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Supporting Information

A small molecule inhibitor of TopBP1 exerts anti-MYC activity and synergy with PARP inhibitors

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Materials and Methods

Cell Culture, Transfection and shRNA Expression Vectors

MDA-MB-468, BT549, MDAH-2774, TOV-112D, A2780cis, HEK293T and AML12 (alpha mouse liver 12) mouse hepatocytes were maintained in DMEM with 10% fetal bovine serum (FBS). MCF10A was maintained in DMEM/F12 with 5% horse serum, 2.5 mM glutamine, 0.5 μg/ml hydrocortisone, 10 μg/ml insulin, 100 ng/ml cholera toxin and 20 ng/ml EGF. OVCAR8, H2170, HCC95 and HCC2814 were maintained in RPMI with 10% FBS. A2780cis cell line (1) (ECACC 93112517) was purchased from PHE-Culture collections through Sigma. AML12 cells are hepatocytes isolated from the normal liver of a 3-month-old mouse and were obtained through ATCC (CRL-2254). HCC95 and HCC2814 cell lines were purchased from UT Southwestern. All other cell lines were purchased from ATCC. The validated pLKO.1 lentiviral expression vectors of MIZ1 shRNA (#1:TRCN0000012954 and #2:TRCN0000012955) were purchased from Sigma. The pLKO.1-Scrambled vector was purchased from Addgene (Plasmid #1864). The pLKO-shTopBP1 expression vectors were purchased from RNAi Consortium. The lentivirus harboring TopBP1 shRNA, MIZ1 shRNA or scrambled shRNA was produced in Lenti-X^{TM} HEK293T (Takara), and was then transduced into MDA-MB-468, MDAH-2774 or H2170. After selection with puromycin (3 μg/ml) for a week, stable cell lines were established for further experiments.

In Vitro Peptide Binding

In vitro peptide binding was performed as previously described (2). Briefly, GST-TopBP1-BRCT7/8 in *Escherichia coli* strain BL21(DE3)pLysS was induced by 0.1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) and purified according to the standard protocol. The GST tag of GST-TopBP1-BRCT7/8 was removed by PreScission protease (Pharmacia). Two purified (> 98%)

biotinylated peptides that contain TopBP1-S1159 residue, including pP, Btn-REERARLApSer^{1159}NLQWPS and nP, Btn-REERARLASNLQWPS, were synthesized by Sigma Genosys. Purified TopBP1-BRCT7/8 was incubated with compound 2H3 or 5D4 in NETN-A buffer (50 mM NaCl, 1 mM EDTA (pH 8.0), 20 mM Tris-HCl (pH 8.0) and 0.5% NP-40) at 4°C for 2 h, followed by the addition of purified pP or nP peptide at 4°C overnight. The biotinylated peptides were then pulled down with streptavidin-Sepharose (Amersham). The beads were washed four times with NETN-B buffer (100 mM NaCl, 1 mM EDTA (pH 8.0), 20 mM Tris-HCl (pH 8.0) and 0.5% NP-40) and then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting using an anti-TopBP1 rabbit antibody (1:1000, BL893, Bethyl Laboratories).

GST Pulldown Assay

Purified TopBP1-BRCT7/8 was incubated with compound 5D4 in NETN-A buffer at 4°C for 1.5 h, followed by the addition of 1 μg GST-mutp53-R273H(DBD) (3), GST-p53(DBD) or GST at 4°C for another 7 h. GST, GST-mutp53-R273H(DBD) or GST-p53(DBD) beads were washed four times with NETN-B buffer and then subjected to SDS-PAGE analysis to detect the associated TopBP1-BRCT7/8 using a rabbit antibody against the C-terminus of TopBP1 (Bethyl).

Cell Viability, Clonogenic Survival, and Caspase-3/7 Activity Assays

Cell viability was determined by MTT assay as described before (4). Alternatively, viable cells were either determined by trypan blue exclusion assay and counted using Countess II Automated Cell Counter (ThermoFisher), or determined by cell counting kit-8 (CCK-8) assay according to the manufactural instruction (APExBIO). Ten μl of WST-8 solution was added to the cells for one

or two hours at 37 ^{o}C, and the amount of WST-8 formazan generated by the activity of dehydrogenases in the living cells was measured by reading the absorbance at OD 450 nm using a microplate reader (BioTek). Clonogenic survival assay was performed in MDA-MB-468 or TOV-112D cells. Cells were treated with 2H3, 5H3 or 5D4 at various concentrations for 24 h. After PBS washing, viable cells were cultured in fresh medium for another four or five days, followed by fixation with 3% formaldehyde and staining with 0.5% crystal violet in 25% methanol. To measure the proliferation rate of tumor spheres, equal amounts of tumor cells (TOV-112D, MDA-MB-468, BT549 or T47D) were seeded on 6-well plates with cell-repellent surface (Greiner-Bio-One). When the diameter of tumor spheres reached 30-50 μm, DMSO vehicle or 5D4 at various concentrations was added every 2-3 days. The images of tumor spheres were captured 24 h after treatment, and the diameter of 40-60 spheres was measured. To determine the viability of PDX tumor spheres, breast cancer PDX tissues were trypsinized and cells were cultured in Complete DMEM/F12 Media for Patient-Derived *In Vitro* and Organoid Cultures as described in NCI Patient-Derived Models Repository (SOP30101). After culturing for ten days, tumor spheres were aliquoted on 48-well plates and treated with DMSO vehicle or 5 μM 5D4 every other day for 6 days, followed by CCK-8 assay to determine the viability of tumor spheres. Apoptosis was determined by the Caspase-Glo® 3/7 activity assay (Promega), which measures the caspase-3/7 activity by cleavage of the luminogenic substrate containing the DEVD sequence, and was normalized to protein concentrations. To investigate the proapoptotic effect of 5D4 on transiently transfected cells, H1299 cells stably expressing mutp53(R273H) were transfected with a pSUPER vector harboring a scrambled shRNA or a TopBP1 shRNA. After 48 h, cells were treated with 5 μM 5D4 for 21 h, followed by caspase-3/7 activity assay.

