10-95% gradient).

Synthesis of intermediate 5

(S)-2-bromo-N-(4-fluorobenzyl)-N-(1,1,1-trifluoropropan-2-yl)acetamide



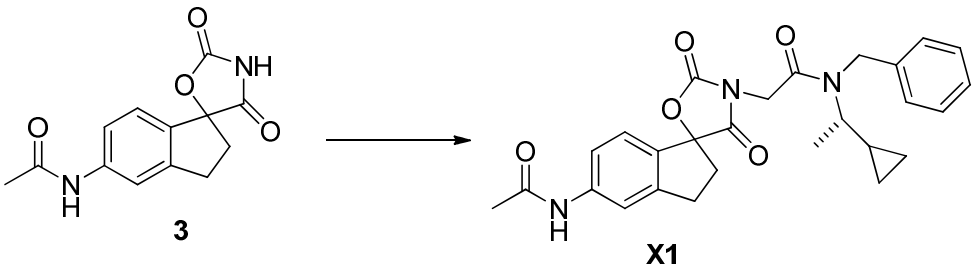
5

(*S*)-1,1,1-trifluoro-N-(4-fluorobenzyl)propan-2-amine:

To a solution of (*S*)-1,1,1-trifluoropropan-2-amine (37 g, 327 mmol) and 1-(bromomethyl)-4-fluorobenzene (74.3 g, 393 mmol) in DMF (500 mL) was added K_2CO_3 (135 g, 981 mmol). The reaction mixture was stirred at room temperature overnight, quenched with water, extracted with EtOAc, washed with water and brine, dried and concentrated under reduced pressure. The crude product was purified by silica gel (methanol: CH_2Cl_2= 1:20) to afford (*S*)-1,1,1-trifluoro-N-(4-fluorobenzyl)propan-2-amine (55 g, 76%) as oil. LC-MS: *m/z* = 222.0 $[M+H]^+$

To a stirring solution of (*S*)-1,1,1-trifluoro-N-(4-fluorobenzyl)propan-2-amine (38.6 g, 175 mmol) in dry CH_2Cl_2 (500 mL) was added 2-bromoacetyl bromide (70.4 g, 350 mmol). The resulting mixture was stirred at RT for 2 h, quenched with $NaHCO_3$, extracted with CH_2Cl_2, dried and concentrated and purified by silica gel (petroleum ether:ethyl acetate = 1:1) to give **5** (49.5 g, 83%) as oil. LC-MS: *m/z* = 342.0/344.0 $[M+H]^+$

Synthesis of Compound R

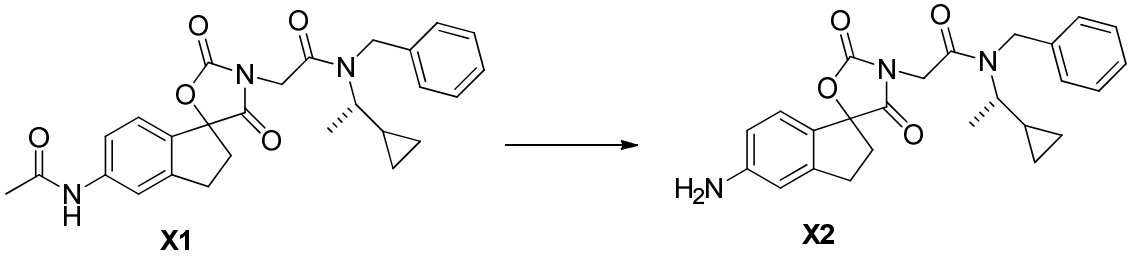


3 **X1**

(S)-2-(5-acetamido-2',4'-dioxo-2,3-dihydrospiro[indene-1,5'-ozazolidine]-3'-yl)-N-benzyl-N-(1-cyclopropylethyl)acetamide (X1):

A solution of intermediate **3** (1 g, 3.85 mmol), (*S*)-*N*-benzyl-2-bromo-N-(1-cyclopropylethyl)acetamide (1.14 g, 3.85 mmol) and K_2CO_3 (1 g, 7.7 mmol) in DMF (20 mL) was stirred for 2 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with sat

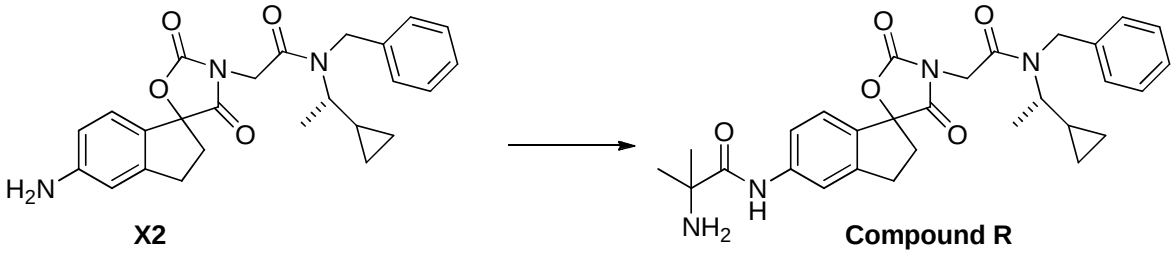
NaCl aqueous and extracted with EtOAc. Combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain crude product, which was purified by silica gel column chromatography to afford compound X1 (1.35 g, 73%) as a yellow liquid.TLC: 35% Petroleum Ether/Ethyl Acetate



X1 **X2**

2-(5-amino-2',4'-dioxo-2,3-dihydrospiro[indene-1,5'-ozazolidine]-3'-yl)-N-benzyl-N-((S)-1-cyclopropylethyl)acetamide (X2):

Compound **X1** (1.35 g, 2.85 mmol) was dissolved with MeOH/THF/H_2O (10 mL/10 mL/10 mL), and 37% HCl (4 mL) was added. The mixture was heated to 60 degrees C and stirred for 2 hours. After consumption of compound X1 (by LC-MS), the solvent from the reaction mixture was removed under reduced pressure. The residue was diluted with sat NaCl aqueous and extracted with EtOAc. Combined organic extracts were washed with sat. Aq. NaHCO3, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain crude product, which was purified by silica gel column chromatography to afford intermediate **X2** (1 g, 81%) as a yellow liquid. TLC: 35% Petroleum Ether/Ethyl Acetate. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.65 (d, J = 18.2 Hz, 1H), 7.51 – 7.08 (m, 7H), 6.56 – 6.41 (m, 2H), 4.75 – 4.20 (m, 5H), 4.13 – 3.54 (m, 1H), 3.52 – 2.74 (m, 2H), 2.74 – 2.33 (m, 1H), 1.42 – 1.00 (m, 3H), 1.02 – 0.84 (m, 1H), 0.56 – 0.16 (m, 4H). LC-MS: *m/z* = 434.10$[M+H]^+$ (91.53% purity, 214 nm)



