PROTEIN KINASE C δ IS A THERAPEUTIC TARGET IN MALIGNANT MELANOMA WITH NRAS MUTATION OR BRAF INHIBITOR-RESISTANCE

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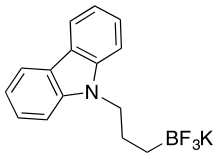
Supporting Information

Preparation of BJE6-154

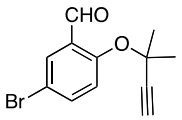
Unless otherwise noted, all reagents were obtained from commercial suppliers and were used without further purification. All air or moisture sensitive reactions were performed under a positive pressured of argon in flame-dried glassware. Tetrahydrofuran (THF), toluene, diethyl ether (Et_2O), dichloromethane, benzene (PhH), acetonitrile (MeCN), triethylamine (NEt_3), pyridine, diisopropyl amine, methanol (MeOH), dimethylsulfoxide (DMSO), and N,N-dimethylformamide (DMF) were obtained from a dry solvent system (Ar degassed solvents delivered through activated alumina columns, positive pressure of argon). Column chromatography was performed on Merck silica gel Kieselgel 60 (230-400 mesh). ^{1}HNMR and ^{13}CNMR spectra were recorded on Varian 300, or 400 MHz spectrometers. Chemical shifts are reported in ppm relative to $CHCl_3$ at δ 7.27 (^{1}HNMR) and δ 77.23 (^{13}CNMR). Mass spectra were obtained on Fisons VG Autospec. IR spectra were obtained from thin films on a NaCl plate using a Perkin-Elmer 1600 series FT-IR spectrometer.

Synthesis of 9-(3-(trifluoro-»^{4}-boranyl)propyl)-9*H*-carbazole, potassium salt:

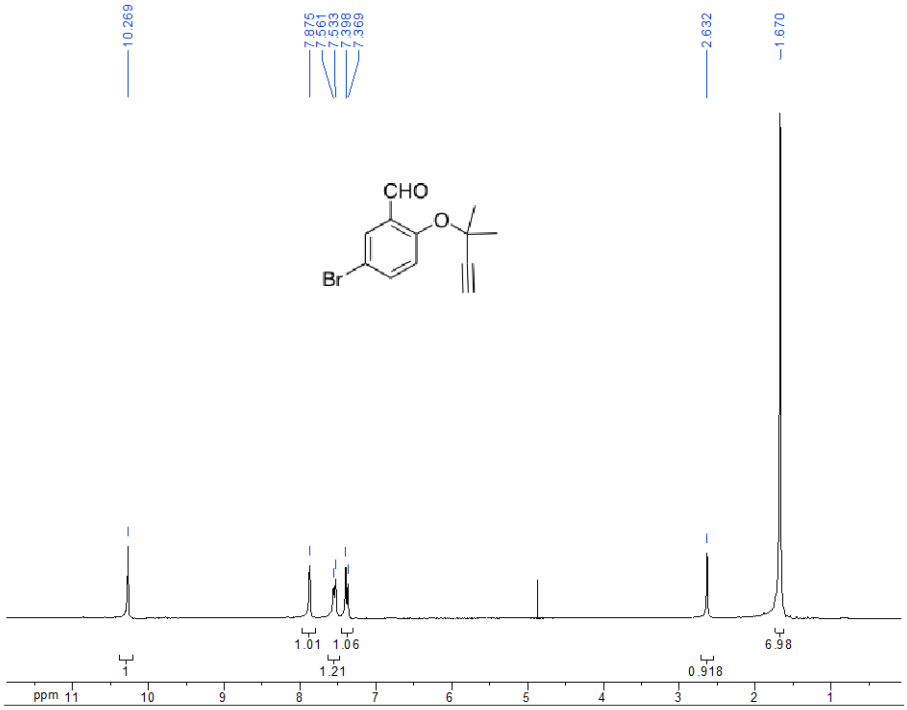


To 3.83 mL (26.5 mmol, 5.5 equiv) 2,5-dimethylhexa-2,4-diene dissolved in 10.0 mL dry THF in a flame dried 100 mL round bottomed flask at 0°C was added 12.06 mL of a 1.0M solution of BH_3 dissolved in THF. The reaction was stirred at 0°C for 3hr before the addition of 1.00 g (4.82 mmol, 1 equiv.) 9-allylcarbazole dissolved in a minimum amount of dry THF. The reaction was allowed to warm to ambient temperature with stirring over 3hr before being cooled to 0°C. To this mixture was added 1.7 mL deionized H_2O. The reaction was then stirred for 1.5hr at ambient temperature before the addition of 4.3 mL of a 37% solution of $CH_2O_{(aq)}$. The reaction was stirred at ambient temperature for 16hr before being added to brine, extracted into EtOAc, dried over Na_2SO_4, and concentrated. The resulting residue was taken up in a mixture of 17.0 mL acetone and 6.5 mL H_2O before the addition of 1.51 g (19.3 mmol, 4 equiv.) KHF_2. The resulting mixture was stirred at ambient temperature for 4hr before being concentrated under reduced pressure. The resulting residue was recrystallized from acetone and Et_2O yielding 1.10 g (72%) of a white crystalize solid which was utilized below without characterization or further purification.

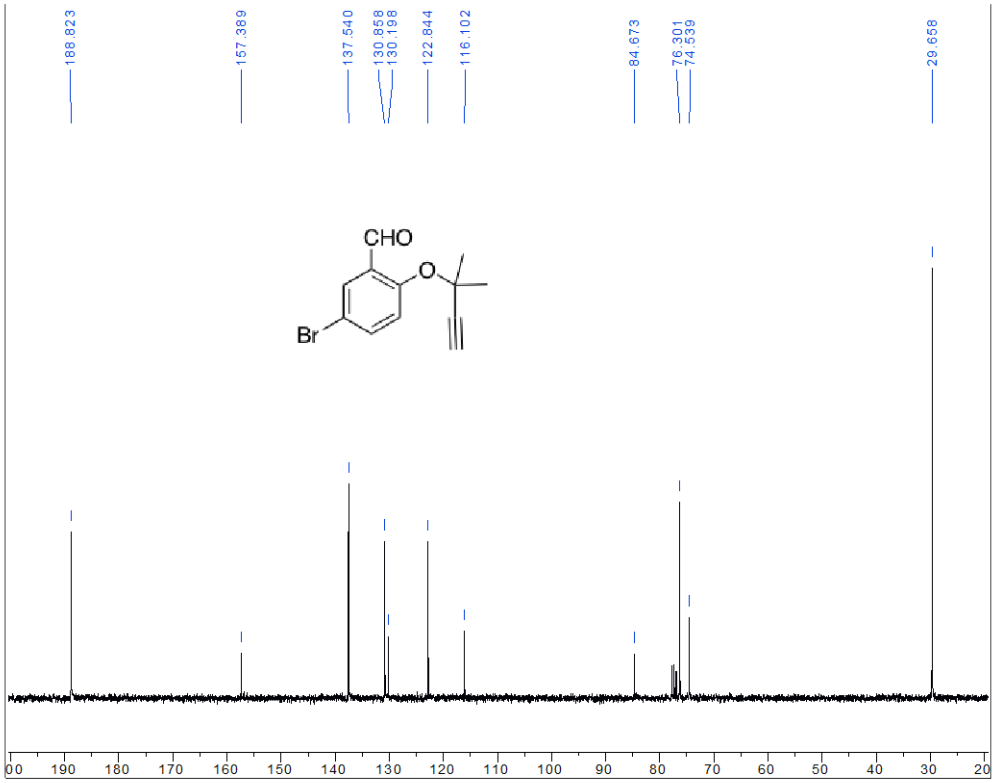
Synthesis of 6-bromo-2,2-dimethyl-2*H*-chromene-8-carbaldehyde: To a 100 mL flame dried round bottomed flask containing 5.54 mL (57.2 mmol, 1.15 equiv) 2-methylbut-3-yn-2-ol dissolved in 50 mL dry MeCN at 0°C was added 11.1 mL (74.6 mmol, 1.5 equiv.) DBU followed by the dropwise addition of 8.08 mL (57.2 mmol, 1.15 equiv.) freshly distilled TFAA. The reaction was stirred at 0°C for 30 min before being added via cannula to a 250 mL round bottomed flask containing 10.0 g (49.7 mmol, 1 equiv.) 5-bromo-2-hydroxybenzaldehyde, 9.65 mL (64.6 mmol, 1.3 equiv.) DBU, and 8.5 mg (0.050 mmol, 0.001 equiv.) $CuCl_2$-$2H_2O$ dissolved in dry MeCN at -5°C. The reaction was stirred for 16hr at ambient temperature before being concentrated under reduced pressure. The resulting residue was taken up in EtOAc, washed once with H_2O, once with 1 M HCl, and once with brine before being dried over Na_2SO_4, and concentrated. This residue was subjected to silica gel flash chromatography eluting with 19 : 1 to 4 : 1 hex/EtOAc to yield 11.17g (84%) of the desired product as a pale yellow solid.