Immunoblotting, Co-immunoprecipitation, and Immunostaining

Immunoblotting was performed using an antibody specific to p62, Cyclin D1, Cyclin A2, c-Myc, Rad51, HSP90 (Cell Signaling), $p21^{Cip1}$, ACTL6A or GAPDH (Santa Cruz Biotechnology). To detect the effect of 5D4 on the interactions of TopBP1 with its binding partners, MDA-MB-468 cells treated with 2 μM 5D4 or MDAH-2774 cells treated with 3 μM 5D4 for 20 h were harvested in a buffer containing 0.1% Triton-X, 150 mM NaCl, 10% glycerol, 1 mM EDTA and 1 mM EGTA. Endogenous TopBP1 was immunoprecipitated with an anti-TopBP1 mouse monoclonal antibody (Santa Cruz Biotechnology) or control mouse IgG, and was resolved by SDS-PAGE. The co-immunoprecipitated proteins were detected by immunoblotting using a rabbit antibody specific to MIZ1, CIP2A, PLK1, p53, Rad9, RPA2 (Cell Signaling) or Treslin (Bethyl). The immunoblot was stripped and reprobed with an anti-TopBP1 rabbit antibody (Bethyl). Co-immunoprecipitation of BRCA1 with BACH1 was performed in MDA-MB-468 cells after treatment with 2 μM 5D4 or vehicle DMSO for 20 h. Endogenous BRCA1 was immunoprecipitated with an anti-BRCA1 mouse monoclonal antibody (Calbiochem) or control mouse IgG, followed by immunoblotting using a rabbit antibody specific to BACH1 (Cell Signaling). Co-immunoprecipitation of TopBP1 with E2F1 or TopBP1-BRCT7/8 was performed as described before (2). Immunostaining was performed to detect Rad51 foci formation. MDAH-2774 cells were treated with 2 μM CalAM, 4 μM 5D4, 10 μM rucaparib or a combination of rucaparib with CalAM or 5D4 for 16 h. Cells were then fixed with 3% formaldehyde, followed by permeabilization with 0.2% Triton-X and blocking with 2% bovine serum albumin in PBS. Endogenous Rad51 was detected using an anti-Rad51 rabbit antibody (Cell Signaling) followed by the Texas Red X-conjugated rabbit secondary antibody (Invitrogen). Nuclei were stained with Hoechst 33258 (Sigma).

RNA-seq

Total RNA was extracted using Trizol method. Eukaryotic RNA-seq including library preparation and sequencing with 20 million raw reads per sample using Illumina Platform paired-end 150 bp (PE150) was performed at Novogene Co., Ltd. (CA, USA). To map reads to the genome, reference genome and gene model annotation files were downloaded from genome website browser (NCBI/UCSC/Ensembl) directly. Indexes of the reference genome was built using STAR and paired-end clean reads were aligned to the reference genome using STAR (v2.5). STAR uses the method of Maximal Mappable Prefix (MMP) which can generate a precise mapping result for junction reads. To quantify the levels of gene expression, HTSeq v0.6.1 was used to count the read numbers mapped of each gene. And then FPKM (Fragments Per Kilobase of transcript per Million mapped reads) of each gene was calculated based on the length of the gene and reads count mapped to this gene (5). FPKM considers the effect of sequencing depth and gene length for the reads count at the same time.

Chromatin-Immunoprecipitation (ChIP)

Treated cells were fixed, harvested, and nuclei were isolated as previously described (6). Nuclei were suspended in shearing buffer (0.1 mM EDTA, pH 8.0, 0.1 mM EGTA, 10 mM Tris-HCl, pH 6.8, 100 mM NaCl, 0.1% sodium deoxycholate, 0.5% N-lauroylsarcosine, protease inhibitors) and sonicated until an average fragment size of 800 bp was reached. Chromatin concentrations were calculated based on processed aliquots, and equal chromatin amounts were used in subsequent immunoprecipitation. All chromatin immunoprecipitations, washes, and downstream processing were performed as described previously (6). IP samples were incubated with 3 μg MIZ1 antibody (Santa Cruz, sc-136985) or 3 μg control mouse IgG antibody (ThermoFisher), and precipitated

with protein G magnetic dynabeads (Invitrogen). Samples were analyzed via qPCR, with ChIP primer sequences as indicated below. Primer sequence for Gene Desert was designed by Active Motif.

ChIP Primer Pairs for Gene Promoter Regions (5' to 3')

	Forward Primer	Reverse Primer
p21	GTGGCTCTGATTGGCTTTCTG	CTGAAAACAGGCAGCCCAAG
Gene Desert	TGAGCATTCCAGTGATTTATTG	AAGCAGGTAAAGGTCCATATTTC
β-actin	CACCAACTGGGACGACAT	ACAGCCTGGATAGCAACG

***In Vivo* Xenograft Experiments**

Freshly grown MDA-MB-468 breast cancer cells (5 million cells per site in 100 μl PBS or MDAH-2774 ovarian cancer cells (2 million cells per site in 100 μl PBS) were injected subcutaneously into the right side of the flank of the 5- to 6-week-old NOD scid IL2 receptor γ chain knockout (NSG) female mice. For patient-derived xenograft (PDX) experiment, breast cancer PDXs BCM2665 and BCM3107 (from BCM PDX Core) were implanted into the mammary fat pads of female NSG mice. When tumors were measureable, the mice were randomly divided into treatment and control groups. 5D4 was dissolved in DMSO and given intraperitoneally at 40 mg/kg, once every three days for three doses (in MDA-MB-468 and MDAH-2774 xenograft experiments) or twice weekly for three or four weeks (in BCM2665 and BCM3107 PDX experiments). Talazoparib was dissolved in 10% Dimethylacetamide (DMAc)/4% DMSO/ PBS, and given intraperitoneally at 0.5 mg/kg, daily x 5 per week (7). The control group mice were injected with vehicle. The mice were monitored twice a week. The mouse weight and tumor size were measured on the indicated day with a caliper and calculated based on the formula π/6 (length x depth x width). The evaluator

was blinded to the group allocation during monitoring. Animals were euthanized on the indicated dates, and tumors were harvested, weighed and further processed for histopathological analysis. All experiments were performed under a Baylor College of Medicine Institutional Animal Care and Use Committee (IACUC)-approved protocol and all experiments confirm to IACUC standards and ethical regulations.