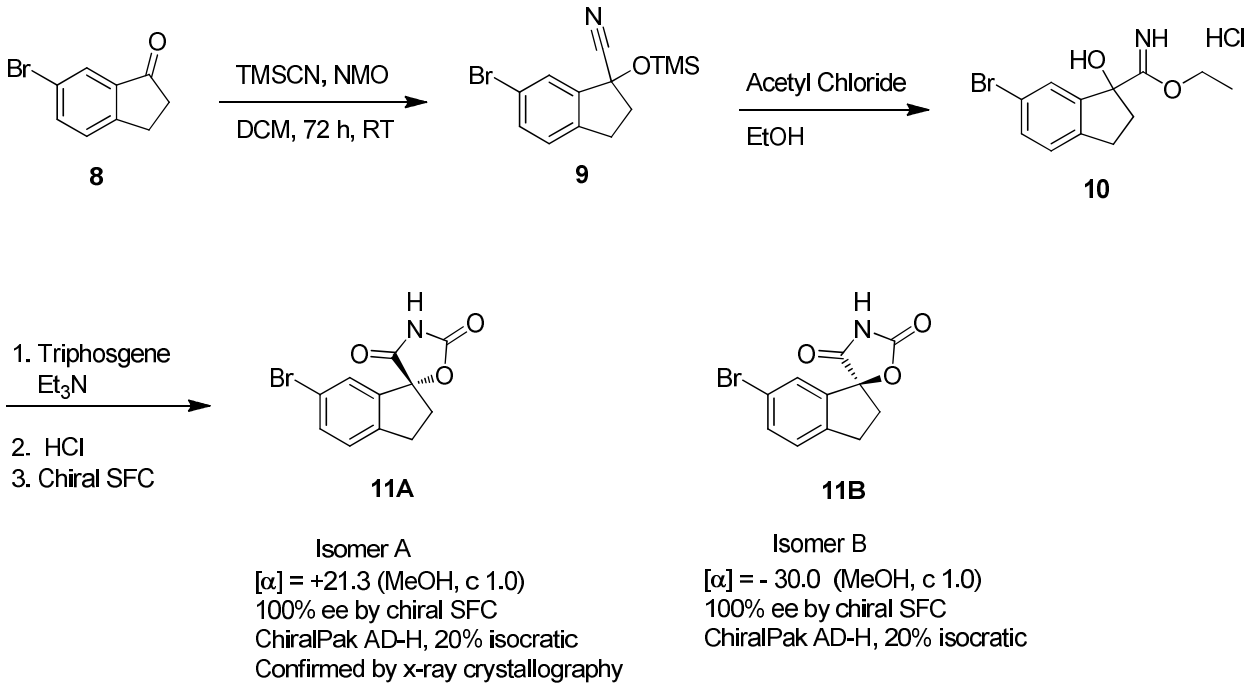
X2 **Compound R**

(S)-2-amino-N-(3'-(2-(benzyl(1-cyclopropylethyl)amino)-2-oxoethyl)-2',4'-dioxo-2,3-dihydrospiro[indene-1,5'-ozazolidine]-5-yl)-2-methylpropanamide - Compound R:

To a stirring solution of 2-amino-2-methylpropanoyl chloride hydrochloride salt (22 mg, 0.14 mmol) in Pyridine (0.5 mL) and CH_2Cl_2 (0.5 ml) was added compound X2 (30 mg, 0.07 mmol) at room temperature and the reaction mixture was stirred at room temperature overnight. The mixture was then

concentrated and purified with Preparative-TLC(10% MeOH/ CH_2Cl_2) to afford **compound R** (25 mg, 69%) as white solid. TLC: 10% MeOH/ CH_2Cl_2 (Rf: 0.2). LC-MS: *m*/*z* = 519[M+H]$^+$ (98% purity). ^{1}H NMR (400 MHz, CD_3OD-d4) δ 7.74 (s, 1H), 7.56 – 7.23 (m, 7H), 4.84 – 4.74 (m, 2H), 4.68 – 4.55 (m, 1H), 4.45 (t, J = 4.6 Hz, 1H), 3.90-3.82 (m, 1H), 3.20-2.12 (m, 1H), 2.84 – 2.76 (m, 1H), 2.63-2.55 (m, 1H), 1.70 (d, J = 2.5 Hz, 6H), 1.28 (dd, J = 21.8, 6.7 Hz, 3H), 1.03-0.9 (m, 2H), 0.68-0.53 (m, 2H), 0.40-0.25 (s, 3H). HRMS (m/z): [M+H]+ calcd for C29H35N4O5, 519.2602 ; found, 519.2602.

Synthesis of A-486



6-bromo-1-((trimethylsilyl)oxy)-2,3-dihydro-1H-indene-1-carbonitrile 6-bromo-2,3-dihydro-1H-inden-1-one. (**9**)

(50 g, 237 mmol) of **8** was dissolved in CH_2Cl_2 (237 ml) under nitrogen and NMO (8.33 g, 71.1 mmol) was added followed by TMS-CN (CAUTION: TOXIC, 38.1 ml, 284 mmol). The resulting black solution was stirred for 5 days. Saturated Na_2CO_3 (aq, 200 ml) was added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (2x) and the combined organics were dried (anhydrous $MgSO_4$), filtered and concentrated by rotary evaporation. The residue was purified by regular phase flash column chromatography (800 g silica gel, 0-15% Hep / EA) to give 6-bromo-1-((trimethylsilyl)oxy)-2,3-dihydro-1H-indene-1-carbonitrile (40 g, 129 mmol, 54.4 % yield) as an oil, which solidified in the freezer. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72 – 7.62 (m, 2H), 7.41 (dd, *J* = 7.9, 0.8 Hz, 1H), 3.17 – 2.92 (m, 2H), 2.83 (ddd, *J* = 13.1, 7.7, 5.3 Hz, 1H), 2.47 (ddd, *J* = 13.3, 7.9, 6.1 Hz, 1H), 0.26 (s, 9H).

ethyl 6-bromo-1-hydroxy-2,3-dihydro-1H-indene-1-carbimidate hydrochloride (**10**)

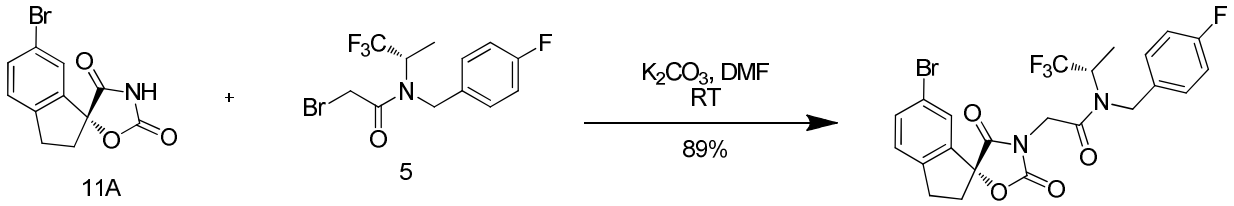
6-bromo-1-((trimethylsilyl)oxy)-2,3-dihydro-1H-indene-1-carbonitrile (15 g, 48.3 mmol) was taken up in ethanol (161 ml) and cooled to 4 °C. Acetyl chloride (138 ml, 1.9 mol) was added drop-wise via addition funnel at a rate as to keep the temperature below 20 °C. The reaction was allowed to stir for 48 h upon which time it was concentrated by rotary evaporation giving a light yellow solid. The solids were

triturated with diethyl ether (2x) to give ethyl 6-bromo-1-hydroxy-2,3-dihydro-1H-indene-1-carbimidate hydrochloride (13.5 g, 42.1 mmol, 87%) as a white solid that was used in the next step without further purification.