^{1}HNMR (300MHz, $CDCl_3$) δ 10.27(s, 1H), 7.88 (s, 1H), 7.55 (d, *J* = 8.4, 1H), 7.38 (d, *J* = 8.4, 1H), 2.63 (s, 1H), 1.67 (s, 6H);); ^{13}CNMR (75MHz, $CDCl_3$) δ 188.8, 157.4, 137.5, 130.9, 130.2, 122.8, 116.1, 84.7, 76.3, 74.5, 29.7; IR (NaCl, film) 3294, 1687, 1588, 1471 cm^{-1}; HRMS (+TOF) 267.0015 calcd for $C_{12}H_{12}BrO_2$ $[M+H]^+$, found: 267.0016; R_f = 0.38 (9 : 1 hex./ EtOAc). Ref. KAM1-415, BJE6-094

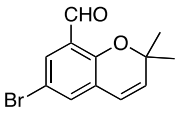


^{1}H NMR (300 MHz) $CDCl_3$

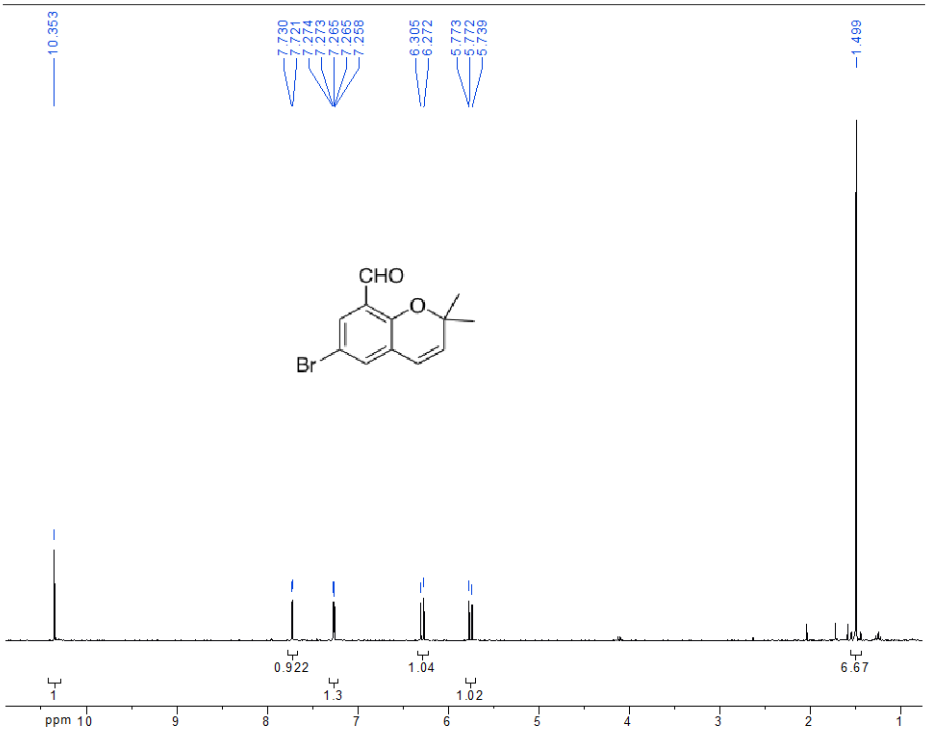


^{13}C NMR (75 MHz) $CDCl_3$

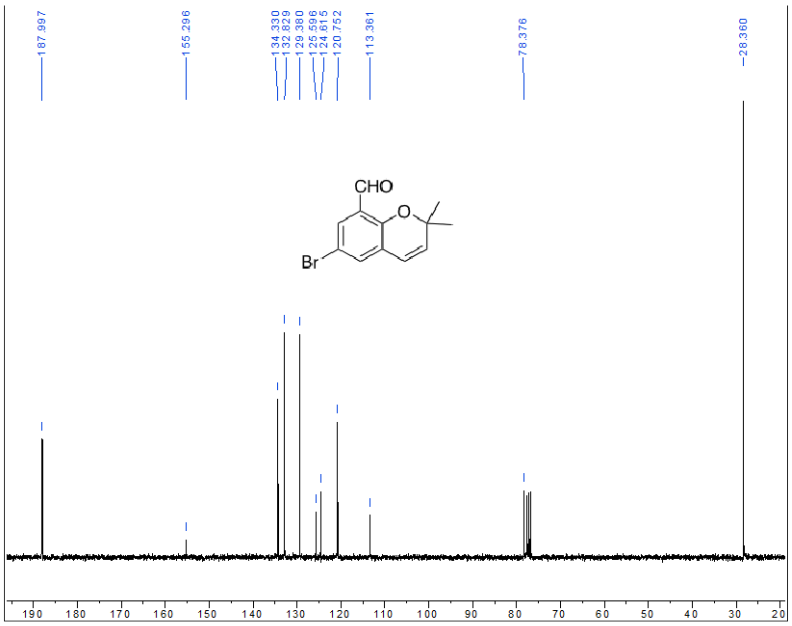
Synthesis of 6-bromo-2,2-dimethyl-2*H*-chromene-8-carbaldehyde: To an 80 mL microwave reaction vessel containing 4.00 g (14.8 mmol, 1 equiv.) 5-bromo-2-((2-methylbut-3-yn-2-yl)oxy)benzaldehyde dissolved in 60 mL dry MeCN was added 66.0 mg (0.300 mmol, 0.02 equiv.) BHT. The reaction was heated in a microwave reactor to 180°C for 20 min before being concentrated and purified by silica gel flash chromatography eluting with 19 : 1 hex./EtOAc to yield 2.10 g (53%) of the desired product as a yellow oil.



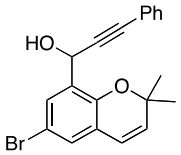
^{1}HNMR (300MHz, $CDCl_3$) δ 10.35 (s,1H), 7.73 (d, *J* = 2.7, 1H), 7.27 (dd, *J* = 2.7, 0.3, 1H), 6.29 (d, *J* = 9.9, 1H), 5.75 (d, *J* = 9.9, 1H), 1.50 (s, 3H); ^{13}CNMR (75MHz, $CDCl_3$) δ 188.0, 155.3, 134.3, 132.8, 129.4, 125.6, 124.6, 120.8, 113.4, 78.4, 28.4; IR (NaCl, film) 2863, 1678, 1574 cm^{-1}; HRMS (+TOF) 267.0015 calcd for $C_{12}H_{12}BrO_2$ $[M+H]^+$, found: 267.0012; R_f = 0.33 (9 : 1 hex./ EtOAc).Ref. KAM1-419, BJE6-105