Histology and Immunohistochemistry

PDX tumors and mouse small intestines were placed in cassettes and fixed in 10% neutral buffered formalin. Next day the samples were sent to The Pathology and Histology Core of Baylor College of Medicine for further processing. The paraffin-embedded sections were stained with H&E, Ki-67, or an anti-cleaved PARP1 antibody using standard operating protocols.

Cellular Thermal Shift Assay (CETSA)

CETSA was performed following the published protocol (8). MDA-MB-468 cells were treated with 10 μM 5D4 at 37 °C for 2 h. After washing with PBS three times, cells were resuspended in PBS supplemented with protease inhibitor cocktail. The cells were then aliquoted and heated individually at different temperatures ranging from 42-48.4 °C for 3 min, followed by cooling down for 3 min at room temperature. The cell suspensions were freeze-thawed three times using liquid nitrogen. After centrifugation at 20,000 x g for 20 min at 4 °C to remove the cell debris, the supernatants were transferred to new microtubes and analyzed by SDS-PAGE followed by immunoblotting. For xenograft experiments, mice were injected with the last dose of 5D4 or vehicle control one day before euthanization and tumor harvesting. The frozen tumors were thawed on ice, and homogenized in cold PBS followed by three cycles of freeze-thawing using liquid

nitrogen. Tissue lysates were separated from the cellular debris by centrifugation at 20,000 x g for 20 min at 4 °C. The supernatants were diluted with PBS supplemented with protease inhibitor cocktail, aliquoted, and then heated at different temperatures ranging from 42-47.6 °C for 3 min, followed by cooling down for 3 min at room temperature. Soluble fractions were isolated by centrifugation and analyzed as mentioned above.

Combination Index Analysis

To determine and quantify a dose-effect relationship between TopBP1i and PARPi, cells were treated with different concentrations of TopBP1i, PARPi or both at a constant concentration ratio between TopBP1i and PARPi. The combinatorial effect was evaluated using the combination index method as previously described (9, 10). The mode of interaction (synergy, antagonism, or additivity) was determined using CompuSyn software program (CompuSyn, Inc., Paramus, NJ). Combination index (CI) is a quantitative measurement of drug interaction (CI = 1, additive effects), (CI < 1, synergy), (CI > 1, antagonism).

Statistical Analysis

We ran linear mixed model analysis in JASP version 0.17.2.1. We performed two-tailed *t* test for comparisons of treatment groups. Data were expressed as mean ± SD from at least three biological replicates, or mean ± SEM (or SD) from at least three independent experiments. *P* values less than 0.05 were considered statistically significant. RNA-Seq data in TCGA database were extracted from cBioPortal server (https://www.cbioportal.org/) and GEPIA2 server (http://gepia2.cancer-pku.cn/#index). The expression of 200 MYC target genes in gene set Hallmark_MYC_targets_v1 was used to correlate with TopBP1 expression in TCGA database. Pearson correlation coefficients

were calculated to evaluate correlations. Gene set enrichment analysis (GSEA) was performed using GSEA software.

Supplementary References:

1. Behrens BC*, et al.* (1987) Characterization of a cis-diamminedichloroplatinum(II)-resistant human ovarian cancer cell line and its use in evaluation of platinum analogues. *Cancer Res* 47(2):414-418.
2. Chowdhury P*, et al.* (2014) Targeting TopBP1 at a convergent point of multiple oncogenic pathways for cancer therapy. *Nat Commun* 5:5476.
3. Liu K, Lin FT, Graves JD, Lee YJ, & Lin WC (2017) Mutant p53 perturbs DNA replication checkpoint control through TopBP1 and Treslin. *Proc Natl Acad Sci U S A* 114(19):E3766-E3775.
4. Lee YJ*, et al.* (2019) CGRRF1, a growth suppressor, regulates EGFR ubiquitination in breast cancer. *Breast Cancer Res* 21(1):134.
5. Mortazavi A, Williams BA, McCue K, Schaeffer L, & Wold B (2008) Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat Methods* 5(7):621-628.
6. Paik JC, Wang B, Liu K, Lue JK, & Lin WC (2010) Regulation of E2F1-induced apoptosis by the nucleolar protein RRP1B. *J Biol Chem* 285(9):6348-6363.
7. Hsu EC*, et al.* (2020) Trop2 is a driver of metastatic prostate cancer with neuroendocrine phenotype via PARP1. *Proc Natl Acad Sci U S A* 117(4):2032-2042.
8. Jafari R*, et al.* (2014) The cellular thermal shift assay for evaluating drug target interactions in cells. *Nat Protoc* 9(9):2100-2122.
9. Chou TC & Talalay P (1984) Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Regul* 22:27-55.
10. Zhang N, Fu JN, & Chou TC (2016) Synergistic combination of microtubule targeting anticancer fludelone with cytoprotective panaxytriol derived from panax ginseng against MX-1 cells in vitro: experimental design and data analysis using the combination index method. *Am J Cancer Res* 6(1):97-104.

Supporting Information Dataset Legends

Supporting Information Dataset 1: ZINC IDs and docking scores of initial hits.

(See separate SI Dataset 1 excel file.)

Supporting Information Dataset 2: 2H3 analogs FAFDrug3 toxicity profile.

(See separate SI Dataset 2 excel file.)

