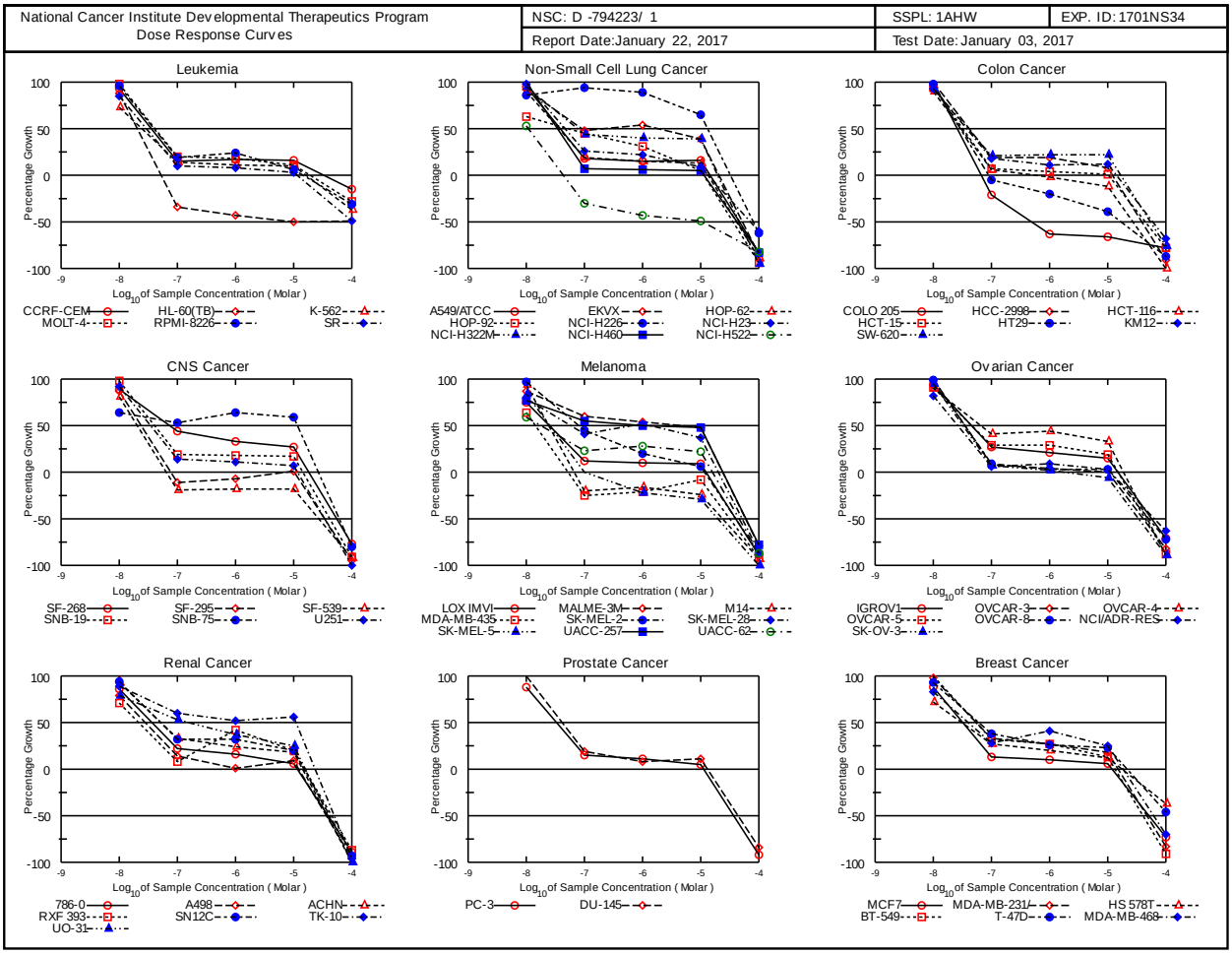
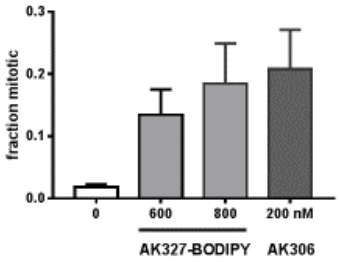
SUPPLEMENTARY MATERIAL