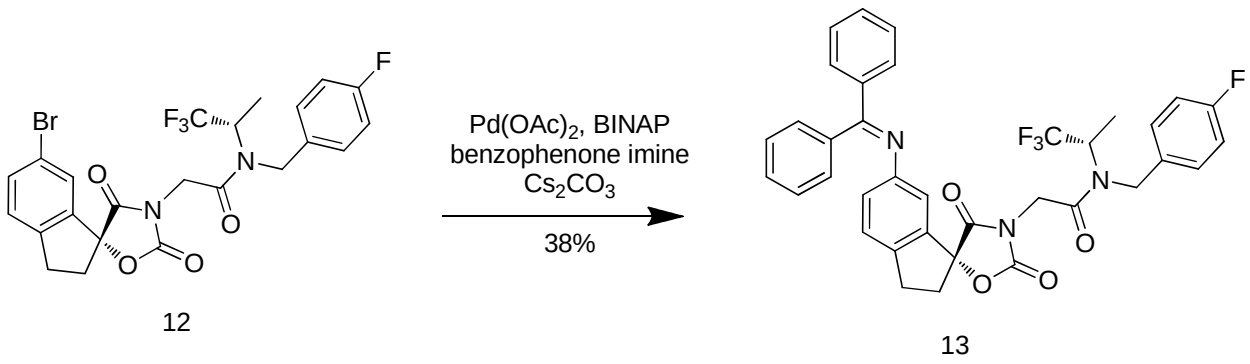
(Compounds 11A and 11B). (R)-6-bromo-2,3-dihydrospiro[indene-1,5'-oxazolidine]-2',4'-dione and (S)-6-bromo-2,3-dihydrospiro[indene-1,5'-oxazolidine]-2',4'-dione.

A 500 ml flask was charged with ethyl 6-bromo-1-hydroxy-2,3-dihydro-1H-indene-1-carbimidate hydrochloride (12.52 g, 39.1 mmol) and tetrahydrofuran (150 ml). To the resulting suspension was added triethylamine (16.33 ml, 117 mmol) and the solution was cooled in an ice bath to 2 °C. The bis(trichloromethyl) carbonate (4.64 g, 15.62 mmol) [CAUTION: WEIGH THE TRIPHOSGENE IN BALANCE ENCLOSURE. VERY TOXIC CHEMICAL] was then added portionwise over 17 min [CAUTION: THE TRIPHOSGENE ADDITION IS STRONGLY EXOTHERMIC] keeping the temperature below 15 °C. After 1 h, the reaction was complete and was cooled back down to 5 °C and carefully diluted portion-wise with 2N aqueous HCl (100 ml). This was initially very exothermic as the mixture got very thick. Stirring was increased and as more acid was added, the solid finally dissolved. As the temperature dropped back to 15 °C, the bath was removed and the mixture was stirred for 30 min and then extracted with ethyl acetate (2x). The combined organics were dried over anhydrous Na_2SO_4, filtered and concentrated by rotary evaporation. The residue was purified by regular phase flash column chromatography (CombiFlash RF, silica gel Gold Rf cartridge (220 g) eluted with a 10 - 50 % ethyl acetate in heptanes gradient) to give racemic 6-bromo-2,3-dihydrospiro[indene-1,5'-oxazolidine]-2',4'-dione (10.9 g, 39.2 mmol, 70%). The enantiomers were separated by chiral SFC (ChiralPak AD-H, 20% isocratic) providing (R)-6-bromo-2,3-dihydrospiro[indene-1,5'-oxazolidine]-2',4'-dione as a single enantiomer (3.9 g, 13.8 mmol, 35.4%) as a white solid. 100% ee determined by Chiral SFC (ChiralPak AD-H, 20% MeOH isocratic); $[\alpha]_D^{23}$ = +21.3 (*c* 1.0, MeOH); ^1H NMR (501 MHz, DMSO-d_6) δ 12.18 (s, 1H), 7.71 (d, *J* = 1.9 Hz, 1H), 7.61 (dd, *J* = 8.1, 1.9 Hz, 1H), 7.44 – 7.28 (m, 1H), 3.14 – 3.02 (m, 1H), 3.02 – 2.90 (m, 1H), 2.66 (ddd, *J* = 14.4, 8.5, 5.9 Hz, 1H), 2.59 – 2.39 (m, 2H). **The structure of the *R* enantiomer was determined by x-ray crystallography.** (S)-6-bromo-2,3-dihydrospiro[indene-1,5'-oxazolidine]-2',4'-dione as a single enantiomer (3.9 g, 13.8 mmol, 35.4%) as a white solid. 100% ee determined by Chiral SFC (ChiralPak AD-H, 20% MeOH isocratic); $[\alpha]_D^{23}$ = - 30.0 (*c* 1.0, MeOH); ^1H NMR (501 MHz, DMSO-d_6) δ 12.18 (s, 1H), 7.71

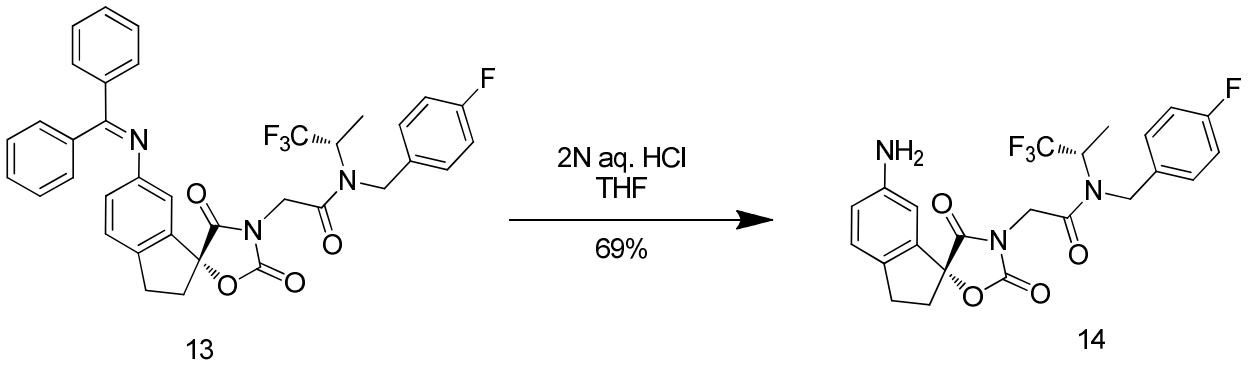
(d, *J* = 1.9 Hz, 1H), 7.61 (dd, *J* = 8.1, 1.9 Hz, 1H), 7.44 – 7.28 (m, 1H), 3.14 – 3.02 (m, 1H), 3.02 – 2.90 (m, 1H), 2.66 (ddd, *J* = 14.4, 8.5, 5.9 Hz, 1H), 2.59 – 2.39 (m, 2H).