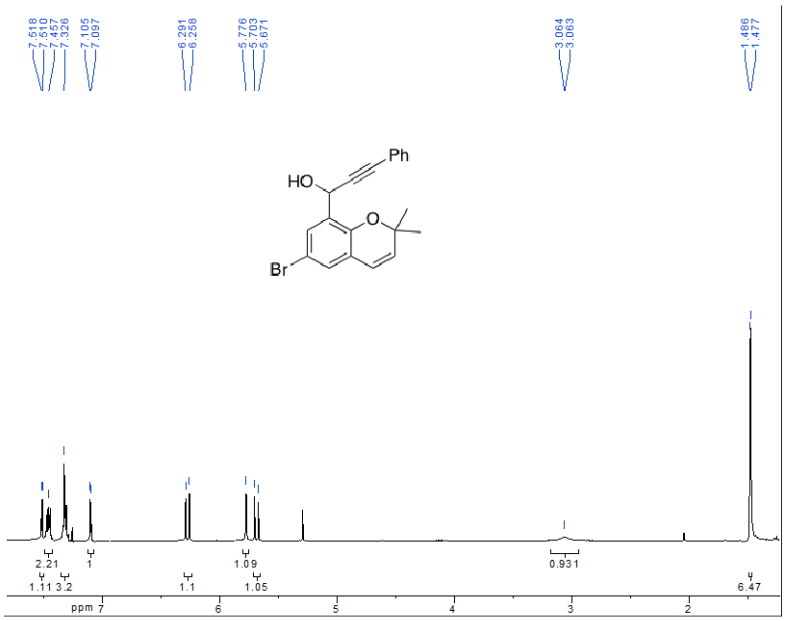
^{1}H NMR (300 MHz) $CDCl_3$



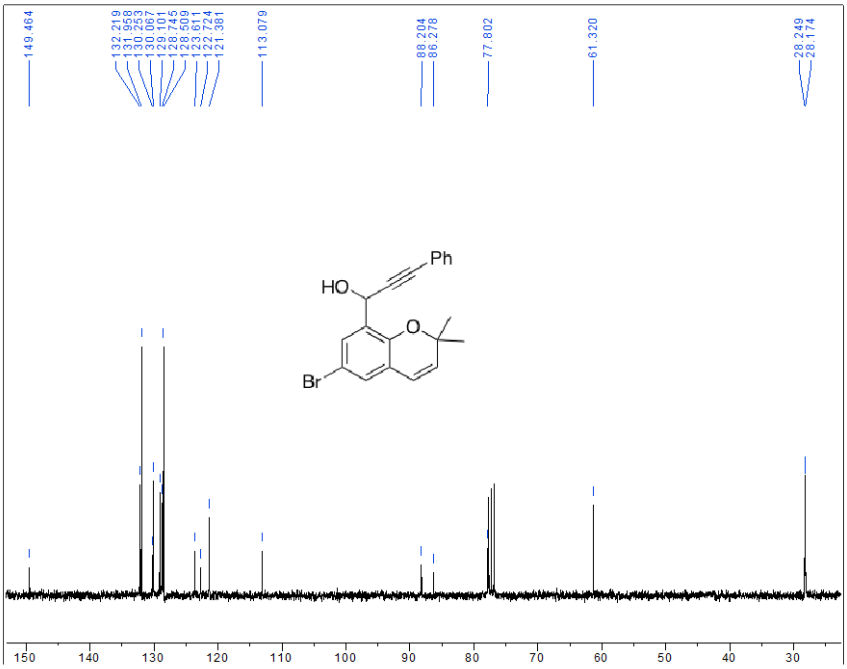
^{13}C NMR (75 MHz) $CDCl_3$



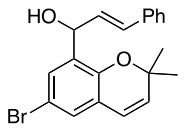
Synthesis of 1-(6-bromo-2,2-dimethyl-2*H*-chromen-8-yl)-3-phenylprop-2-yn-1-ol: To a flame dried 100 mL round bottomed flask containing 745 mg (7.30 mmol, 1.3 equiv.) ethynylbenzene dissolved in 30 mL dry THF at -78^{o}C was dropwise added 4.2 mL (6.7 mmol, 1.2 equiv) of a 1.6 M solution of *n*-BuLi in hexanes. The reaction was allowed to stir at -78^{o}C for 30 min before the addition of 1.50 g (5.62 mmol, 1 equiv.) 6-bromo-2,2-dimethyl-2*H*-chromene-8-carbaldehyde dissolved in 10 mL dry THF. After stirring for 30 min at -78^{o}C the reaction was poured into saturated $NH_4Cl_{(aq.)}$, extracted into EtOAc, dried over Na_2SO_4, and concentrated. The resulting residue was purified by silica gel flash chromatography eluting with 4 : 1 hex./EtOAc yielding 2.1 g (99%) of the desired product as a yellow oil.
^{1}HNMR (300MHz, $CDCl_3$) δ 7.52 (d, *J* = 2.4, 1H), 7.46 (m, 2H), 7.33 (m, 3H), 7.10 (d, *J* = 2.4, 1H), 6.27 (d, *J* = 9.9, 1H), 5.78 (s, 1H), 5.69 (d, *J* = 9.6, 1H), 3.06 (bs, 1H), 1.49 (s, 3H), 1.48 (s, 3H); ^{13}CNMR (75MHz, $CDCl_3$) δ 149.5, 132.2, 132.0, 130.3, 130.1, 129.1, 128.7, 128.5, 123.6, 122.7, 121.4, 113.1, 88.2, 86.3, 77.8, 61.3, 28.2, 28.2; IR (NaCl, film) 3428 br, 2230 cm^{-1}; HRMS (+TOF) 351.0379 calcd. for $C_{20}H_{16}BrO$ $[M-H_2O]^+$, found: 351.0389; R_f = 0.40 (4 : 1 hex./ EtOAc). Ref. KAM1-420, BJE6-107



^{1}H NMR (300 MHz) $CDCl_3$

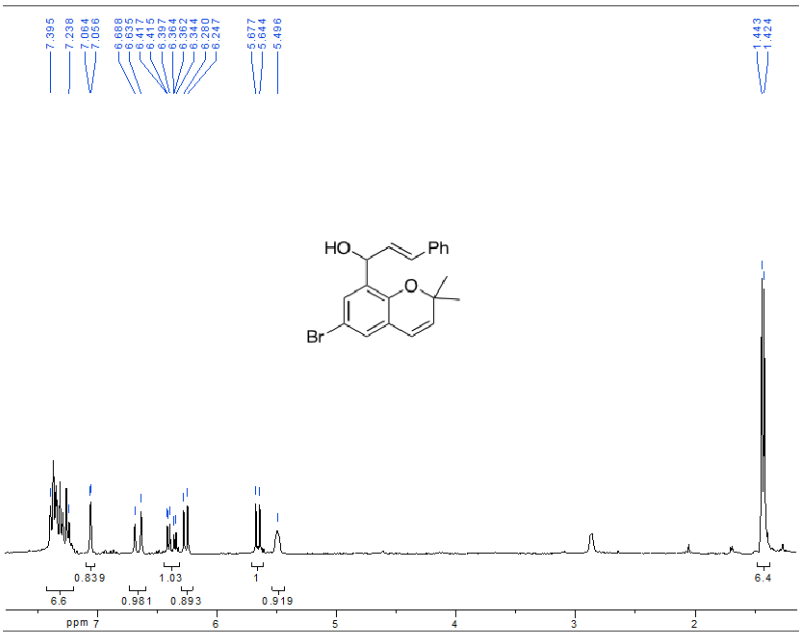


^{13}C NMR (75 MHz) $CDCl_3$

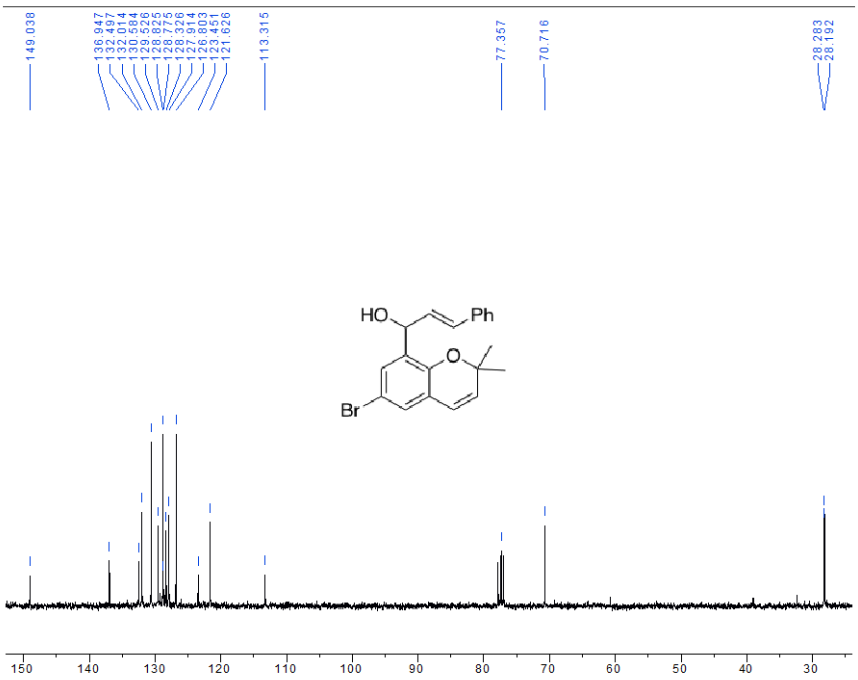


Synthesis of (*E*)-1-(6-bromo-2,2-dimethyl-2*H*-chromen-8-yl)-3-phenylprop-2-en-1-ol: To a 10 mL round bottomed flask containing 100 mg (0.271 mmol, 1 equiv.) 1-(6-bromo-2,2-dimethyl-2*H*-chromen-8-yl)-3-phenylprop-2-yn-1-ol dissolved in 1.5 mL dry THF was added 12 mg (0.33 mmol, 1.2 equiv.) $LiAlH_4$. The reaction was heated to reflux for 1 hr and cooled to ambient temperature. The reaction was quenched by addition of H_2O followed by 15% $NaOH_{(aq.)}$ and then EtOAc. The organic layer was separated and then filtered through a short silica gel plug before being concentrated to yield 100 mg (99%) of the desired product as a yellow oil.

^{1}HNMR (300MHz, $CDCl_3$) δ 7.40-7.24 (m, 6H), 7.06 (d, *J* = 2.4, 1H), 6.70 (d, *J* = 15.9, 1H), 6.38 (dd, *J* = 15.9, 5.4, 1H), 6.26 (d, *J* = 9.9, 1H), 5.66 (d, *J* = 9.9, 1H), 5.50 (m, 1H), 1.44(s, 3H), 1.42 (s, 3H); ^{13}CNMR (75MHz, $CDCl_3$) δ 149.0, 136.9, 132.5, 132.0, 130.6, 129.5, 128.8, 128.8, 128.3, 127.9, 126.8, 123.5, 121.6, 113.3, 77.4, 70.7, 28.3, 28.2; IR (NaCl, film) 3416 br cm^{-1}; HRMS (+TOF) 353.0536 calcd for $C_{11}H_{15}O_3$ $[M\text{-}H_2O]^+$, found: 353.0548; R_f = 0.26 (9 : 1 hex./ EtOAc). Ref. KAM1-421, BJE110