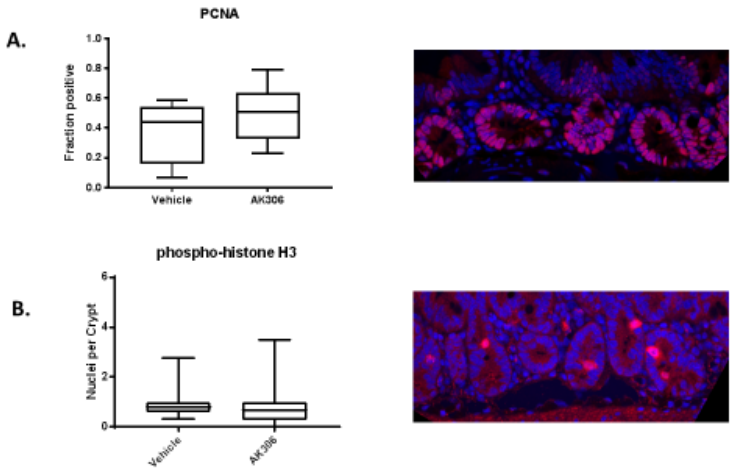
SUPPLEMENTARY FIGURES



Supplementary Figure 1: Results from AK306 testing on the NCI60 cancer cell line panel. Testing was done by the National Institutes of Health Developmental Therapeutics Program.



Supplementary Figure 2: AK3-BODIPY arrests YAMC cells in mitosis at 600 and 800 nM. YAMCs were treated with compound overnight and then assayed for mitotic arrest by staining for phospho-histone H3

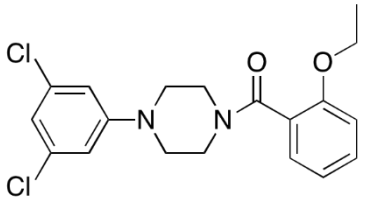


Supplementary Figure 3: Tumor-bearing *Apc*$^{\Delta 14/+}$ mice were treated with five injections of AK306 (30 mg/kg) over the course of three days. Animals were sacrificed and tissue analyzed for PCNA staining **(A)** and phospho-histone H3 staining **(B)**. Representative staining images are shown in the right panels, with quantified staining shown in the left panels.

SUPPLEMENTARY INFORMATION

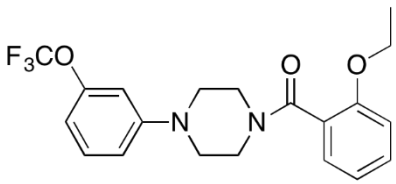
Chemical synthesis procedures and analytical data

Procedure A: Synthesis of 1-(3,5-dichlorophenyl)-4-(2-ethoxybenzoyl)piperazine (AK306)



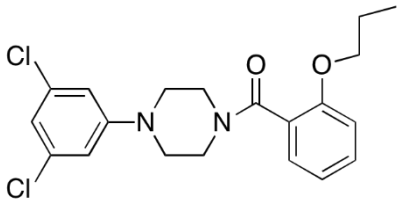
To solid 1-(2-Ethoxybenzoyl)piperazine (100 mg, 0.427 mmol), 1-bromo-3,5-dichlorobenze (482 mg, 2.13 mmol), palladium acetate (2.87 mg, 12.8 µmol), BINAP (15.9 mg, 25.6 µmol) and sodium tert-butoxide (41.0 mg, 0.427 mmol) was added toluene (0.33 M) and the reaction was stirred for 21 hr at 65°C. Reaction worked up with sodium bicarbonate and extracted with dichloromethane (2 X 10 mL). Organic layer washed twice with brine (15 mL), dried with sodium sulfate, and filtered through celite. Filtrate was concentrated and purified by gradient flash chromatography (Hexane/EtOAC 95:5 followed by 80:20) to yield AK306 (112 mg, 30% yield) as a pale yellow solid; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.40 (ddd, *J* = 8.3, 7.4, 1.7 Hz, 1H), 7.33 – 7.31 (dd, *J* = 5.7 Hz, 1H), 7.08 – 7.02 (td, *J* = 7.45, 0.7 Hz 1H), 6.96 (d, *J* = 8.3 Hz, 1H), 6.89 (t, *J* = 1.6 Hz, 1H), 6.79 (d, *J* = 1.6 Hz, 2H), 4.12 (dd, *J* = 14.9, 7.2 Hz, 2H), 4.07 – 3.88 (m, 2H), 3.54 – 3.36 (m, 2H), 3.32 (t, *J* = 5.2 Hz, 2H), 3.26 – 3.08 (m, 2H), 1.43 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.0, 154.6, 152.4, 135.6, 130.7, 128.3, 125.7, 121.1, 119.6, 114.4, 112.0, 77.3, 77.0, 76.8, 64.0, 48.9, 48.6, 46.3, 41.3, 14.9; HRMS (DART, M^++H) *m/z* 379.0951 (calculated for $C_{19}H_{21}Cl_2N_2O_2$, 379.0980).