(R)-6-bromo-2,3-dihydrospiro[indene-1,5'-oxazolidine]-2',4'-dione (**11A**) (0.705 g, 2.5 mmol), (S)-2-bromo-N-(4-fluorobenzyl)-N-(1,1,1-trifluoropropan-2-yl)acetamide (0.855 g, 2.500 mmol) (**5**) and potassium carbonate (0.691 g, 5.00 mmol) were combined in DMF (12.50 ml) and the mixture was stirred at RT for 4 hours. At this time LCMS indicates no remaining isoxazolidinedione starting material. The reaction mixture was diluted with water (50 mL) and extracted with EtOAc (3 x 30 mL). Extracts were dried (Na_2SO_4) and concentrated then purified by flash chromatography (gradient from 0 to 30% EA) to give the product **12** (1.215 g, 89%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, T = 90 ^{o}C) δ 7.62 (d, *J* = 1.9 Hz, 1H), 7.58 (dd, *J* = 8.1, 1.9 Hz, 1H), 7.35 (d, *J* = 8.3 Hz, 1H), 7.36-7.28 (br s, 2 H), 7.13 (br t, *J* = 7.6 Hz, 2H), 5.20 (br s, 1H), 4.83 (d, *J* = 17.6 Hz, 1H), 4.62 (br s, 2H), 4.35 (br s, 1H), 3.19 – 3.07 (m, 2H), 2.71 – 2.63 (m, 1H), 2.55 – 2.48 (m, 1H), 1.36 (d, *J* = 7.0 Hz, 3H). MS (ESI+) m/z 565.0 $(M+Na)^+$



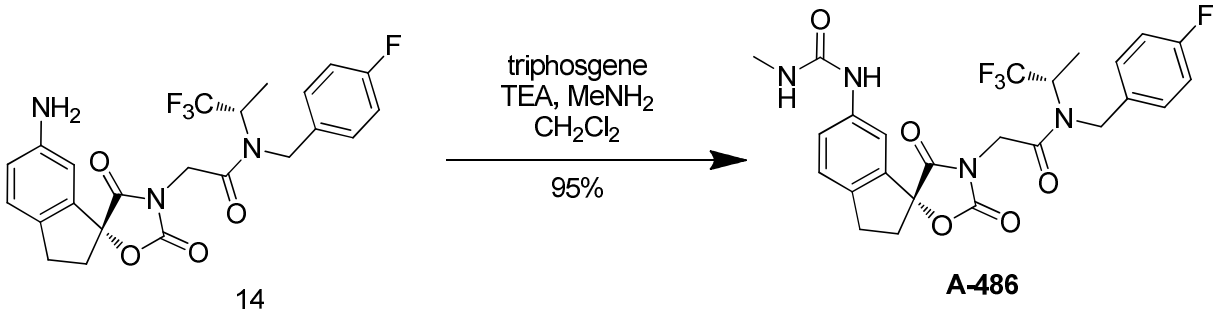
A flask was charged with 2-((R)-6-bromo-2',4'-dioxo-2,3-dihydrospiro[indene-1,5'-oxazolidin]-3'-yl)-N-(4-fluorobenzyl)-N-((S)-1,1,1-trifluoropropan-2-yl)acetamide (1.2 g, 2.21 mmol) and then degassed under a stream of nitrogen for 30 min. Toluene (12 ml, from a fresh Sure-Seal™ bottle) and diphenylmethanimine (0.560 g, 3.09 mmol) were added to the solids via syringe and the degassing was continued for another 10 min. Separately, diacetoxypalladium (0.020 g, 0.088 mmol), BINAP (0.124 g, 0.199 mmol) and cesium carbonate (1.007 g, 3.09 mmol) were placed into a dried, nitrogen-flushed 3-neck flask equipped with a condenser and degassed under a stream of nitrogen for 40 min. Toluene (8 ml) was added and the mixture was stirred for 10 min. The bromide solution from above was added to the palladium catalyst suspension via syringe and the resulting mixture was heated to 100 °C overnight. The color changed from orange to deep red to gold as the reaction progressed. The reaction mixture was cooled to RT and then passed through a membrane filter and directly loaded onto a column. The product was purified by flash chromatography (gradient from 0 to 60% EA-heptane) to give the product **13** (541 mg, 38%) as a light yellow foam.^{1}H NMR (400 MHz, DMSO-$d_{6,}$ T = 90 ^{o}C) δ 7.60-7.56 (br m, 2H), 7.50-7.18 (br m, 8H), 7.17-6.99, (br m, 4H), 7.12 (d, *J* = 8.0 Hz, 1H), 6.77 (d, *J* = 1.9 Hz, 1H), 6.72 (dd, *J* = 8.1, 2.0 Hz, 1H), 5.19 (br s, 1H), 4.82 (d, *J* = 17.5 Hz, 1H), 4.60 (br s, 2H), 4.33 (br s, 1H), 3.03 (dt, *J* = 15.3, 7.4 Hz, 1H), 2.91 (ddt, *J* = 12.9, 8.6, 4.4 Hz, 1H), 2.60 (ddd, *J* = 14.9, 8.7, 6.4 Hz, 1H), 2.41 (ddd, *J* = 14.5, 8.3, 4.3 Hz, 1H), 1.37 – 1.33 (d, J = 7.1 Hz, 2H). MS (ESI+) m/z 644.2 $(M+H)^{+}$



13 14

2-((R)-6-((diphenylmethylene)amino)-2',4'-dioxo-2,3-dihydrospiro[indene-1,5'-oxazolidin]-3'-yl)-N-(4-fluorobenzyl)-N-((S)-1,1,1-trifluoropropan-2-yl)acetamide (541 mg, 0.841 mmol) was dissolved in THF (8 ml) and 2N aq. HCl (3 ml, 6.00 mmol) was added. The reaction was stirred for 30 min at RT, at which time TLC (50% EA-heptane) indicates completion. The reaction mixture was diluted with sat $NaHCO_3$ (70

mL) and stirred for 30 min then extracted with EtOAc (3 x 30 mL). Extracts were dried (Na_2SO_4) and concentrated then purified by flash chrom (gradient from 0 to 60% EA) to give 280 mg (69%) of the product **14** , a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, T = 120 ^{o}C), δ 7.33 (dd, *J* = 8.5, 5.5 Hz, 2H), 7.12 (t, *J* = 8.8 Hz, 2H), 7.01 (d, *J* = 8.1 Hz, 1H), 6.68 (dd, *J* = 8.1, 2.2 Hz, 1H), 6.55 (d, *J* = 2.1 Hz, 1H), 5.19 (d, *J* = 8.9 Hz, 1H), 4.83 (d, *J* = 17.6 Hz, 1H), 4.71 (s, 2H), 4.65 – 4.54 (m, 2H), 4.35 (d, *J* = 16.8 Hz, 1H), 3.03 – 2.94 (m, 1H), 2.89 (td, *J* = 7.8, 7.2, 4.8 Hz, 1H), 2.61 (ddd, *J* = 14.3, 8.4, 5.9 Hz, 1H), 2.40 (ddd, *J* = 14.2, 8.3, 4.8 Hz, 1H), 1.36 (d, *J* = 7.0 Hz, 3H). MS (ESI-) m/z 478.1 $(M-H)^-$