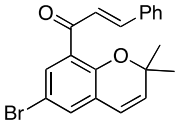


^{1}H NMR (300 MHz) $CDCl_3$



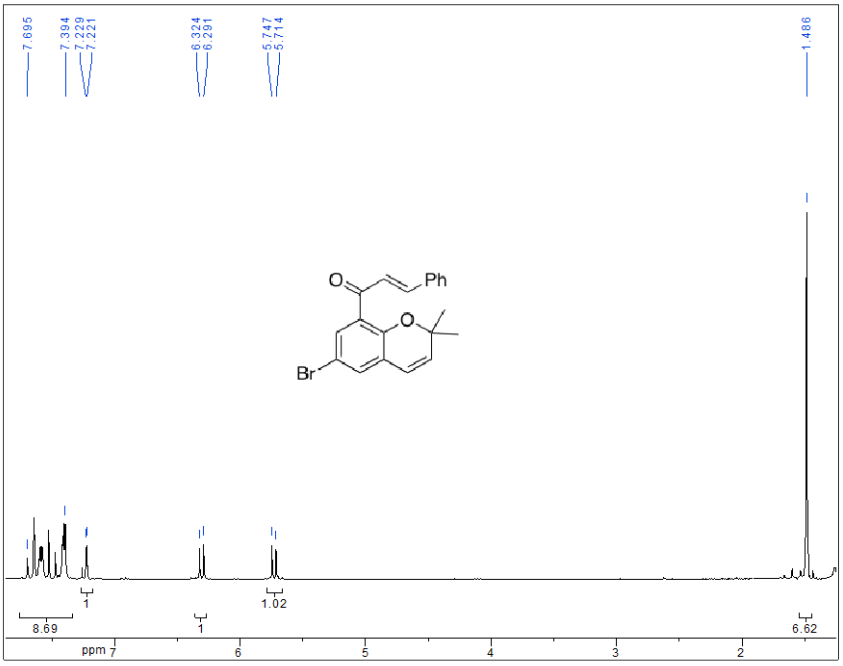
^{13}C NMR (75 MHz) $CDCl_3$

Synthesis of (*E*)-1-(6-bromo-2,2-dimethyl-2*H*-chromen-8-yl)-3-phenylprop-2-en-1-one: To a 50 ml round bottomed flask containing 1.41 g (3.80 mmol, 1 equiv.) (*E*)-1-(6-bromo-2,2-dimethyl-2*H*-chromen-8-yl)-3-phenylprop-2-en-1-ol dissolved in 20 mL dry CH_2Cl_2 was added 1.98 g (22.8 mmol, 6 equiv.) MnO_2 and the reaction was stirred at ambient temperature for 2 hr. The reaction was filtered through celite and concentrated. The resulting residue was purified by silica gel flash chromatography eluting with 19 : 1 to 4 : 1 hex./EtOAc yielding 1.25 g (89%) of the desired product as a yellow oil.

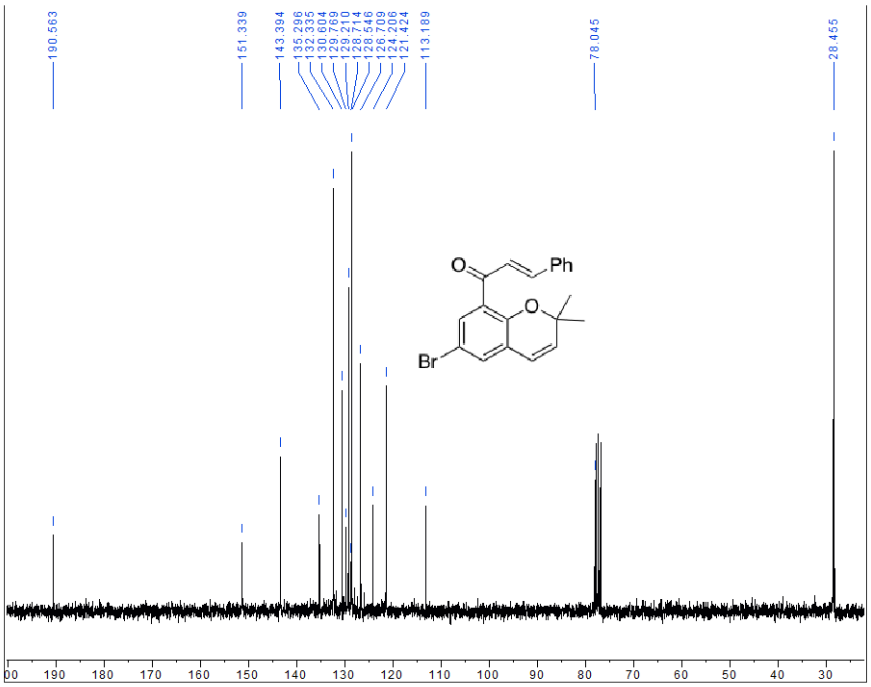


^{1}HNMR (300MHz, $CDCl_3$) δ 7.70-7.39 (m, 8H), 7.22 (d, *J* = 2.4, 1H), 6.31 (d, *J* = 9.9, 1H), 5.72 (d, *J* = 9.9, 1H), 1.49 (s, 6H); ^{13}CNMR (75MHz, $CDCl_3$) δ 190.6, 151.3, 143.4, 135.3, 132.3, 130.6, 129.8, 129.2, 128.7, 128.5, 126.7, 124.2,

121.4, 113.2, 78.0, 28.5; IR (NaCl, film) 1654, 1603, 1435 cm^{-1}; HRMS (+TOF) 369.0485 calcd for $C_{20}H_{18}BrO_2$ $[M+H]^+$, found: 369.0489; R_f = 0.50 (4 :1 hex./ EtOAc). Ref. KAM1-422, BJE6-113

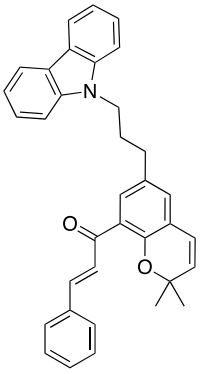


^{1}H NMR (300 MHz) $CDCl_3$

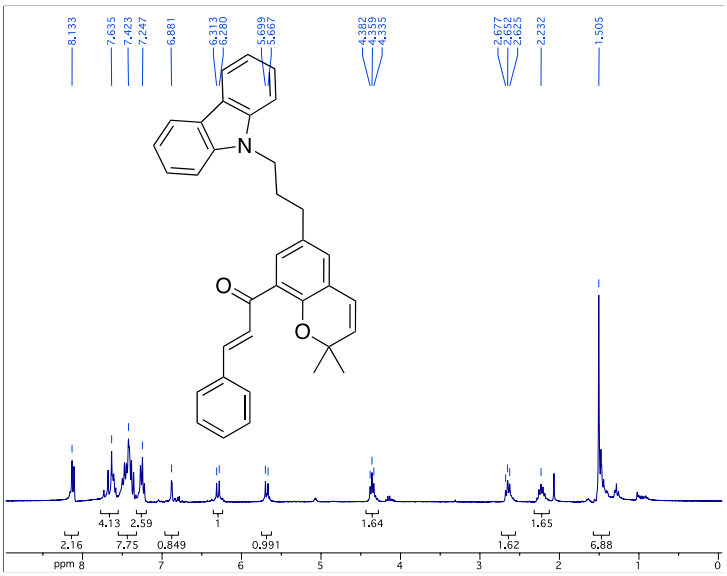


^{13}C NMR (75 MHz) $CDCl_3$

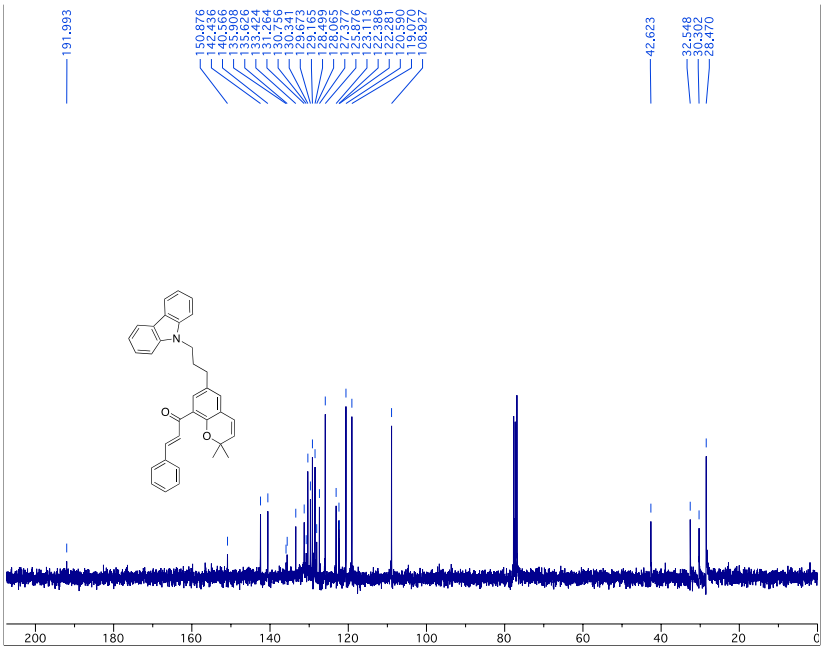
Synthesis of (*E*)-1-(6-(3-(9*H*-carbazol-9-yl)propyl)-2,2-dimethyl-2*H*-chromen-8-yl)-3-phenylprop-2-en-1-one: To a 10 mL round bottomed flask containing 76 mg (0.206 mmol, 1 equiv.) (*E*)-1-(6-bromo-2,2-dimethyl-2*H*-chromen-8-yl)-3-phenylprop-2-en-1-one and 65 mg (0.206 mmol, 1 equiv.) of 9-(3-(trifluoro-»[4]-boranyl)propyl)-9*H*-carbazole, potassium salt was added 8 mg (0.010 mmol, 0.05 equiv.) $PdCl_2$(dppf)-CH_2Cl_2 and 201 mg (0.618 mmol, 3 equiv.) Cs_2CO_3 followed by 1.5 mL dry PhMe and 0.5 mL H_2O. The reaction was heated to 80°C for 12 hr, filtered through cotton and concentrated. Purification by silica gel flash chromatography eluting with 9 : 1 hex./EtOAc to 1 : 1 hex./EtOAc yielded 46 mg (45%) of the desired product as a yellow oil.