Procedure B: Synthesis of 1-(2-ethoxybenzoyl)-4-[3-(trifluoromethoxy)phenyl]piperazine (AK307)



To solid 1-(2-Ethoxybenzoyl)piperazine (100 mg, 0.427 mmol), , palladium acetate (2.87 mg, 12.8 µmol), BINAP (15.9 mg, 25.6 µmol) and sodium tert-butoxide (41.0 mg, 0.427 mmol) was added 1-bromo-3(trifluoromethoxy)benzene (514 mg, 217 µL, 2.13 mmol) and toluene (0.33 M). The reaction was stirred for 2.5 hr at 95°C. Reaction washed with sodium bicarbonate (3 X 10 mL) and then filtered through celite. Filtrate was concentrated and purified by gradient flash chromatography (Hexane/EtOAC 95:5 followed by 80:20 and 75:25) to yield AK307 (70 mg, 42% yield) as a pale yellow solid; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.39 (ddd, *J* = 8.3, 7.6, 1.7 Hz, 1H), 7.34 – 7.31 (m, 1H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.04 (td, *J* = 7.4, 0.8 Hz, 1H), 6.95 (d, *J* = 8.3 Hz, 1H), 6.87 (dd, *J* = 8.4, 1.9 Hz, 1H), 6.80 – 6.74 (m, 2H), 4.12 (dq, *J* = 14.2, 7.1 Hz, 2H), 4.07 – 3.92 (m, 2H), 3.56 – 3.37 (m, 2H), 3.33 (t, *J* = 5.3 Hz, 2H), 3.28 – 3.08 (m, 2H), 1.43 (t, *J* = 7.0 Hz, 3H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.0, 154.6, 152.4, 150.3, 150.3, 130.6, 130.2, 128.2, 125.7, 121.0, 114.4, 112.0, 112.0, 108.9, 64.0, 49.3, 49.0, 46.4, 41.4, 14.9; HRMS (DART, M^++H) *m/z* 395.1573 (calculated for $C_{20}H_{22}F_3N_2O_3$, 395.1583).

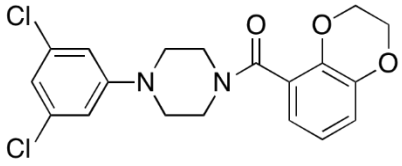
Procedure C: Synthesis of 1-(3,5-dichlorophenyl)-4-(2-propoxybenzoyl)piperazine (AK311)



To solid 1-(3,5-dichlorophenyl)piperazine hydrochloride (100 mg, 432.7 µmol), 2-propoxy benzoic acid (117 mg, 649 µmol), DCC (133 mg, 649 µmol), DMAP (7.93 mg, 6.49 µmol) was added triethylamine (52.5 mg; 72.4 µL, 519 µmol) and reaction-grade dichloromethane (43 mM). The reaction was stirred for 3 hr at room temperature. Reaction concentrated and purified by gradient flash chromatography (Hexane/EtOAC 95:5 followed by 80:20) to yield AK311 (44.4 mg; 26% yield) as a pale yellow solid; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.39 (ddd, *J* = 8.3, 7.5, 1.7 Hz, 1H), 7.33 – 7.28 (m, 1H), 7.04 (td, *J* = 7.45, 0.5 Hz, 1H), 6.95 (d, *J* = 8.3 Hz, 1H), 6.88 (t, *J* = 1.7 Hz, 1H), 6.78 (d, *J* = 1.7 Hz, 2H), 4.07 – 3.92 (m, 4H), 3.54 – 3.36 (m, 2H), 3.31 (q, *J* = 5.8 Hz,

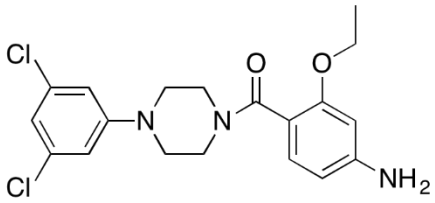
2H), 3.25 – 3.03 (m, 2H), 1.82 (h, *J* = 7.3 Hz, 2H), 1.04 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.0141, 154.8236, 152.3995, 135.5869, 130.6629, 128.1294, 125.6768, 121.0424, 119.5535, 114.3495, 112.1526, 70.0452, 48.8709, 48.5478, 46.2404, 41.2462, 22.6515, 10.5644; HRMS (DART, M^++H) *m/z* 393.1173 (calculated for $C_{20}H_{23}Cl_2N_2O_2$, 393.1137).