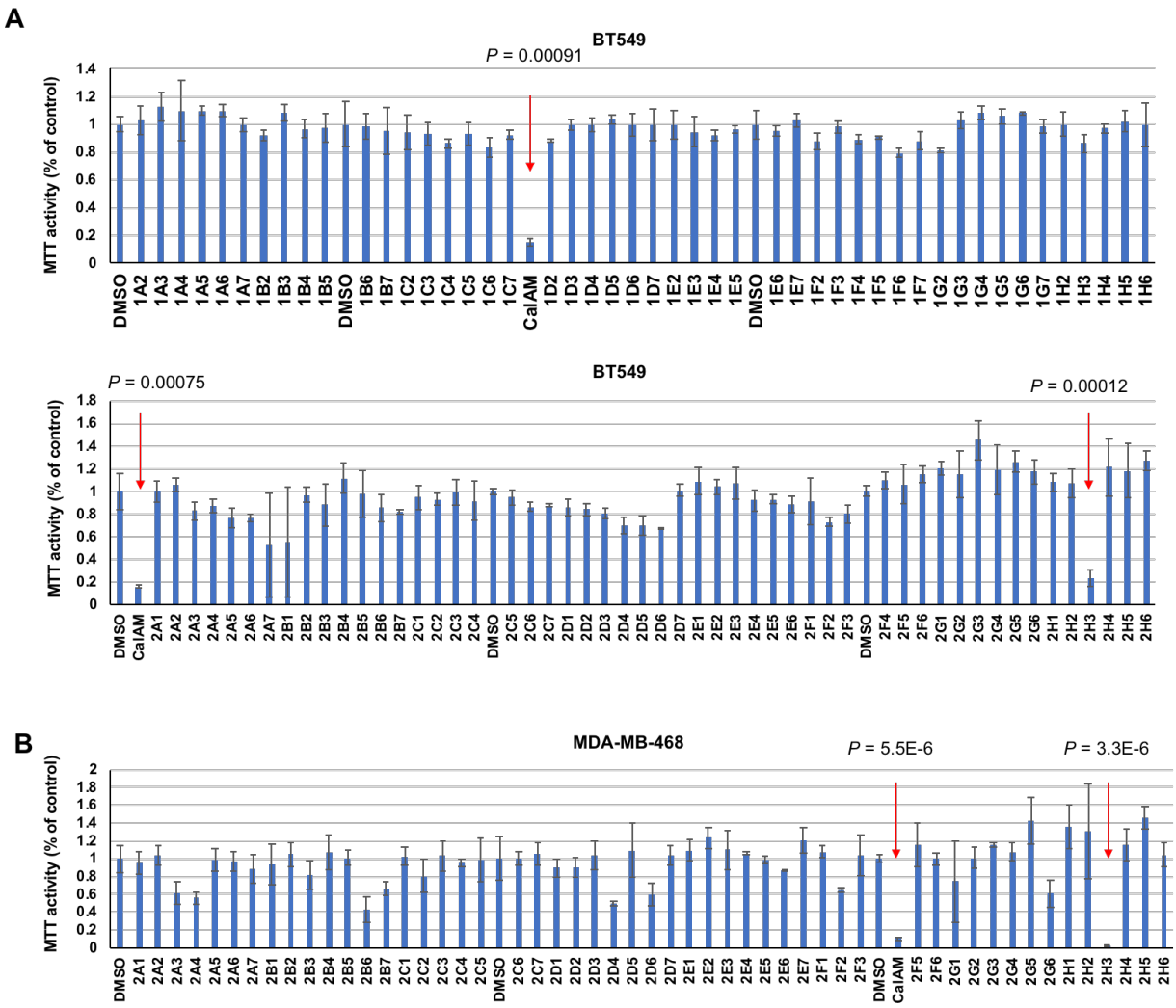
Supporting Information Dataset 3: Summary of 2H3 analogs SAR study.

(See separate SI Dataset 3 excel file.)

Supplementary Table S1: Calculated parameters of Lipinski's rule of five for the compounds.

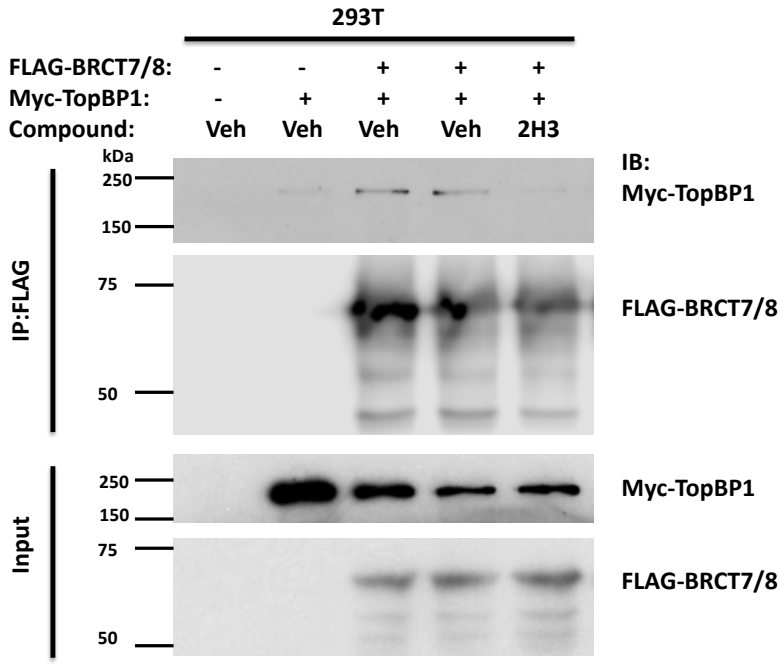
Calculated parameters of Lipinski's rule of five for the compounds

Parameter	reference value	Calcein AM	2H3	5D4	5H3
H bond donors	<= 5	0	1	2	2
H bond acceptors	<= 10	25	5	5	4
MW	< 500	994.857	426.564	467.637	494.663
log P	<= 5	3.49	4.801	2.773	3.497
number of atoms	20-70	117	64	69	72
polar surface area	<= 140 Å2	305 Å2	46.23 Å2	44 Å2	54.6 Å2
rotatable bonds	<= 10	32	6	7	6
#Rule of 5 violations		2	0	0	0