^{1}HNMR (300MHz, $CDCl_3$) δ 8.13 (m, 2H), 7.64 (m, 4H), 7.42 (m, 8H), 7.25 (m, 2H), 6.88 (s, 1H), 6.29 (d, *J* = 9.9, 1H), 5.68 (d, *J* = 9.9, 1H), 4.36 (t, *J* = 7.2, 2H), 2.65 (t, *J* = 8.1, 2H), 2.23 (m, 2H), 1.51 (s, 6H); ^{13}CNMR (75MHz, $CDCl_3$) δ 192.0, 150.9, 142.4, 140.6, 135.9, 135.6, 133.4, 131.3, 130.8, 130.3, 129.7, 129.2, 128.5, 128.1, 127.4, 125.9, 123.1, 122.4, 122.3, 120.6, 119.1, 108.9, 42.6, 32.5, 30.3, 28.5; HRMS (+TOF) 498.2433 calcd for $C_{35}H_{32}NO_2$ $[M+H]^+$, found: 498.2431; R_f = 0.14 (9 : 1 hex./ EtOAc). Ref. BJE6-118

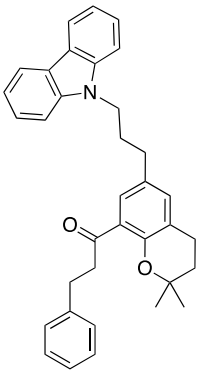


^{1}H NMR (300 MHz) $CDCl_3$



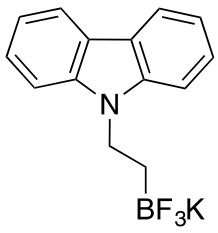
^{13}C NMR (75 MHz) $CDCl_3$

Synthesis of 1-(6-(3-(9*H*-carbazol-9-yl)propyl)-2,2-dimethylchroman-8-yl)-3-phenylpropan-1-one (BJE6-154): To a 10 mL round bottomed flask containing 20.0 mg (0.0401 mmol, 1 equiv.) (*E*)-1-(6-(3-(9*H*-carbazol-9-yl)propyl)-2,2-dimethyl-2*H*-chromen-8-yl)-3-phenylprop-2-en-1-one dissolved in 1 mL dry THF was added a spatula tip of 10% Pd/C. H_2 gas was bubbled through the mixture for 2 min and the reaction was stirred at ambient temperature under balloon pressure of $H_{2(g)}$ for 2 hr before being filtered through celite and concentrated to yield 18 mg (90%) of the desired product as a colorless film. The NMR and mass spectra were fully consistent with the structure of the desired product.

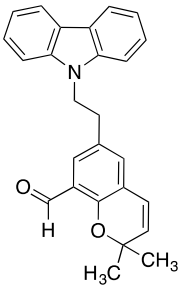


Preparation and Spectra for B106

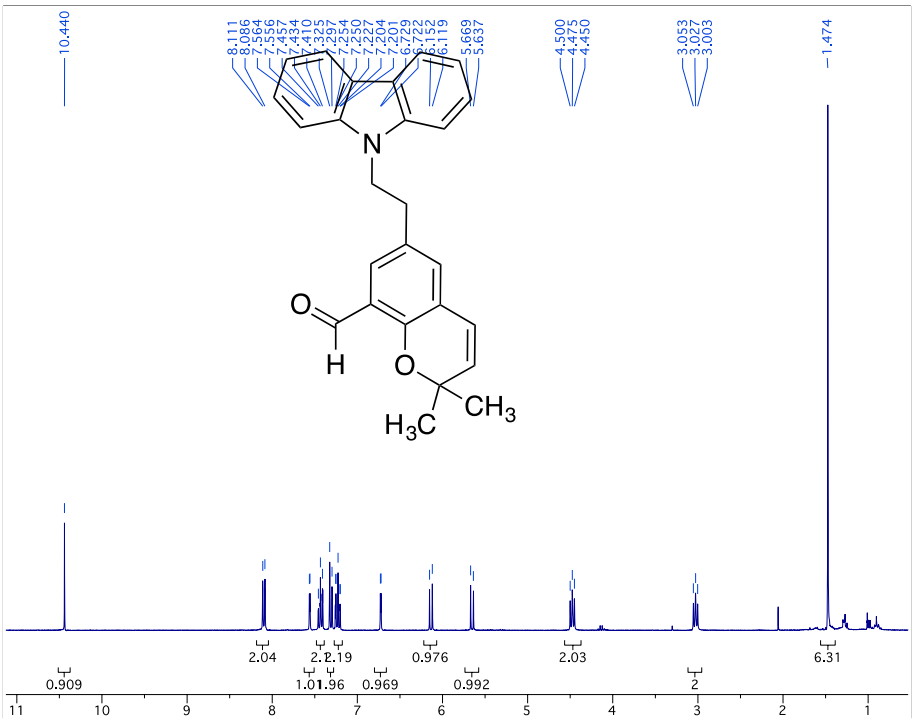
Synthesis of Molander Salt *1* (9-(2-(trifluoro-»^{4}-boranyl)ethyl)-9*H*-carbazole, potassium salt): To 4.11 mL (28.4 mmol, 5.5 equiv) 2,5-dimethylhexa-2,4-diene dissolved in 10.0 mL dry THF in a flame dried 100 mL round bottomed flask at 0°C was added 12.9 mL of a 1.0M solution of BH_3 dissolved in THF. The reaction was stirred at 0°C for 3hr before the addition of 1.00 g (5.17 mmol, 1 equiv.) 9-vinylcarbazole dissolved in a minimum amount of dry THF. The reaction was allowed to warm to ambient temperature with stirring over 3hr before being cooled to 0°C. To this mixture was added 1.7 mL deionized H_2O. The reaction was then stirred for 1.5hr at ambient temperature before the addition of 4.3 mL of a 37% solution of $CH_2O_{(aq)}$. The reaction was stirred at ambient temperature for 16hr before being added to brine, extracted into EtOAc, dried over Na_2SO_4, and concentrated. The resulting residue was taken up in a mixture of 17.0 mL acetone and 6.5 mL H_2O before the addition of 1.62 g (20.7 mmol, 4 equiv.) KHF_2. The resulting mixture was stirred at ambient temperature for 4hr before being concentrated under reduced pressure. The resulting residue was recrystallized from acetone and Et_2O yielding 1.06 g (68%) of a white crystalize solid which was utilized below without characterization or further purification.



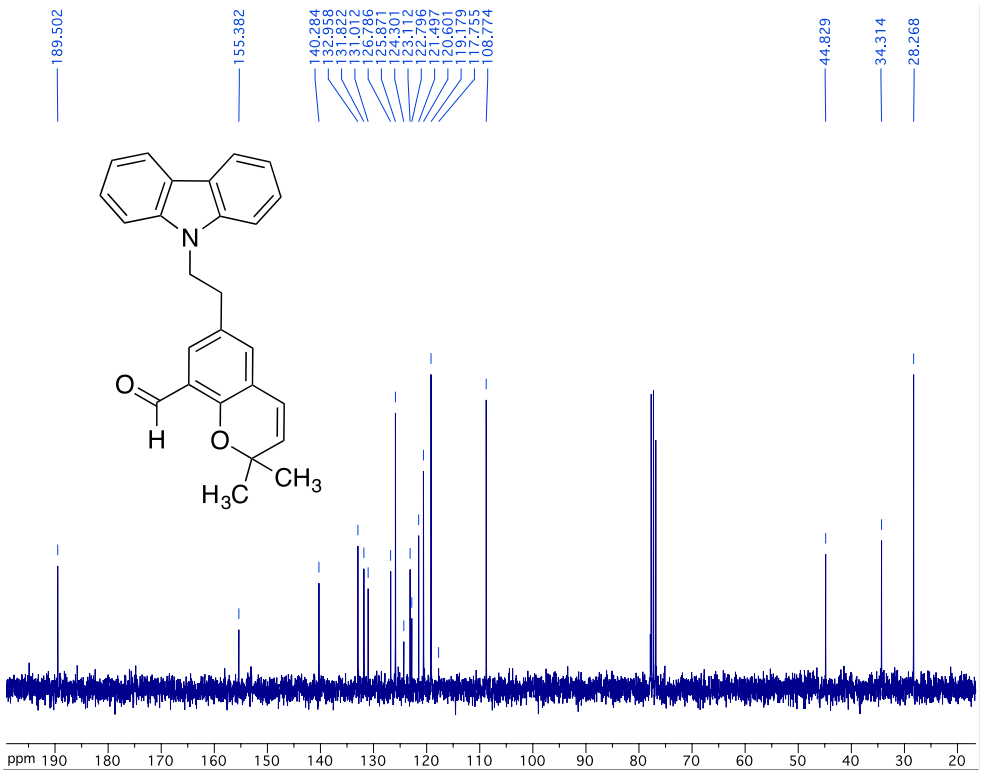
Synthesis of 6-(2-(9*H*-carbazol-9-yl)ethyl)-2,2-dimethyl-2*H*-chromene-8-carbaldehyde (BJE6-106): To a flame dried 10 mL round bottomed flask containing 57.0mg (0.213 mmol, 1 equiv.) 6-bromo-2,2-dimethyl-2*H*-chromene-8-carbaldehyde, 64.0mg (0.213 mmol, 1 equiv.) Molander Salt *1* (9-(2-(trifluoro-»^{4}-boranyl)ethyl)-9*H*-carbazole, potassium salt), 8.0 mg (0.011 mmol, 0.05 equiv.) $PdCl_2$(dppf)-CH_2Cl_2, and 208 mg (0.639 mmol, 3 equiv.) anhydrous Cs_2CO_3 was added 1.5 mL dry toluene and 0.5 mL deionized H_2O. The reaction was heated to 80°C for 16hr, added to brine, extracted into EtOAc, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel flash chromatography eluting with 19 : 1 hex./EtOAc to 1 : 1 hex./EtOAc yielding 36 mg (44%) of the desired product as a colorless oil.