Procedure D: 1-(3,5-dichlorophenyl)-4-(2,3-dihydro-1,4-benzodioxine-5-carbonyl)piperazine (AK313)



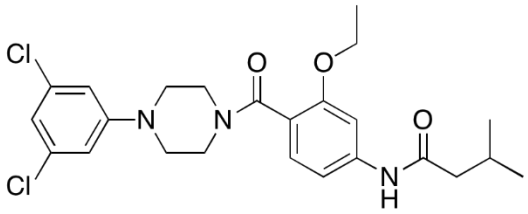
To solid 1-(3,5-dichlorophenyl)piperazine hydrochloride (100 mg, 432.7 µmol), 1,4-benzodioxan-5-carboxylic acid (78.0 mg, 649 µmol), DCC (133 mg, 649 µmol), DMAP (7.93 mg, 6.49 µmol) was added triethylamine (52.5 mg; 72.4 µL, 519 µmol) and reaction-grade dichloromethane (43 mM). The reaction was stirred for 1 hr at room temperature. Reaction washed with 10 mL sodium bicarbonate and extracted with EtoAc (2 X 15 mL). Combined organic extracts dried with sodium sulfate and filtered through celite. Filtrate was concentrated and purified by gradient flash chromatography (Hexane/EtOAC 95:5 followed by 80:20) to yield AK313 (11.0 mg; 6.47% yield) as a pale yellow solid; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.95 (dd, *J* = 8.1, 1.9 Hz, 1H), 6.92 (t, *J* = 7.7 Hz, 1H), 6.89 – 6.84 (m, 2H), 6.78 (d, *J* = 1.8 Hz, 2H), 4.31 (s, 4H), 3.96 (t, *J* = 4.3 Hz, 2H), 3.60 – 3.40 (m, 2H), 3.31 (t, *J* = 4.6 Hz, 2H), 3.17 (d, *J* = 14.5 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.0053, 152.3506, 143.7262, 139.9195, 135.6035, 125.1622, 121.7198, 119.9806, 119.6406, 118.4328, 114.4030, 64.5307, 64.2366, 48.9565, 48.4878, 46.3644, 41.3062; HRMS (DART, M^++H) *m/z* 393.0737 (calculated for $C_{19}H_{19}Cl_2N_2O_3$, 393.0773).

Procedure E: Synthesis of 4-[4-(3,5-dichlorophenyl)piperazine-1-carbonyl]-3-ethoxyaniline (AK327)



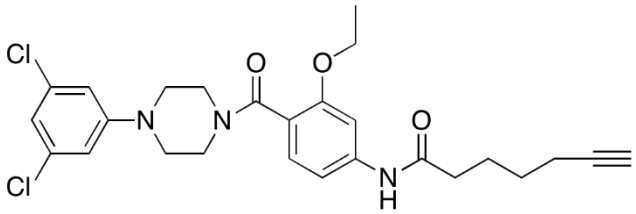
To a solution of Nickel (II) chloride hexahydrate (280 mg, 1.18 mmol) in methanol (0.1 M) was added sodium borohydride (134 mg, 3.54 mmol) in three portions to yield a black mixture. Reaction was degassed with Argon and stirred for 30 minutes at room temperature. Vessel then cooled to 4^{o}C with ice and 1-(3,5-dichlorophenyl)-4-(2-ethoxy-4-nitrobenzoyl)piperazine (500 mg, 1.18 mmol) dissolved in methonal/THF. An additional 12 equivalents of sodium borohydrie (267 mg, 7.08 mmol) was added and the reaction was stirred on ice for 10 minutes. Reaction filtered through celite. Filtrate washed with sodium bicarb (15 mL) and extracted from with dichloromethane (3 X 10 mL). Organic washes combined, dried with sodium sulfate, filtered through cotton and concentrated to yield AK327 (446 mg, 96% yield) as a pale yellow solid; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.13 (d, *J* = 8.0 Hz, 1H), 6.87 (t, *J* = 1.7 Hz, 1H), 6.78 (d, *J* = 1.7 Hz, 2H), 6.33 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.24 (d, *J* = 2.0 Hz, 1H), 4.04 (s, 3H), 3.94 (s, 3H), 3.49 (d, *J* = 28.2 Hz, 2H), 3.29 (s, 2H), 3.16 (d, *J* = 35.8 Hz, 2H), 1.40 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.5, 156.1, 152.5, 149.1, 135.6, 130.0, 119.5, 115.7, 114.3, 107.4, 98.7, 63.9, 49.0, 48.5, 46.5, 41.5, 14.9; HRMS (DART, M^++H) *m/z* 394.1095 (calculated for $C_{19}H_{22}Cl_2N_3O_2$, 394.1089).