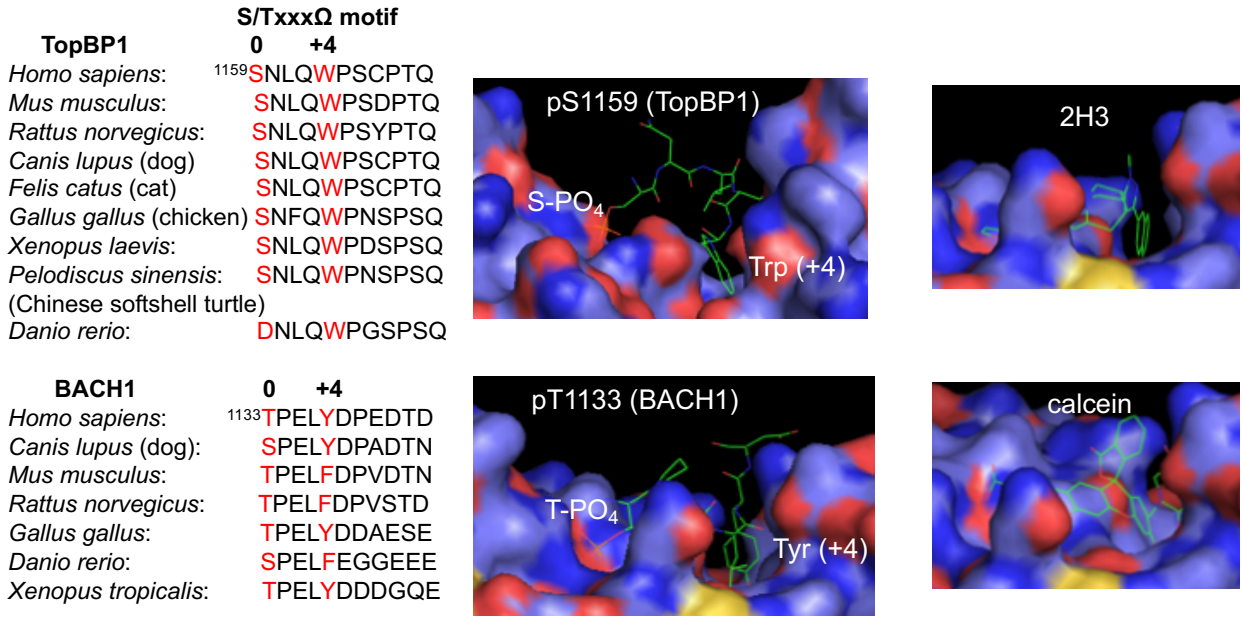
Supplementary Figure S1. Compound screening identifies Cpd 2H3 as a lead compound that exhibits anti-proliferative activity in breast cancer cells.

BT549 (**A**) or MDA-MB-468 (**B**) cells were treated with vehicle DMSO, CalAM (5 μM) or one of the compounds (20 μM) for 48 h. Cell viability was determined by MTT assay. Data shown are the mean ± SD done in triplicates. Arrows indicate the *P* value versus vehicle DMSO.



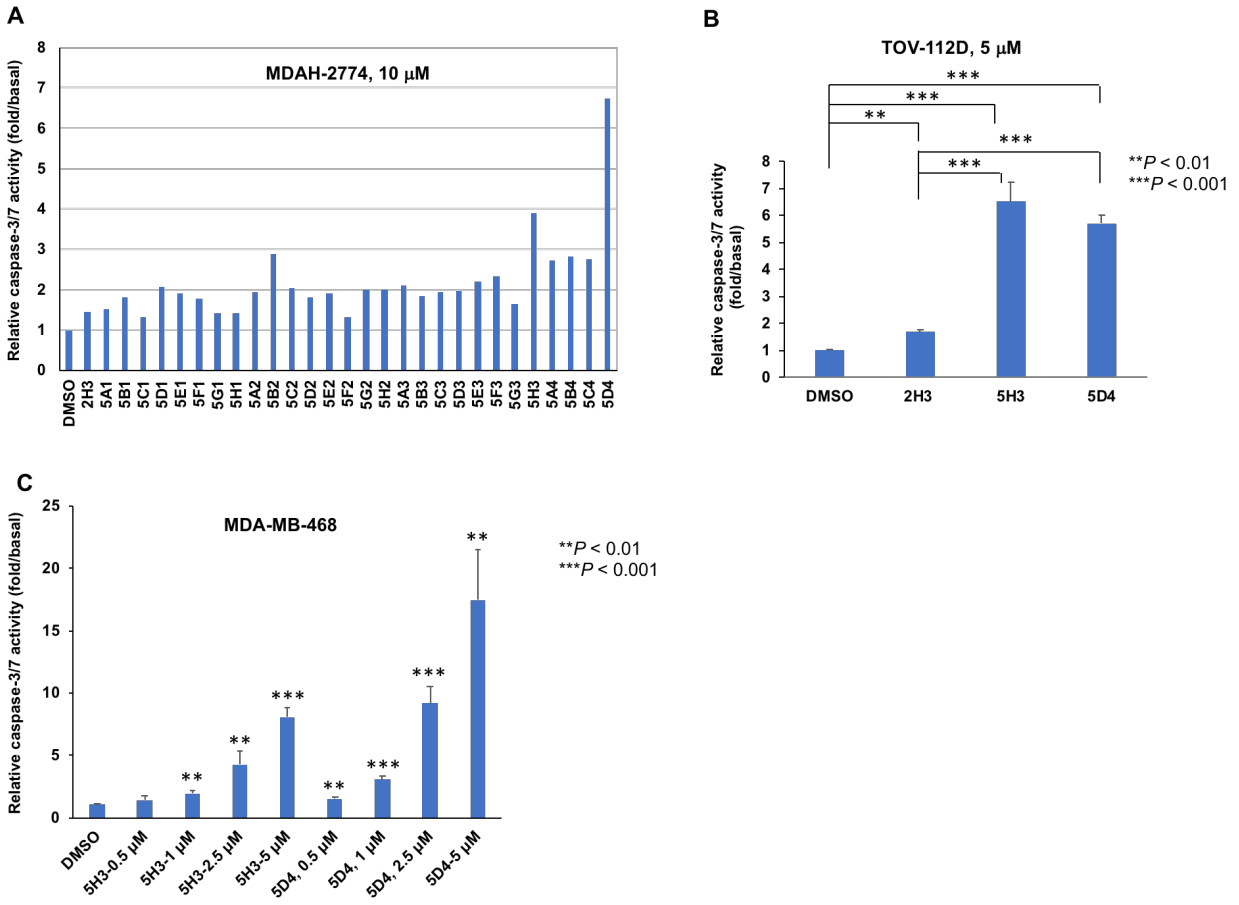
Supplementary Figure S2. Cpd 2H3 blocks the self-association of TopBP1 with TopBP1-BRCT7/8 domains.