^{1}HNMR (300MHz, $CDCl_3$) δ 10.44 (s, 1H), 8.09 (d, *J* = 7.5, 2H), 7.56 (d, *J* = 2.4, 1H), 7.43 (t, *J* = 6.9, 2H), 7.32 (t, *J* = 8.4, 2H), 7.23 (t, *J* = 6.9, 2H), 6.73 (d, *J* = 2.1, 1H), 6.14 (d, *J* = 9.9, 1H), 5.65 (d, *J* = 9.6, 1H), 4.48 (t, *J* = 7.5, 2H), 3.03 (t, *J* = 7.5, 2H), 1.47 (s, 6H); ^{13}CNMR (75MHz, $CDCl_3$) δ 189.5, 155.4, 140.3, 133.0, 131.8, 131.0, 126.8, 125.9, 124.3, 123.1, 122.8, 121.5, 120.6, 119.2, 117.8, 108.8, 44.8, 34.3, 28.3; IR (NaCl, film) 2861, 1679 cm^{-1}; HRMS (+TOF) 382.1802 calcd for $C_{26}H_{23}NO_2$ $[M+H]^+$, found: 382.1799; R_f = 0.25 (9 : 1 hex./ EtOAc).
Ref. BJE6-106



^{1}H NMR Spectrum (300MHz, $CDCl_3$)



^{13}C NMR (75 MHz) $CDCl_3$

Pharmacophore Modeling of Novel PKC–delta inhibitors.

Numerous docking studies were conducted to predict how mallotoxin/rottlerin binds to PKC-delta. Since a structure of PKC-delta was not available, rottlerin was docked into the catalytic binding site of several different PKC crystal structures. The structure of PKC-theta complexed with staurosporine (pdb code 1XJD) was selected as the most suitable model.

The TFIT program within FLO molecular modeling software [1] was used to construct pharmacophores, as we have previously described.[2-4] An ensemble of low energy conformers was computed for mallotoxin/rottlerin and staurosporine, optimized to simultaneously minimize the internal energy and maximize the match of chemically similar atoms (**Fig. S1**). There has been excellent agreement between the bio-active conformations of inhibitors calculated by TFIT and that found experimentally.[5,6]

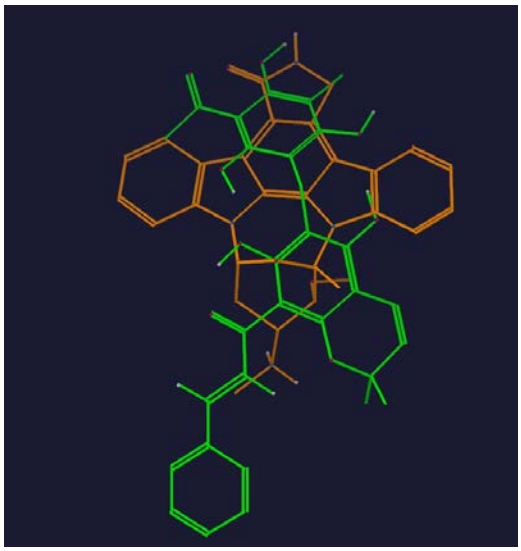


Fig. S1: "Best-fit" superimposition of staurosporine (brown and mallotoxin/rottlerin (green) structures using TFIT.

Dockmin+ is an energy minimization procedure developed for the FLO+ molecular modeling package, which uses a molecular mechanics force field to find the nearest minimum to the original structure. Then dockmin+ uses a scoring function to evaluate the ligand/binding site interactions. The scoring function used by the FLO+ suite of programs uses a potential function containing the following terms: Contact Energy; Hydrogen Bond energy, Entropy, Polar Desolvation, Internal Free Energy, and Repulsion. It is known from crystal structures of many kinase/inhibitor complexes that the kinase active site is flexible. Therefore, regions known to be flexible were allowed to be free during the docking procedures. Eventually a reasonable binding mode of rottlerin bound to PKC-delta was obtained (**Fig. S2**). However, because rottlerin is structurally so different from typical kinase inhibitors whose binding mode is now know from x-ray data, the binding mode of rottlerin obtained in this study was taken as an initial hypothesis.

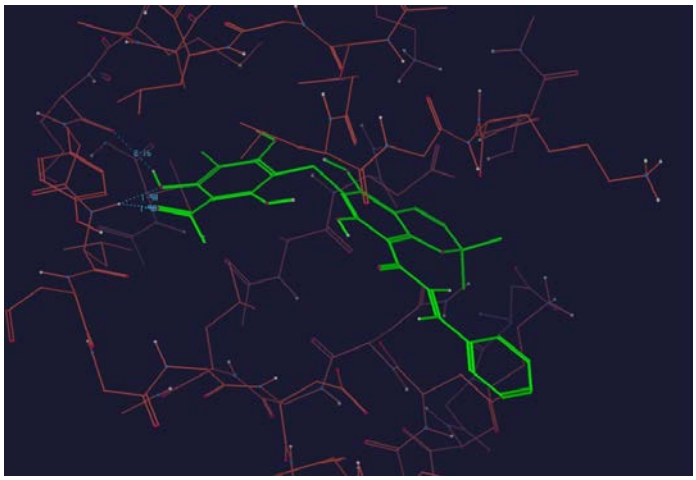


Figure S2: Model of rottlerin bound to PKC-delta

Lzm is the graphical interface used in part of the FLO+ molecular modeling

suite. Mallotoxin/rottlerin and staurosporine were evaluated using the solvent accessible surface and the atom scores. Looking at a ligand and the solvent accessible surface mesh it becomes immediately obvious to what extent the ligand occupies the binding site and gives a sense of the quality of the protein/ligand interactions. Ligand atoms in ideal van der Waals contact with the binding site atoms lie on or near the surface mesh. Ligand atoms that form hydrogen bonds with binding site atoms, penetrate the mesh.[7] The atom scores assign a numerical energy to each ligand atom showing the contribution that atom makes to the total predicted binding energy. The solvent accessible surface of the mallotoxin-rottlerin binding site is shown in a mesh in **Fig. S3**. It is computed at the van der Waals radius of the binding site atoms plus a probe distance of 1.4 Angstroms.

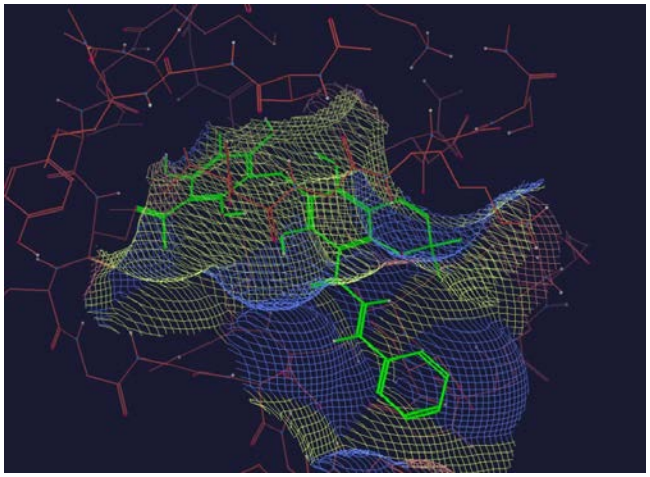
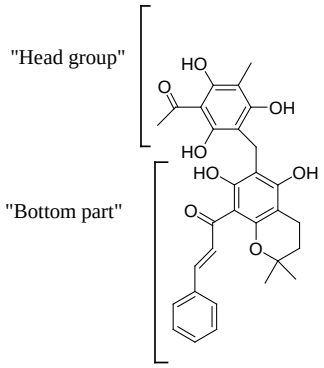


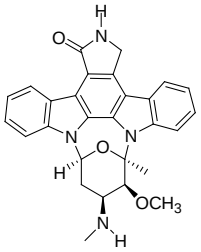
Figure S3: Model of mallotoxin/rottlerin docked in PKC-delta with the solvent accessible surface of the binding site shown as a mesh.

Five molecules were designed using the PKC-delta model developed from the rottlerin docking studies. The strategy was to retain most of the "bottom" part of rottlerin (see figures below) which is assumed to give rottlerin its specificity but to vary the "head group" which is assumed to bind to the hinge region of the kinase active site. Numerous "head groups" from known potent kinase inhibitors were tested in the PKC-theta model, including staurosporine, purine-based inhibitors, a CDK2 inhibitor (pdb code: 1FVT) and an aurora kinase inhibitor (pdb code 2F4J). The criteria for selection was that the resulting molecule should form favorable interactions with the hinge region while the "bottom part" retained interactions with the binding site similar to that of staurosporine (from the x-ray crystal structure) and rottlerin (from our docking studies).

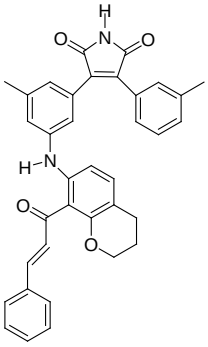
In Structures 1 and 2, the head group resembled that of staurosporine and other bisindoyl maleimide inhibitors. Ease of synthesis was a major factor in the design of this head group.