<u>Procedure F: Synthesis of N-{4-[4-(3,5-dichlorophenyl)piperazine-1-carbonyl]-3-ethoxyphenyl}-3-methylbutanamide (AK330)</u>



To a solution of the hydrochloride salt of AK327 (75 mg, 190 µmol) in dichloromethane (0.1 M) and triethylamine (39 mg; 53.5 µL, 380 µmol) was added isovaleryl chloride (230 mg; 230 µL, 1.9 mmol) dropwise. The solution was stirred at 35°C for 3 hr. Reaction was washed with sodium bicarbonate (15 mL) and extracted from with ethyl acetate (2 X 10 mL). Organic washes were combined, dried with sodium sulfate, filtered through cotton and concentrated. Concentrate was purified by flash chromatography (Hexane/EtOAc 80:20) to yield AK330 (73 mg, 80% yield) as an off-white solid; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.06 (s, 1H), 7.52 (s, 1H), 7.14 (d, *J* = 8.1 Hz, 1H), 6.88 (s, 1H), 6.80 (s, 1H), 6.78 (s, 2H), 4.05 (d, *J* = 29.1 Hz, 2H), 3.96 (s, 2H), 3.45 (d, *J* = 34.4 Hz, 2H), 3.30 (t, *J* = 5.3 Hz, 2H), 3.15 (d, *J* = 34.8 Hz, 2H), 2.26 (s, 3H), 1.39 (t, *J* = 6.9 Hz, 3H), 1.05 (d, *J* = 5.5 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.4, 168.0, 155.2, 152.4, 140.8, 135.6, 128.5, 120.7, 119.7, 114.4, 111.9, 104.2, 64.1, 49.0, 48.5, 46.9, 46.4, 41.4, 26.2, 22.5, 14.8; HRMS (DART, M^++H) *m/z* 478.1672 (calculated for $C_{24}H_{30}Cl_2N_3O_3$, 478.1664).

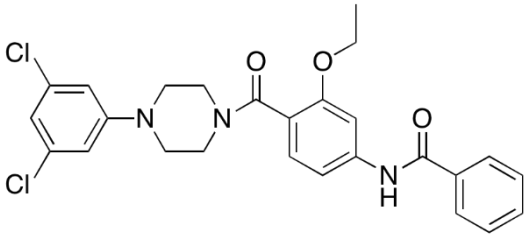
Procedure G: Synthesis of N-{4-[4-(3,5-dichlorophenyl)piperazine-1-carbonyl]-3-ethoxyphenyl}hept-6-ynamide (AK331)



To a solution of DCC (43 mg, 210 µmol) and DMAP (2.3 mg, 19 µmol) in reaction grade dichloromethane (0.1 M) was added the hydrochloride salt of AK327 (75 mg, 190 µmol), triethylamine (39 mg; 54 µL, 380 µmol), and 6-heptynoic acid (48 mg; 48 µL, 380 µmol). Reaction stirred for 12 hrs at room temperature. TLC showed 50% completion so 3 more equivalences of 6-heptynoic acid (72 mg; 72 µL, 570 µmol) and DCC (129 mg, 630 µmol) was added. Solution stirred for 3 hr at 40°C. Reaction filtered through celite, concentrated, purified by gradient flash chromatography (Hexane/EtOAc 70:30 followed by 60:40). NMR showed contaminant believed to be the urea intermediate of DCC and 6-heptynoic acid. To remove urea, product was dissolved