HEK293T cells were transiently transfected with the expression vector(s) of FLAG-TopBP1-BRCT7/8, Myc-TopBP1 or both, followed by treatment with 2H3 (10 μM) or DMSO vehicle control (Veh) for 8 h. Cell lysates were harvested and FLAG-BRCT7/8 was immunoprecipitated with anti-FLAG mouse monoclonal antibody-conjugated agarose, followed by immunoblotting using an anti-Myc rabbit antibody to detect the co-immunoprecipitated Myc-TopBP1. The blot was stripped and reprobed with an anti-FLAG rabbit antibody to detect the immunoprecipitated FLAG-TopBP1-BRCT7/8. The bottom two panels show the expression of Myc-TopBP1 and FLAG-BRCT7/8, respectively, in the whole cell lysates.



Supplementary Figure S3. Structure of TopBP1-BRCT7/8 binding pocket with pS/TxxxΩ phosphopeptides or lead compounds.

Shown in the left panel are conservative phosphopeptides containing the pS/TxxxΩ motif in TopBP1 and BACH1.

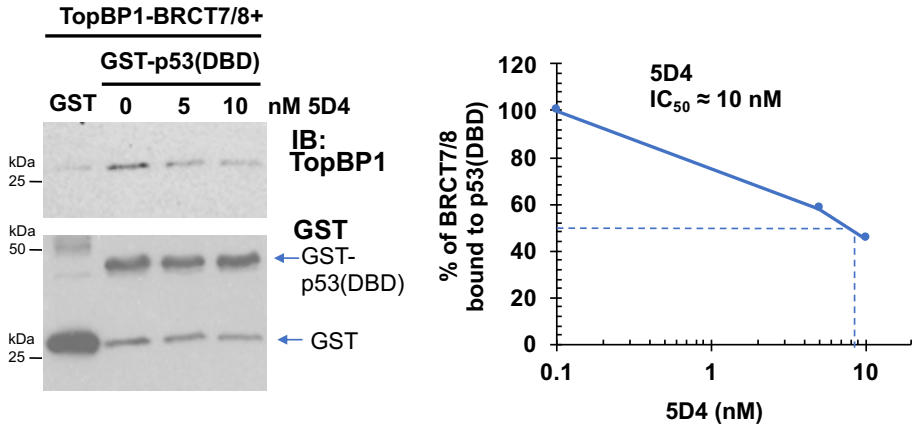


Supplementary Figure S4. Compound screening of 2H3 analogs identifies 5D4 and 5H3 as more potent compounds than 2H3 in the induction of apoptosis in breast and ovarian cancer cells.

A. MDAH-2774 cells were treated with vehicle DMSO or one of the 2H3 analogs (10 μM) for 20 h. Active caspase-3/7 was determined by Caspase-Glo® 3/7 Assay and was normalized to protein concentrations.

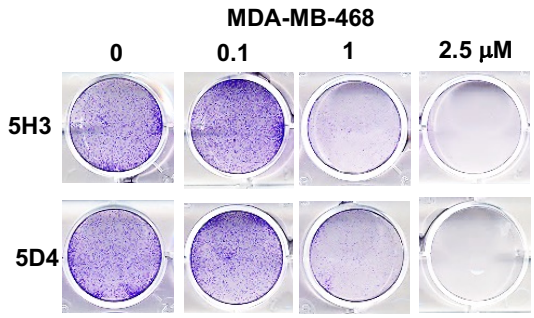
B. TOV-112D cells were treated with vehicle DMSO or 5 μM 2H3, 5H3 or 5D4 for 16 h. Active caspase-3/7 was determined by Caspase-Glo® 3/7 Assay and was normalized to protein concentrations. Data shown are the mean ± SD from four independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$ versus vehicle DMSO (two tailed *t* test).

C. MDA-MB-468 cells were treated with vehicle DMSO or increasing concentrations of 5H3 or 5D4 for 18 h. Active caspase-3/7 was determined by Caspase-Glo® 3/7 Assay and was normalized to protein concentrations. Data shown are the mean ± SD done in triplicates. $^{**}P < 0.01$, $^{***}P < 0.001$ versus vehicle DMSO (two tailed *t* test).