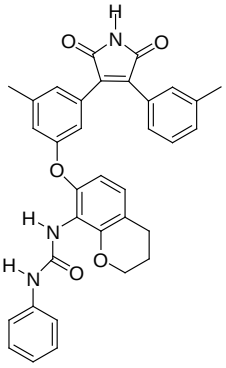
Modifications of these structures that allowed for easier synthesis produced the 2nd and 3rd generation PKCδ inhibitors described previously[8] and in this report.





Rottlerin Staurosporine





Structure 1 Structure 2

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Supplemental Table 1:

PKCδ Inhibitory Activity of BJE6 compounds

	IC_{50} (*in vitro*)[1]			IC_{50} (*in culture*)[3]
Compound	PKCδ IC_{50} (μM)	PKCα IC_{50} (μM)	PKCδ/PKCα (IC_{50} ratio)[2]	
Rottlerin	4.4	75	28	10
KAM-1	3.6	177	56	5
B058	5.8	NA[4]	-	>50
B071	3	NA	-	>100
B095	8	NA	-	>100
B097	9.5	NA	-	>100
B106	0.08	15	200	1
B108	3	NA	-	>100
B109	0.09	75	800	>100
B111	2.5	25	100	40
B112	6	NA	-	>50
B117	4.5	NA	-	>100
B118	10	NA	-	>100
B121	4	NA	-	>100
B125	0.25	20	800	>100
B128	6.5	NA	-	>100
B129	6.5	NA	-	>50
B130	4	NA	-	40
B131	10	NA	-	>100
B136	14	NA	-	>100
B137	3	NA	-	>100
B141	4.8	NA	-	>50
B142	3.5	NA	-	>100
B143	6	NA	-	>100
B146	5	NA	-	>100
B147	5.8	NA	-	20
B148	4	NA	-	>100
B149	0.31	>300	>1000	20
B150	0.625	500	800	30
B151	< 0.05	50	>1000	>100
B152	< 0.05	50	>1000	>100
B153	3	NA	-	>100
B154	23	NA	-	>100

B155	3	NA	-	>100
B156	7.5	NA	-	>100
B157	5	NA	-	>100
B158	4.5	NA	-	>100
B159	7	NA	-	40

[1] IC_{50} measured using recombinant PKCδ or PKCα

[2] Ratio of PKCδ IC_{50} to PKCα IC_{50}

[3] IC_{50} for cytotoxicity on a KRAS-mutant cell line H460.

[4] NA = not assayed (PKCα inhibitory activity was only assayed on compounds with an IC_{50} for PKCδ of <2.5 μM).

METHODS

Reagents. Rottlerin/mallotoxin, PLX4032 (vemurafenib), propidium iodide, and RNase A were purchased from Axxora, LC Labs, Sigma-Aldrich and Fisher Scientific, respectively. Z-VAD-FMK was purchased from R&D Systems and Enzo Life Sciences. Antibodies against phospho-SAPK/JNK (Thr183/Tyr185) (#4668), SAPK/JNK (#9252), phospho-Histone H2A.X (Ser 139) (#2577), Histone H2A (#2578), phospho-SEK1/MKK4) (#4514), SEK1/MKK4 (#9152), phospho-MKK7 (Ser271/Thr275) (#4171), MKK7 (#4172), phospho-c-Jun (Ser63) (#9261), c-Jun (#9165), phospho-ERK1/2 (Thr202/Tyr204) (#4370), phospho-p38 (Thr180/Tyr182) (#4511) and p38 (#9212) were purchased from Cell Signaling Technologies. Antibodies against ERK1 (K-23) and PKCδ (#610398) were purchased from Santa Cruz Biotechnology and BD Biosciences, respectively. Antibodies against α-Tubulin (#T6074), β-Actin (#A1978) and GAPDH (#G8795) were purchased from Sigma-Aldrich. ON-TARGETplus SMART pool siRNA against JNK1 (L-003514), JNK2 (L-003505), H2AX (L-011682) and non-targeting scrambled siRNA #1 (D-001810-01) were purchased from Dharmacon. Silencer Select siRNA against PKCδ (PRKCD) was purchased from Life Technologies.

Synthetic Methods. BJE6-106 and BJE6-154 were synthesized using Molander trifluorborate coupling chemistry,[1,2] with the details and NMR spectra presented elsewhere in Supplemental Information. Briefly: unless otherwise noted, all reagents were obtained from commercial suppliers and were used without further purification. All air or moisture sensitive reactions were performed under a positive pressured of argon in flame-dried glassware. Tetrahydrofuran (THF), toluene, diethyl ether (Et_2O), dichloromethane, benzene (PhH), acetonitrile (MeCN), triethylamine (NEt_3), pyridine, diisopropyl amine, methanol (MeOH), dimethylsulfoxide (DMSO), and N,N-dimethylformamide (DMF) were obtained from a dry solvent system (Ar degassed solvents delivered through activated alumina columns, positive pressure of argon). Column chromatography was performed on Merck silica gel Kieselgel 60 (230-400 mesh). ^{1}HNMR and ^{13}CNMR spectra were recorded on Varian 300, or 400 MHz spectrometers. Chemical shifts are reported in ppm relative to $CHCl_3$ at δ 7.27 (^{1}HNMR) and δ 77.23 (^{13}CNMR). Mass spectra were obtained on Fisons VG Autospec. IR spectra were obtained from thin films on a NaCl plate using a Perkin-Elmer 1600 series FT-IR spectrometer.

Synthesis of Molander Salt (4): (9-(2-(trifluoro-»^{4}-boranyl)ethyl)-9H-carbazole, potassium salt): To 4.11 mL (28.4 mmol, 5.5 equiv) 2,5-dimethylhexa-2,4-diene dissolved in 10.0 mL dry THF in a flame dried 100 mL round bottomed flask at 0^{o}C was added 12.9 mL of a 1.0M solution of BH_3 dissolved in THF. The reaction was stirred at 0^{o}C for 3 hr before the addition of 1.00 g (5.17 mmol, 1 equiv.) 9-vinylcarbazole dissolved in a minimum amount of dry THF. The reaction was allowed to warm to ambient temperature with stirring over 3 hr before being cooled to 0^{o}C. To this mixture was added 1.7 mL deionized H_2O. The reaction was then stirred for 1.5 hr at ambient temperature before the addition of 4.3 mL of a 37% solution of $CH_2O_{(aq)}$. The reaction was stirred at ambient temperature for 16 hr before being added to brine, extracted into EtOAc, dried over Na_2SO_4, and concentrated. The resulting residue was taken up in a mixture of 17.0 mL acetone and 6.5 mL H_2O before the addition of 1.62 g (20.7 mmol, 4 equiv.) KHF_2. The resulting mixture was stirred at ambient temperature for 4hr before

being concentrated under reduced pressure. The resulting residue was recrystallized from acetone and Et_2O yielding 1.06 g (68%) of a white crystalline solid (**4**) (see Figure 2A, Scheme 1) which was utilized below without characterization or further purification.

Synthesis of 6-bromo-2,2-dimethyl-2H-chromene-8-carbaldehyde (2): To a 100 mL flame dried round bottomed flask containing 5.54 mL (57.2 mmol, 1.15 equiv) 2-methylbut-3-yn-2-ol dissolved in 50 mL dry MeCN at 0°C was added 11.1 mL (74.6 mmol, 1.5 equiv.) DBU followed by the dropwise addition of 8.08 mL (57.2 mmol, 1.15 equiv.) freshly distilled TFAA. The reaction was stirred at 0°C for 30 min before being added via cannula to a 250 mL round bottomed flask containing 10.0 g (49.7 mmol, 1 equiv.) 5-bromo-2-hydroxybenzaldehyde (**1**), 9.65 mL (64.6 mmol, 1.3 equiv.) DBU, and 8.5 mg (0.050 mmol, 0.001 equiv.) $CuCl_2$-$2H_2O$ dissolved in dry MeCN at -5°C. The reaction was stirred for 16hr at ambient temperature before being concentrated under reduced pressure. The resulting residue was taken up in EtOAc, washed once with H_2O, once with 1 M HCl, and once with brine before being dried over Na_2SO_4, and concentrated. This residue was subjected to silica gel flash chromatography eluting with 19:1 to 4:1 hex/EtOAc to yield 11.17g (84%) of the desired product (**2**) as a pale yellow solid.

^{1}HNMR (300MHz, $CDCl_3$) δ 10.27 (s, 1H), 7.88 (s, 1H), 7.55 (d, *J* = 8.4, 1H), 7.38 (d, *J* = 8.4, 1H), 2.63 (s, 1H), 1.67 (s, 6H);); ^{13}CNMR (75MHz, $CDCl_3$) δ 188.8, 157.4, 137.5, 130.9, 130.2, 122.8, 116.1, 84.7, 76.3, 74.5, 29.7; IR (NaCl, film) 3294, 1687, 1588, 1471 cm^{-1}; HRMS (+TOF) 267.0015 calcd for $C_{12}H_{12}BrO_2$ $[M+H]^+$, found: 267.0016; R_f = 0.38 (9 : 1 hex./ EtOAc).

Synthesis of 6-bromo-2,2-dimethyl-2H-chromene-8-carbaldehyde (3): To an 80 mL microwave reaction vessel containing 4.00 g (14.8 mmol, 1 equiv.) 5-bromo-2-((2-methylbut-3-yn-2-yl)oxy)benzaldehyde (**2**) dissolved in 60 mL dry MeCN was added 66.0 mg (0.300 mmol, 0.02 equiv.) BHT. The reaction was heated in a microwave reactor to 180°C for 20 min before being concentrated and purified by silica gel flash chromatography eluting with 19:1 hex./EtOAc to yield 2.10 g (53%) of the desired product (**3**) as a yellow oil.