in diethyl ether and placed at 80°C causing the urea to crash out of solution. Ether was filtered through cotton and concentrated to yield AK331 (41 mg, 44% yield) as a white solid; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.74 (s, 1H), 7.56 (s, 1H), 7.19 (d, *J* = 8.0 Hz, 1H), 6.89 (t, *J* = 1.8 Hz, 1H), 6.80 (d, *J* = 1.9 Hz, 1H), 6.79 (d, *J* = 1.8 Hz, 2H), 4.10 (s, 2H), 3.96 (s, 2H), 3.46 (d, *J* = 31.1 Hz, 2H), 3.31 (t, *J* = 5.3 Hz, 2H), 3.16 (d, *J* = 31.9 Hz, 2H), 2.45 (t, *J* = 7.4 Hz, 2H), 2.30 (td, *J* = 7.0, 2.6 Hz, 2H), 2.02 (t, *J* = 2.6 Hz, 1H), 1.90 (p, *J* = 7.5 Hz, 2H), 1.68 (p, *J* = 7.2 Hz, 3H), 1.41 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.5, 168.0, 155.2, 152.4, 140.6, 135.6, 128.5, 120.7, 119.7, 114.4, 111.8, 104.1, 84.1, 68.8, 64.1, 49.0, 48.5, 46.4, 41.4, 36.9, 27.9, 24.5, 18.2, 14.8; HRMS (DART, M^{+}+H) *m/z* 502.1676 (calculated for $C_{26}H_{30}Cl_2N_3O_3$, 502.1664).

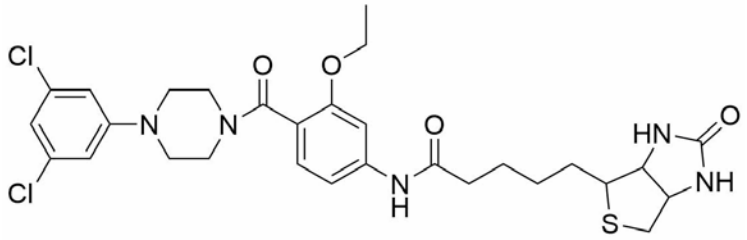
Procedure H: Synthesis of N-{4-[4-(3,5-dichlorophenyl)piperazine-1-carbonyl]-3-ethoxyphenyl}benzamide (AK332)



To a solution of DCC (40 mg, 190 µmol) and DMAP (2.3 mg, 19 µmol) in reaction grade dichloromethane (0.1 M) was added the hydrochloride salt of AK327 (75 mg, 190 µmol), triethylamine (39 mg; 54 µL, 380 µmol), and benzoic acid (46 mg; 380 µmol). Reaction stirred for 18 hrs at 40°C. TLC showed 50% completion so 6 more equivalences of benzoic acid (138, 1.14 mmol) and 1 more equivalence of DCC (40 mg, 190 µmol) was added. Solution stirred for 3 hr at 40°C. Reaction filtered through celite, concentrated, purified by gradient flash chromatography (Hexane/EtOAc 70:30 followed by 60:40). NMR showed contaminant believed to be the urea intermediate of DCC and benzoic acid. To remove urea, product was dissolved in diethyl ether and placed at 80°C causing the urea to crash out of solution. Ether was filtered through cotton and concentrated to yield AK332 (45 mg, 48% yield) as a white solid; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.14 (s, 1H), 7.96 (s, 1H), 7.95 (d, *J* = 1.6 Hz, 1H), 7.72 (d, *J* = 1.9 Hz, 1H), 7.66

– 7.60 (m, 1H), 7.56 (t, *J* = 7.5 Hz, 2H), 7.27 (d, *J* = 8.2 Hz, 1H), 6.97 (dd, *J* = 8.1, 1.9 Hz, 1H), 6.89 (t, *J* = 1.7 Hz, 1H), 6.79 (d, *J* = 1.7 Hz, 2H), 4.15 (d, *J* = 23.5 Hz, 2H), 3.97 (d, *J* = 22.9 Hz, 2H), 3.49 (d, *J* = 41.0 Hz, 2H), 3.31 (t, *J* = 5.3 Hz, 2H), 3.17 (d, *J* = 36.5 Hz, 2H), 1.43 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.8, 165.9, 155.4, 152.4, 140.4, 135.6, 134.6, 132.2, 128.9, 128.8, 127.1, 121.4, 119.7, 114.4, 112.2, 104.4, 64.2, 49.0, 48.6, 46.4, 41.4, 14.8; HRMS (DART, M^++H) *m/z* 498.1380 (calculated for $C_{26}H_{26}Cl_2N_3O_3$, 498.1351).