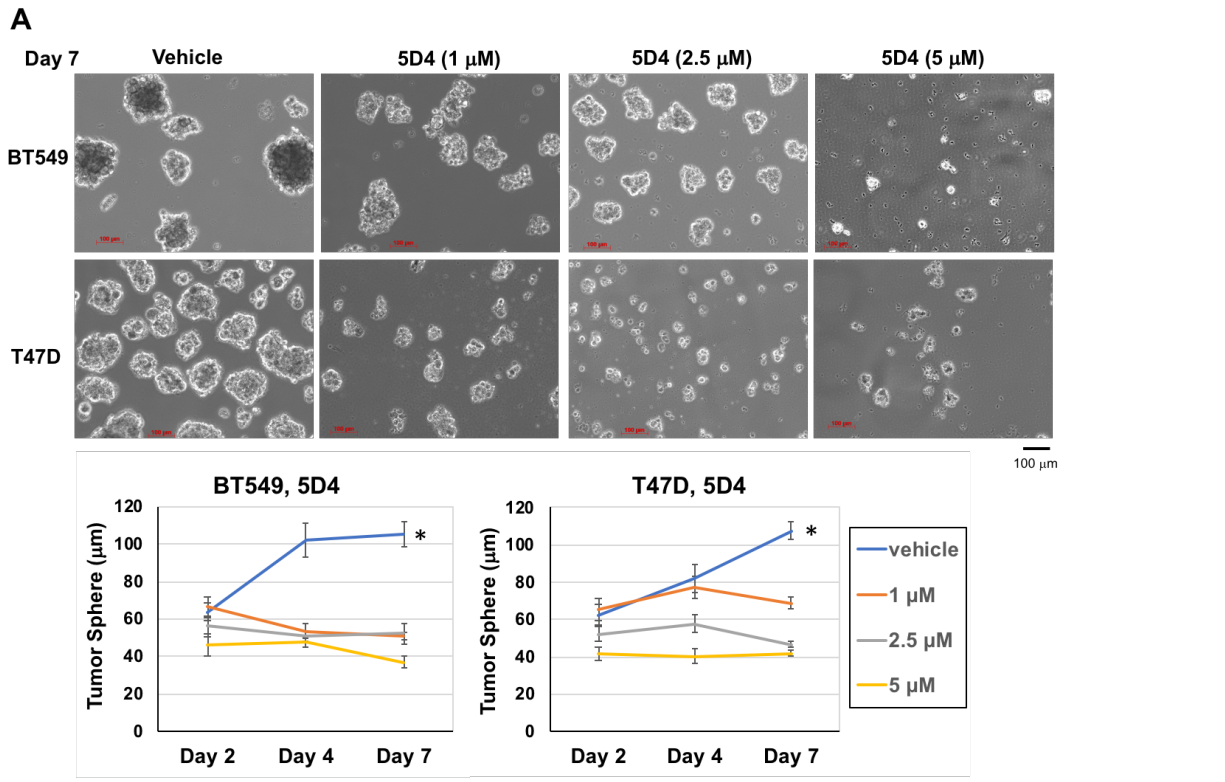
Supplementary Figure S5. Cpd 5D4 inhibits the TopBP1-BRCT7/8 binding to the DBD of wild-type p53.

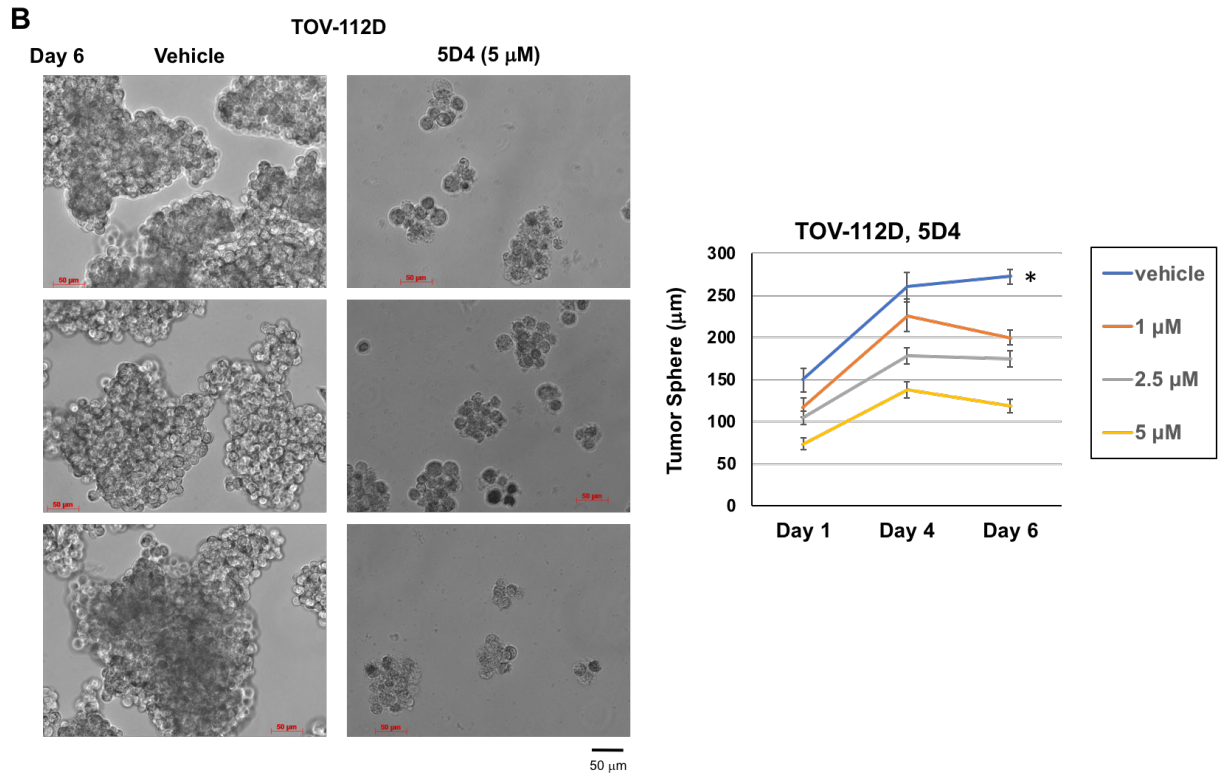
Purified TopBP1-BRCT7/8 was incubated with GST-p53(DBD) in the presence of various concentrations of 5D4, and GST pulldown assay was performed. The pulldown of TopBP1-BRCT7/8 was detected by immunoblotting using an anti-TopBP1 antibody against the C-terminus of TopBP1.



Supplementary Figure S6. Both 5H3 and 5D4 inhibits clonogenic survival of MDA-MB-468 cells.

Equal amounts of cells were treated with vehicle DMSO or different concentrations of 5H3 or 5D4 for 24 h. After washing with PBS, cells were cultured in fresh growth medium for another five days and then fixed with 3% formaldehyde. Viable cells were stained with 0.5% crystal violet. Shown are representative images (n = 2).