14 **A-486**

2-((R)-6-amino-2',4'-dioxo-2,3-dihydrospiro[indene-1,5'-oxazolidin]-3'-yl)-N-(4-fluorobenzyl)-N-((S)-1,1,1-trifluoropropan-2-yl)acetamide (**14**) (0.28 g, 0.584 mmol) was dissolved in CH_2Cl_2 (8.34 ml). Triethylamine (0.244 ml, 1.75 mmol) was added, and then the solution was chilled in an ice-water bath to 0 ^{o}C and triphosgene (0.069 g, 0.234 mmol) was added in a single portion. The reaction was stirred for 2 hr at 0 ^{o}C and then methylamine, 2N in THF (0.876 ml, 1.75 mmol) was added. The cooling bath was removed and the reaction was stirred overnight at RT.

The reaction mixture was diluted with 1N HCl (10 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 10 mL). Extracts were dried (Na_2SO_4) and concentrated then purified by flash chromatography (gradient from 0 to 80% EA-heptane) to give the product **A-486** (297 mg, 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.46 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.36 – 7.29 (m, 3H), 7.21 (d, *J* = 8.3 Hz, 1H), 7.12 (t, *J* = 8.8 Hz, 2H), 5.75 (s, 1H), 5.24 – 5.13 (m, 1H), 4.83 (d, *J* = 17.6 Hz, 1H), 4.61 (d, *J* = 18.1 Hz, 2H), 4.37 (d, *J* = 16.8 Hz, 1H), 3.11 – 2.92 (m, 2H), 2.72 – 2.64 (m, 1H), 2.63 (d, *J* = 4.7 Hz, 3H), 2.50 –

2.42 (m, 1H), 1.36 (d, *J* = 7.1 Hz, 3H). MS (ESI+) m/z 537.1 $(M+H)^+$. ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.79, 167.53, 161.97 (d, J = 243.5 Hz), 156.37, 154.33, 140.72, 138.09, 137.94, 133.78, 128.73 (d, J = 8.1 Hz), 125.99 (q, J = 284.2 Hz), 125.49, 121.99, 115.66 (d, J = 21.6 Hz), 113.73, 94.56, 52.57 – 51.69 (m), 46.24, 42.21, 35.98, 29.47, 26.52, 11.97. HRMS (m/z): $[M+H]^+$ calcd for $C_{25}H_{25}F_4N_4O_5$, 537.1756 ; found, 537.1766.

Online Methods

Chemicals and Antibodies

A-485, A-486, and Compound R were synthesized at AbbVie. Enzalutamide was from SelleckChem (# S1250). Antibodies used in this study are listed in **Supplementary Table 14**.

Radiometric SPA Histone acetyltransferase activity assay

Reactions were performed in a 40 µL volume in polypropylene 96 well 300 µL plates (Greiner) in reaction buffer (100 mM HEPES buffer; pH 7.9, 80 µM EDTA, 40 µg/mL BSA, 100 mM KCl, 1 mM DTT, 0.01% triton X-100 and 5% DMSO). The test compounds were dissolved in DMSO to generate 10 mM stocks and further diluted with 10mM HEPES, pH 7.8 with 20% DMSO to make 4X intermediate concentrations. Compounds were tested from 120 µM to 2 nM in 3-fold dilutions. 10 µL of the compound stock was added to 20 µL of p300 HAT domain enzyme at 10 nM (2x of final concentration) and incubated at room temperature for 30 minutes. The reaction was initiated by adding 10 µL of a 4X substrate mix consisting of a biotinylated synthetic Histone H4 Peptide (Biotin-C6-GRGKGGKGLGKGGAK) at 100 µM, 1.8 µM cold acetyl coenzyme A (Moravek) and 0.6 µM tritiated acetyl coenzyme A (Sigma). The reaction was incubated for one hour at room temperature and terminated with the addition of 160 µL of 0.5 N HCl. The reaction contents were then transferred to a 96 well streptavidin and scintillant-coated microplate (Perkin Elmer), incubated for 1 hour, and counted in TopCount (Perkin Elmer) microplate scintillation counter at one minute per well. IC_{50} values were generated based on percent inhibition calculated from the scintillation counter readings using Prism GraphPad 5.

p300-BHC and CBP-BHC domain expression and purification

p300-BHC domain (residues 1036-1822) and CBP-BHC domain (residues 1072-1859) were synthesized by GenScript with N-terminal 6His-Flag tag and cloned into pFastBac vector. The protein was expressed in

Sf9 cells using the Bac-to-Bac baculovirus system (Invitrogen). Proteins were lysed and purified by FLAG chromatography using 20 mM Tris (pH 8.0), 150 mM KCl, 10% glycerol and 2mM EDTA. SigmaFast protease cocktail S8830 (EDTA free) was used during cell lysis on a French Pressure cell. After elution, FLAG peptide was removed by extensive dialysis against buffer also containing 1mM TCEP. To understand the EDTA impact on biochemical activity assay, another preparation of p300 BHC domain was generated under the same conditions, but omitting EDTA throughout the preparation.

p300 and CBP biochemical activity assay

Acetyltransferase activity assays for p300-BHC and CBP-BHC domains were performed by detecting the acetylation of Lysine residues of a histone H4 synthetic-peptide using a TR-FRET assay. Reactions were performed in a 10 µL volume using an assay buffer containing 100 mM HEPES; pH 7.9, 80 µM EDTA, 40 µg/mL BSA, 100 mM KCl, 1 mM DTT, 0.01% triton X-100. Given the use of EDTA in this assay, it is possible that the structural integrity of the BHC protein used could be affected due to the presence of multiple Zinc containing domains (C/H3, PHD, and RING) in this protein. Thus, the p300-BHC activity assay was also performed with p300-BHC purified in the complete absence of EDTA and in assay buffer in the absence of EDTA. Each compound of interest was dissolved in DMSO and dispensed at 50 nL by a Labcyte Echo (Labcyte, Sunnyvale, CA) into white 384 well low- volume plates (Perkin Elmer 6008289) in 3-fold dilutions from 50 µM to 0.00075 µM. p300-BHC or CBP-BHC protein at 0.6 nM was pre-incubated with A-485 or A-486 for 30 minutes. The reaction was initiated by adding 5 µL of a biotinylated synthetic Histone-H4 peptide (Anaspec AS-65097) at 2 µM and acetyl coenzyme A (Sigma-Aldrich A2056) at 0.5 µM. Following incubation for 1 hour at room temperature in a humidified chamber, the reaction was terminated with 10 µL of 3 nM LANCE Ultra Europium-anti-acetyl-Histone H4 Lysine antibody (Perkin Elmer TRF0412-D), 900 nM LANCE Ultra ULight-Streptavidin (Perkin Elmer TRF0102-D) in LANCE Detection Buffer (PerkinElmer CR97-100). TR-FRET measurements were obtained using a Perkin Elmer