^{1}HNMR (300MHz, $CDCl_3$) δ 10.35 (s, 1H), 7.73 (d, *J* = 2.7, 1H), 7.27 (dd, *J* = 2.7, 0.3, 1H), 6.29 (d, *J* = 9.9, 1H), 5.75 (d, *J* = 9.9, 1H), 1.50 (s, 3H); ^{13}CNMR (75MHz, $CDCl_3$) δ 188.0, 155.3, 134.3, 132.8, 129.4, 125.6, 124.6, 120.8, 113.4, 78.4, 28.4; IR (NaCl, film) 2863, 1678, 1574 cm^{-1}; HRMS (+TOF) 267.0015 calcd for $C_{12}H_{12}BrO_2$ $[M+H]^+$, found: 267.0012; R_f = 0.33 (9:1 hex./ EtOAc).

Synthesis of 6-(2-(9H-carbazol-9-yl)ethyl)-2,2-dimethyl-2H-chromene-8-carbaldehyde (B106): To a flame dried 10 mL round bottomed flask containing 57.0mg (0.213 mmol, 1 equiv.) 6-bromo-2,2-dimethyl-2*H*-chromene-8-carbaldehyde (**3**), 64.0mg (0.213 mmol, 1 equiv.) Molander Salt (**4**) (9-(2-(trifluoro-»^{4}-boranyl)ethyl)-9*H*-carbazole, potassium salt), 8.0 mg (0.011 mmol, 0.05 equiv.) $PdCl_2$(dppf)-CH_2Cl_2, and 208 mg (0.639 mmol, 3 equiv.) anhydrous Cs_2CO_3 was added 1.5 mL dry toluene and 0.5 mL deionized H_2O. The reaction was heated to 80°C for 16hr, added to brine, extracted into EtOAc, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel flash chromatography eluting with 19 : 1 hex./EtOAc to 1 : 1 hex./EtOAc yielding 36 mg (44%) of the desired product (**B106**) as a colorless oil.

^{1}HNMR (300MHz, $CDCl_3$) δ 10.44 (s, 1H), 8.09 (d, *J* = 7.5, 2H), 7.56 (d, *J* = 2.4, 1H), 7.43 (t, *J* = 6.9, 2H), 7.32 (t, *J* = 8.4, 2H), 7.23 (t, *J* = 6.9, 2H), 6.73 (d, *J* = 2.1, 1H), 6.14

(d, J = 9.9, 1H), 5.65 (d, J = 9.6, 1H), 4.48 (t, J = 7.5, 2H), 3.03 (t, J = 7.5, 2H), 1.47 (s, 6H); ^{13}CNMR (75MHz, $CDCl_3$) δ 189.5, 155.4, 140.3, 133.0, 131.8, 131.0, 126.8, 125.9, 124.3, 123.1, 122.8, 121.5, 120.6, 119.2, 117.8, 108.8, 44.8, 34.3, 28.3; IR (NaCl, film) 2861, 1679 cm^{-1}; HRMS (+TOF) 382.1802 calcd for $C_{26}H_{23}NO_2$ $[M+H]^+$, found: 382.1799; R_f = 0.25 (9:1 hex./ EtOAc).
Ref. BJE6-106

Cell culture, siRNA transfection, plasmid stable transfection & PLX4032-resistant sub cell lines. Human melanoma cell lines WM1366, WM1361A, WM852, FM28, FM6, SKMEL2 and SKMEL28, SBcl2, A375 and SKMEL5 were originally obtained from ATCC or the European Searchable Tumour Line Database (ESTDAB) and authenticated by these agencies using DNA fingerprinting quality control. Their respective RAS or BRAF mutations previously identified in the literature were verified by sequencing. SBcl2 and A375 and its derivative lines were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. SKMEL5 was maintained in minimum essential medium (MEM) supplemented with 10% fetal bovine serum. All media were additionally supplemented with L-glutamine 2 mM, penicillin 100 units/ml and streptomycin 100 μg/ml. Primary human melanocytes were grown in medium 254 (Invitrogen).

siRNA transfection was performed by reverse transcription using Lipofectamine RNAiMax (Invitrogen) according to the product protocol, and media was changed the following day of transfection. PLX4032-resistant cell sublines were established according to the method described in [3]. Briefly, A375 and SKMEL5 cells were plated at low cell density and treated with PLX4032 at 1 μM or 0.5 μM, respectively. The concentration of PLX4032 was gradually increased up to 4μM (A375) or 2 μM (SKMEL5) over the course of a 3-4 week period, and clonal colonies were picked. Derived sublines of A375 and SKMEL5 were maintained in PLX4032-containing medium at 1-2 μM (A375) or 0.5 μM (SKMEL5).

Cell proliferation & Caspase assays. Cell proliferation assays (MTS assay) and caspase assays were performed with CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay kit and Caspase-Glo 3/7 Assay Systems (Promega) according to the manufacturers' protocols. Briefly, for the assays employing inhibitors, cells were plated in a 96-well plate (500-4000 cells per well depending on the cell lines and duration of the experiment), exposed to inhibitors 24 hr later and cultured for the durations indicated in the individual figure legends. For the assays employing siRNA, cells were plated the day of siRNA transfection, cultured for the duration indicated in the figure legends, and if indicated, treated with inhibitors or vehicle. After the indicated treatment times, assay reagent was added and cell plates were incubated for 1 hr at 37°C (MTS assay) or 30 min at RT (caspase assay). Absorbance at 490 nm (MTS assay) or luminescence (caspase assay) were measured using microplate readers for quantification. Human primary melanocytes were plated in triplicate on a 12-well plate. Test compounds at different concentrations or vehicle (DMSO) were added on the second day, and viable cells were enumerated 72 rh after addition of compounds. Cell survival was evaluated against the DMSO-treated controls.

Clonogenic colony assay. For the experiments depicted in Figure 2E, cells were treated with drugs for the time indicated in the figure, and then the same number of viable cells from each treatment was replated at low cell density and cultured in medium without inhibitors for 8 days, at which time colony formation was quantitated. Cell colonies were stained with ethidium bromide for visualization on an ImageQuant LAS 4000 (GE Healthcare) and colonies enumerated.

DNA fragmentation assays. Cells were harvested and fixed in 1 ml of a 35% ethanol/DMEM solution at 4°C for 30 min. Cells were then stained with a solution containing 25 μg/ml of propidium iodide/ml and 50 μg/ml of RNase A in PBS and incubated in the dark at 37°C for 30 min for flow cytometric analysis. The proportion of cells in the sub-G1 population, which contain a DNA content of less than 2N (fragmented DNA), was measured as an indicator of apoptosis.

Immunoblotting. Whole cell lysates were prepared in a buffer containing 20 mM Hepes (pH 7.4), 10% glycerol, 2 mM EDTA, 2 mM EGTA, 50 mM β-glycerophosphate and 1% Triton-X100, 1 mM dithiothreitol (DTT) and 1 mM sodium vanadate, supplemented with Halt Protease and Phosphatase Inhibitor Cocktail (100X) (Thermo Scientific). Lysates were subjected to SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked at RT for 1-1.5 hr with 5% BSA or 5% non-fat dry milk in TBS-T (10 mM Tris [pH 7.5], 100 mM NaCl, 0.1% Tween 20) and probed with the appropriate primary antibodies (1:500-1:10,000) overnight. After washing, the blots were incubated with horseradish peroxidase-conjugated secondary antibodies (1:2000-1:10,000) and visualized using the ECL system (GE Healthcare) on an ImageQuant LAS 4000. As loading controls, either α-tubulin, β-actin or GAPDH was selected, depending on the molecular weight of the proteins being studied in each experiment or the acrylamide percentage of the gels. In some experiments, photographs of the same gel processed from the same experiment, are presented in two separate panels. In some cases, lanes not directly relevant to the particular panel have been deleted for clarity, and this is indicated in the respective figure legends.

Quantitative real-time PCR. RNA was extracted with RNeasy Mini kit purchased (Qiagen) according to the manufacturer's protocol. 1 μg of RNA was used to synthesize cDNA in a 20 μl reaction volume employing SuperScript III First-Strand Synthesis System (Invitrogen) or QuaniTect Reverse Transcription Kit (Qiagen) according to the product protocol. Quantitative real-time PCR was performed with SYBR Green PCR Master Mix (Applied Biosystems (now under Life Technologies)) according to the manufacturer's protocol. Briefly, cDNA was diluted to a final concentration of 25 ng per reaction, added to a primer set (5 μM) and SYBR Green PCR Master Mix to a final volume of reaction mixture of 20 μl, and run on an Applied Biosystems 7500 Fast Real-Time PCR system using the following thermal cycling protocol: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 sec and 60°C for 1 min. The relative amount of an mRNA of interest was calculated by normalizing the Ct value of the mRNA to the Ct of the internal control (β-actin). Primer sequences were: H2AX Forward: 5'-CAACAAGAAGACGCGAATCA-3', H2AX Reverse: 5'-CGGGCCCTCTTAGTACTCCT-3', β-actin Forward: 5'-

GCTCGTCGTCGACAACGGCTC-3', β-actin Reverse: 5'-CAAACATGATCTGGGTCATCTTCTC-3'

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