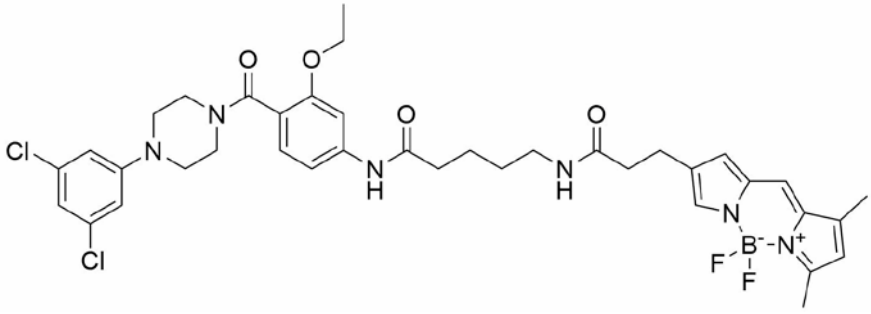
Procedure I: *Synthesis of N-{4-[4-(3,5-dichlorophenyl)piperazine-1-carbonyl]-3-ethoxyphenyl}-5-{2-oxo-hexahydro-1H-thieno[3,4-d]imidazolidin-4-yl}pentanamide*



(AK328)

To solid AK327 (20 mg, 50.7 µmol) and NHS-Biotin (20.8 mg, 60.9 µmol) was added DMF and reaction stirred for 6 days at 50°C. Reaction was concentrated and purified by flash chromatography (dichloromethane/methanol 95:5) to yield AK328 (2.84 mg, 9.0% yield) as a pale yellow solid.

Procedure J: *Synthesis of 2,2-difluoro-10,12-dimethyl-4-{2-[(4-{[4-(piperazine-1-carbonyl)phenyl]carbamoyl}butyl)carbamoyl]ethyl}-1λ^5,3-diaza-2-boratricyclo[7.3.0.0^{3,7}]dodeca-1(12),4,6,8,10-pentaen-1-ylium-2-uide* (**AK3-BODIPY)**



To solid *5-amino-N-{4-[4-(3,5-dichlorophenyl)piperazine-1-carbonyl]-3-ethoxyphenyl}pentanamide* (12.7 mg, 25.6 µmol) and solid BDP FL NHS ester (5 mg, 12.8 µmol) was added DMF (0.06 M) and the reaction was stirred at 37oC for 3 days. The resulting red solution was azeotroped with toluene (3 X 3 mL) and placed under high vacuum for 2 days to remove DMF. Resulting red solid was brought up in 65% acetonitrile/H2O and purified by high performance liquid chromatography (acetonitrile/H2O 60:40, RT: 27.5 minutes) to yield **AK327-BODIPY** (8.36 mg, 85% yield) as a red solid; 1H NMR (500 MHz, Methanol-d4) δ 7.56 (s, 1H), 7.43 (s, 1H), 7.22 (d, J = 8.2 Hz, 1H), 7.17 (d, J = 8.3 Hz, 1H), 7.00 (d, J = 3.9 Hz, 1H), 6.94 (s, 2H), 6.86 (s, 1H), 6.35 (d, J = 4.0 Hz, 1H), 6.23 (s, 1H), 4.13 (dd, J = 15.7, 7.4 Hz, 2H), 3.91 (d, J = 16.8 Hz, 2H), 3.47 (d, J = 17.9 Hz, 2H), 3.26 (td, J = 7.3, 3.9 Hz, 6H), 2.64 (t, J = 7.6 Hz, 2H), 2.54 (s, 3H), 2.43 (t, J = 7.4 Hz, 2H), 2.30 (s, 3H), 1.73 (p, J = 7.4 Hz, 2H), 1.60 (p, J = 6.9 Hz, 2H), 1.41 (t, J = 7.0 Hz, 3H);13C NMR (126 MHz, MeOD) δ 174.6611, 174.5104, 170.1334, 161.3429, 158.5193, 156.5639, 154.1410, 145.8185, 142.8600, 136.6847, 129.5251, 125.7510, 121.5593, 121.3640, 119.8875, 117.6686, 115.2654, 112.9186, 104.8771, 65.1810, 49.8042, 47.8356, 42.7247, 39.9266, 37.5049, 36.0435, 29.8611, 25.6681, 23.9818, 15.1211, 14.8621, 11.1836.