Envision with laser excitation at 335 nm and emission at 665 nm and 620 nm. For acetyl-CoA competition experiments, the assay was run as above except the acetyl-CoA concentration was varied from 0.078 to 10 µM. IC_{50} values for inhibition were calculated using a sigmoidal fit of the concentration/inhibition response curves using Prism GraphPad 5.

p300 AlphaLISA Peptide Binding Assay

Binding of P300-BHC to a histone H4 synthetic-peptide was assessed using AlphaLISA (Perkin Elmer) technology. Assays were performed in a 40 µL volume in white 384 well assay plates (Perkin Elmer 6007290) with an assay buffer containing 100 mM HEPES, pH 7.9, 80 µM EDTA, 40ug/mL BSA, 100 mM KCl, 1 mM DTT, 0.01% triton X-100. Ten µL of a biotin-labeled H4 peptide (Anaspec AS-65097) was added to 10 µL of P300-BHC (for a final concentration of 15 nM and 115 nM respectively) and incubated for one hour at room temperature. To demonstrate a decrease in AlphaLISA signal by competing with the biotin-labeled peptide, a 10 µL 2-fold mix of an unlabeled H4 peptide (Anaspec AS-62499) and biotin labeled H4 peptide (final concentration of 300 µM and 115 nM respectively) was added to 20 µL of P300-BHC (final concentration of 15nM) and incubated for 1 hour at room temperature. 20 µL of a 2-fold mix containing Nickel- chelate AlphaLISA acceptor beads (Perkin Elmer AL108) and AlphaScreen streptavidin donor beads (Perkin Elmer 6760002) was added (final concentration of 20 µg/mL each) to the enzyme-peptide complex and incubated at room temperature for 1.5 hours. AlphaLISA counts were obtained using a Perkin Elmer Envision with laser excitation at 680 nm and emission at 615 nm.

Thermal shift assay

Thermal shift assays were performed on the Roche LightCycler 480 instrument using p300-BHC purified with EDTA in the lysis buffer (above). Sypro Orange dye was purchased from Invitrogen as 5000X stock. The assay was performed in 20mM HEPES, pH 7.5, 50mM NaCl, 1mM TCEP, 2% DMSO, and 1:500

dilution of the dye at a protein concentration of 1.5 uM. 50x stock DMSO samples of Lys CoA, (CPC Scientific, CA) and A-485 were prepared so the final DMSO concentration was 2%, (v/v). All samples were run in quadruplicate.

Δp300 HAT domain protein expression and purification for Surface Plasmon Resonance (SPR) and X-ray crystallography

Δp300 HAT domain protein construct (residues 1287 to 1652 with internal loop deletion of amino acids 1523-1554 plus K1637R and M1652G mutations fused to Vent Intein chitin binding domain) was expressed in E. coli BL21-CodonPlus(DE3)-RIL cells and purified on Chitin resin (New England Biolabs, Ipswich, MA) following the previously described protocol [1] with minor modifications. To make the final HAT domain (1287-1666) and compound complex, compound was added to a final 100 µM during the ligation step with the C-terminal 14-mer peptide (CMLVELHTQSQDRF). Ligation was done in the absence of air on a rocker at 21˚C overnight. The next morning the ligated protein was eluted from the chitin column and dialyzed into a buffer containing 20 mM HEPES (pH 7.2), 50 mM NaCl, and 1 mM TCEP. The protein complex was further purified using a Tosoh SP5PW (2X15cm) column. Elution was achieved with buffer 20 mM Tris (pH 8.0), 400 mM NaCl, and 1 mM TCEP over 7.5 column volumes. 20 µM compound was added to the eluted protein pool. The protein was then dialyzed overnight into the buffer containing 20 mM HEPES (pH7.2), 50 mM NaCl, 1 mM TCEP, and 20 µM compound and concentrated to 8.4 mg/ml for X-ray. For surface plasmon resonance (SPR) studies, 14mer peptide plus the Avi-tag (CMLVELHTQSQDRFGGSGGLNDIFEAQKIEWHW) was used for the ligation with Δp300 HAT (1287-1652)-Vent Intein chitin binding domain protein to make the Δp300 HAT (1287-1666)-Avi tagged protein.

Surface Plasmon Resonance (SPR)

The binding kinetics of compounds to p300-HAT domain protein were determined by surface plasmon resonance-based measurements made on Biacore T200 instruments (GE Healthcare) at 25 °C using a biotin-neutravidin capture assay approach. The recombinant domains of human p300-HAT were expressed as described above and enzymatically biotinylated with BirA using the BIR-A kit from Avidity Technologies, (Aurora, CO) per the manufacturer's instructions. For neutravidin chip preparation running buffer was buffer HBS-EP+ (10 mM Hepes, pH7.4, 150 mM NaCl, 3 mM EDTA, 0.05% Tween 20; GE Healthcare, cat # BR-1006-69). NeutrAvidin Protein (Thermo Fisher Scientific Inc., cat # 31000) was diluted to 12.5 µg/mL in 10 mM sodium acetate (pH 5.5) and directly immobilized across a CM5 biosensor chip (GE Healthcare, cat#29-1496-03) using standard amine coupling procedures to a level of approximately 7,000 RU. For test surface preparation, biotinylated p300 protein was captured by neutravidin in running buffer containing 1 mM TCEP to approximately 3000RU; neutravidin only surfaces served as primary reference, all surfaces were then blocked by injection of free biotin. Binding kinetic measurements were made in the assay buffer HBS-EP+ containing 1mM TCEP, 3% DMSO. A-485 was assayed in single-cycle kinetics mode wherein each assay cycle consisted of the following: five consecutive analyte injections (either a 5 point, 4-fold increasing compound concentration series ending at 6uM top dose or a buffer only series) over both reference and test surface, 60 s at 80 µL/min, after which the dissociation was monitored for 600 seconds at 80 µL/min. Washout cycles were included between each compound series. A-486 was assayed in consecutive cycle mode for an 11point, 2-fold concentration series ending at 100 µM top dose and buffer only wherein the assay consisted of 30 s association phase followed by a 30 s dissociation phase at 80 µL/min. During the assay, all measurements were referenced against the capture surface alone (i.e. neutravidin with no captured p300) and buffer-only injections were used for double referencing. DMSO correction cycles were included to correct for minor DMSO differences and excluded volume effects. Data were processed and

fitted globally to a 1:1 binding model using Biacore T200 Evaluation software to determine the binding kinetic rate constants, k_a ($M^{-1}s^{-1}$) and k_d (s^{-1}), and the equilibrium dissociation constant K_D (M) or steady-state binding levels were fitted to determine the equilibrium dissociation constant K_D (M) alone.

Crystallography

Crystals were obtained using the vapor diffusion method with a 1:1 mixture of crystallization reagents (25% (w/v) PEG3,350, 0.2M Sodium Chloride, 0.1M BIS-Tris buffer pH 5.5) and Δp300/A-485 complex sample mentioned above. Needle crystals initially formed after incubating the crystallization plates at 17 °C overnight. Later, thin plate crystals appeared on top of needle crystals which grew to their full size within one week. These plate crystals were separated from the needles and flash frozen into liquid nitrogen using the reservoir solution plus 20% glycerol as the cryo-protectant. Diffraction data were collected to 1.95Å using beamline 17ID at the Advanced Photon Source at 1.000 Å and 100K. Data were reduced and scaled with autoPROC [2] software having a completeness of 100% and Rmerge=0.058. The crystal belongs to spacegroup $C222_1$ with unit cell dimensions a=45.19, b=102.94, c=168.44, α=β=γ=90°. The structure was solved by molecular replacement using 3BIY as the search model with the program Phaser. [3] The dictionary for the ligand was generated using the program AFITT [4]. Iterative rounds of map fitting and refinement were performed using the programs Coot [5] and either Refmac [6] or Buster. [7] Analysis of the structure showed that 99% of the residues are in the preferred or allowed portions of the Ramachandran diagram. Data collection and refinement statistics are shown in **Extended Data Fig. 2.**

Cell Culture

Cell lines were obtained from the vendors as detailed in **Supplementary Table 10**. The cells were tested for mycoplasma using MycoAlert Detection Kit (Lonza, Walkersville, MD), authenticated using GenePrint 10 STR Authentication Kit (Promega, Madison, WI), and grown in media as recommended by the

supplier. For DHT stimulation, LnCaP-FGC cells were starved of androgens in phenol red free media containing 10% charcoal stripped FBS (ThermoFisher cat # 12676-029) for 72 h, treated with 5-fold dilutions of A-485 or enzalutamide (Enz) starting at 10 μM for 30 min, and then further treated with 5 nM DHT for the indicated times. VCaP Cells were starved of androgens for 24 h and then treated as above except that 1 nM DHT was added. 22Rv1 cells were starved of androgens for 72 h as per LnCap-FGC cells and treated as above in the absence of DHT via adding only the ethanol diluent.

High Content Microscopy

U2OS and PC-3 cells were plated in Collagen I coated 96-well view plates (Perkin Elmer Cat #: 6005810) while LnCaP-FGC cells were plated in uncoated plates (Perkin Elmer Cat #: 6005810) overnight. Cells were then treated with an 8 point half-log dose response of A-485 or A-486 starting at 10 μM for the indicated times. Cells were fixed in 10% formaldehyde (Polysciences, Inc. #04018) at room temperature for 10 min, washed in PBS, and then permeablized in 0.1% Triton X-100 for 10 min. Cells were then blocked in 1% BSA for 1 h and incubated with the incubated antibodies in antibody dilution buffer (0.3% BSA in PBS) overnight at 4 °C. Cells were washed three times in PBS and then incubated with a mixture of Alexa Fluor488-conjugated goat anti-rabbit IgG antibodies (Life Technologies, #A-11029), Alexa Fluor555-conjugated goat anti-mouse IgG (Life Technologies, #A-21424) antibodies, and Hoechst 33342 (Life Technologies, #H3570) for 1 h at room temperature. After washing four times in PBS, plates were scanned within 24 h of processing on a ThermoFisher CellInsight using the target activation algorithm acquiring 15 fields per well. Fluorescence intensities were quantified using the average mean intensity function. EC_{50} values for H3K27Ac, H3K18Ac, and H3K9Ac inhibition were calculated using a sigmoidal fit of the concentration/inhibition response curves using Prism GraphPad 5.

Analysis of CBP/p300 acetylation

HeLa cells were SILAC labeled with heavy isotopes of arginine and lysine (^{13}C6,^{15}N4-arginine and ^{13}C6,^{15}N2-lysine, Cambridge Isotope Laboratories). The cells were treated with A-485 (10 µm) or vehicle control for 16 hours. Cell were lysed in ice-cold lysis buffer [50mM Hepes, pH7.5, 150mM NaCl, 1mM EDTA, 1% NP-40, 0.1% sodium deoxycholate, 1x complete protease inhibitor cocktail (Roche)]. Proteins were proteolyzed with trypsin, and acetylated peptides were analyzed as described previously [8]. Briefly, the peptide samples were analyzed by online nanoflow liquid chromatography coupled tandem mass spectrometry (LC-MS/MS) using a Proxeon easy nLC system connected to a Q-Exactive HF mass spectrometer (Thermo Scientific), as reported previously [9]. The raw data was computationally processed using MaxQuant [10] and searched against the UniProt database (downloaded July 6, 2015) using the integrated Andromeda search engine (http://www.maxquant.org/).

RNA isolation and quantitative reverse transcription PCR (qRT-PCR)

Cells were treated with compounds in 96 well plates for the indicated times and then washed once in PBS. Plates were then sealed and put at -80 °C for 24 h. RNA was then isolated via the 96 well RNA spin kit (GE Health 25-0500-75) according to manufacturer's instructions. cDNA was generated using the Superscript III First-Strand Synthesis SuperMix (Thermo Fisher 18080-400) according to manufacturer's instructions. Samples were then run on a Bio-Rad CFX384 machine using Taqman supermix (Applied Biosciences 4427788), cDNA, and Primetime PCR assays (Integrated DNA Technologies, IDT) for the indicated genes (probes listed in **Supplementary Table 15**). Data analysis was performed using Bio-Rad CFX 3.1 Manager and further processed via Prism GraphPad 5. IC_{50} values for inhibition of gene expression were calculated using a sigmoidal fit of the concentration/inhibition response.

Cell Proliferation assay

Cell lines were plated in 96 well or 384 well plates and allowed to adhere for 24 h. The cells were then treated with compounds for 3, 4, or 5 days. Experiments were run in triplicate and the fraction of viable cells was determined using the CellTiter-Glo Luminescent Cell Viability Assay (Promega, #G7572) according to the manufacturer's recommendations. The plates were read in a Perkin Elmer Envision using a Luminescence protocol with a 0.5 second integration time. To generate dose response curves, the data was normalized to percent cellular viability by setting the average of the staurosporine and DMSO control wells to 0% and 100% viability respectively. The replicate values for each dilution point were averaged and the EC_{50} values for each compound generated in Prism GraphPad 5 by fitting normalized data to a sigmoidal curve model of linear regression. For Thymidine incorporation assays, cells were treated with compounds for 1, 2, 3, or 4 days. Twenty four hours prior to the time point, tritiated thymidine was added and cells were incubated for an additional 24 h. Genomic DNA was then isolated on filter plates using Cell Harvester (PerkinElmer). Filter plates were oven dried at 37 °C overnight and then sealed on the bottom. Econscint scintillation fluid (40 µl) was added and the plates were then sealed over the top and counted in a TopCount (Perkin Elmer) microplate scintillation counter.

Western Blotting

Cells were lysed in ice cold cell lysis buffer [Cell Lytic M, Sigma #C2978 supplemented with protease inhibitors (Roche #11836153001), phosphatase inhibitors (Roche #04906837001) and benzonase (EMDMillipore, #71206)] and processed for western blotting. Protein samples were resolved in 10% Bis-Tris polyacrylamide gels (Bio-Rad #345-0113) and transferred to nitrocellulose membranes. Visualization was performed on an Odyssey infrared imaging system (LI-COR Biosciences).

Chromatin immunoprecipitation assay (ChIP)

LnCaP-FGC cells were plated at a density of $3x10^6$ in 10 cm dishes in 10ml of CS-FBS media. After 3 days, cells were treated with compounds for 6 h. For DHT stimulation experiments, cells were treated with compounds for 30 minutes prior to addition of 5 nM DHT. Cells were fixed with formaldehyde (1% final) (ThermoFisher #28908) at room temperature for 10 min and quenched with glycine (0.125M final) for 5 min. The cells were then washed 2X with cold PBS and cell pellets were flash frozen at -80 °C until processed for ChIP. Briefly, cells were resuspended in 100ul Lysis Buffer (1% SDS, 10 µM EDTA, 50 µM Tris pH 8.0, 5 mM NaButyrate), vortexed 4 times during 10 min incubation on ice and sonicated using a Diagenode Bioruptor 300 at 4 rounds of 5 cycles of 30 sec with 45 sec rest. Samples were centrifuged for 15min at 20,000 g at 4 °C and 400 µl of Buffer B (0.01% SDS, 16.7 µM Tris pH 8.0, 1 µM EDTA, 167 µM NaCl, 1.1% Triton X-100, 5 mM NaButyrate) was added to the supernatant. Samples were then processed on a Diagenode IP Star according to the manufacturer's instructions. Immunoprecipitations (IPs) used 2 µg H2K27Ac (Active Motif #39685) or 5 µg AR (Millipore #06-608) antibodies and 50 µl of Protein G or A Dynabeads, respectively (Life Technology A10002D and 10004D) with 5 µg/ml BSA. After IP, immunocomplexes were resuspended in elution buffer (20 mM NaHCO3, 1% SDS, 150 mM NaCl) and cross-links were reversed overnight at 65 °C, followed by 1 h RNase A and proteinase K digestion at 45 °C. DNA was purified using ChIP DNA Clean & Concentrator (Zymo). qPCR was performed with SYBR (Perfecta, Quanta), using specific primers (listed in **Supplementary Table 16**) on a Bio-Rad CFX384 and analyzed using DDCt method in Microsoft Excel using negative control regions GAD67 or MYOD.

RNA isolation and Gene Expression Microarray

Cell pellets were lysed using Qiazol lysis reagent (Qiagen, Hilden, Germany) followed by RNA extraction with Qiagen RNeasy mini kit columns according to manufacturer protocols. RNA was subsequently used for Affymetrix gene expression microarray analysis. RNA was converted to biotin labeled cRNA using the

3' IVT plus kit. The labeled cRNA was fragmented and loaded onto Human Genome U133 Plus 2.0 Arrays. Hybridization, washing, staining and scanning of the arrays were performed with Affymetrix equipment. All procedures were performed according to manufacturer protocols (Affymetrix, Santa Clara, CA).

Gene expression analysis, statistics and bioinformatics

Rosetta Resolver® gene expression data analysis system (IBM) was used to normalize raw microarray data, calculate gene expression index and determine significant changes between different experimental conditions. p-value ≤ 0.01 and fold change ≥ 2 or ≤ -2 were used as the statistical cut-offs to select differentially expressed genes from two independent biological replicates. The selected differentially expressed genes were subjected to bioinformatics analysis using Ingenuity® Pathway Analysis (IPA®), where the significantly Upstream Regulator effects were selected. The significance of a relatively activate or inactive regulatory effect was measured by Z-score, where Z-scores ≥ 2 or ≤ -2 were the statistical cut-offs for significance [http://www.ingenuity.com/products/ipa].

***In Vivo* Pharmacology**

All animal studies were conducted in accordance with the guidelines established by the internal Institutional Animal Care and Use Committee. Tumor volumes did not exceed 15% of total body weight per IACUC guidelines.

LuCap-77 CR xenograft efficacy studies

The LuCap-77 CR prostate PDX model was provided by Dr. Eva Corey, University of Washington, (Seattle, Washington, USA). Donor tumors were dissociated and injected as a brie (1:2) into the right flank of 16 week old male C.B.-17 SCID mice on day 0 in a volume of 0.2 ml. Tumors were size matched on day 26 post-inoculation with a mean tumor volume of 211 ± 3 (SEM) mm^3 with dosing beginning on day 28. No

mice on study were excluded from the analysis. Mice were randomized into treatment groups using Studylog software (Studylog Systems, Inc., South San Francisco, CA) based on tumor volume. Tumor volume was calculated twice weekly. Measurements of the length (L) and width (W) of the tumor were taken via electronic caliper and the volume was calculated according to the following equation: $V = L \times W^2/2$ using Study Director version 3.1 (Studylog Systems, Inc., South San Francisco). Partial blinding was used. A different technician formulated and dosed compounds while the main investigator randomized and measured tumor volumes during the study. Tumor growth inhibition was calculated according to the following equation: TGI% = (mean tumor volume of the control group – mean tumor volume of the treated group) / mean tumor volume of the control group x 100.

LuCap-77 CR xenograft PD studies

LuCap-77 CR xenograft tumors were established in SCID mice and animals were dosed with A-485 as described above in "LuCap-77 CR xenograft growth studies" for 7 days. Three hours post the final dose, tumors were harvested and snap frozen on dry ice. For RNA isolation, tumors were homogenized in lysis solution from the 96 well RNA spin kit (GE Health 25-0500-75) using a Precellys 24 homogenizer (Bertin) and further processed according to manufacturer's instructions. For western blotting, tumors were homogenized in ice cold lysis buffer (as described in "Western blotting") and centrifuged at 20,000 g for 20 min. The supernatant was then processed for western blotting as described above.

Statistical analyses

For animal studies, a one sided t-test was used to determine the number of animals needed to obtain 80% power at alpha = 0.05. For this, if there is 48% tumor reduction compared to that of vehicle group measured at the same time, n=8 animals per group will give 80% power. A two-tailed paired students t-test using Microsoft Excel was utilized for statistical significance analyses.

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