A
Day 7
Vehicle
5D4 (1 μM)
5D4 (2.5 μM)
5D4 (5 μM)
BT549
T47D
100 μm
BT549, 5D4
T47D, 5D4
Tumor Sphere (μm)
120
100
80
60
40
20
0
Day 2
Day 4
Day 7
*
vehicle
1 μM
2.5 μM
5 μM


B
TOV-112D
Day 6
Vehicle
5D4 (5 μM)
50 μm
TOV-112D, 5D4
Tumor Sphere (μm)
300
250
200
150
100
50
0
Day 1
Day 4
Day 6
*
vehicle
1 μM
2.5 μM
5 μM

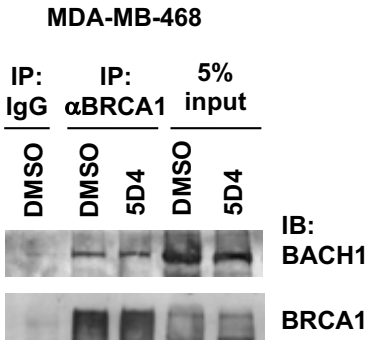
Supplementary Figure S7. Treatment with Cpd 5D4 inhibits the growth of breast and ovarian cancer tumor spheres.

Equal amounts of breast cancer cells (BT549 or T47D) (A) or TOV-112D ovarian cancer cells (B) were seeded on 6-well plates with cell-repellent surface. When the diameter of tumor spheres reached 30-50 μm, spheres were treated with vehicle DMSO or different concentrations of 5D4 on day 0, 3 and 6 (A) or day 0, 3 and 5 (B). The images were captured 24 h after each treatment, and the diameter of tumor spheres was measured. Data shown are the mean ± SEM of 40-60 spheres (*$P < 0.05$ versus 5D4-treated cells). The images shown were captured either on day 7 or 6. Scale bars are 100 μm in (A) and 50 μm in (B).

A

BRCT domain	PDB ID	docking score
TopBP1-BRCT7/8	3AL3	-9.2
TopBP1-BRCT0/1/2	6HM5	-6.4
TopBP1-BRCT4/5	3UEN	-5.7
TopBP1-BRCT6	3JVE	-5.8
BRCA1 tandem BRCT	1L0B	-6.3
MCPH1-BRCT2/3	3U3Z	-5.8
MDC1 tandem BRCT	2ETX	-5.2
BARD1 tandem BRCT	3FA2	-6.4
PTIP tandem BRCT	3SQD	-5.2
XRCC1 tandem BRCT	6WH2	-6
Lig IV tandem BRCT	3II6	-6.1
53BP1 tandem BRCT	5ECG	-6.1
ECT2 tandem BRCT	6L30	-5.5

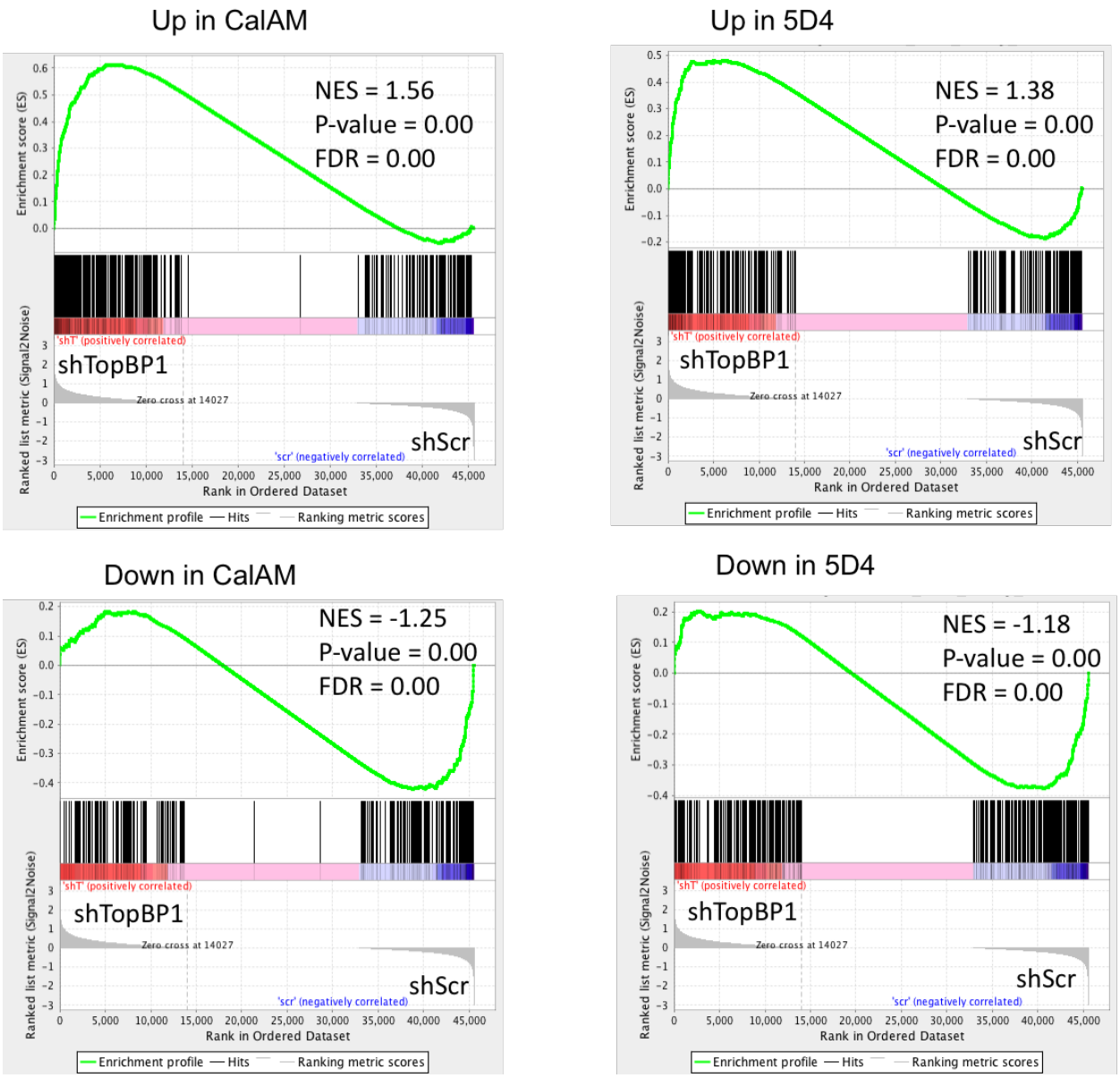
B



Supplementary Figure S8.

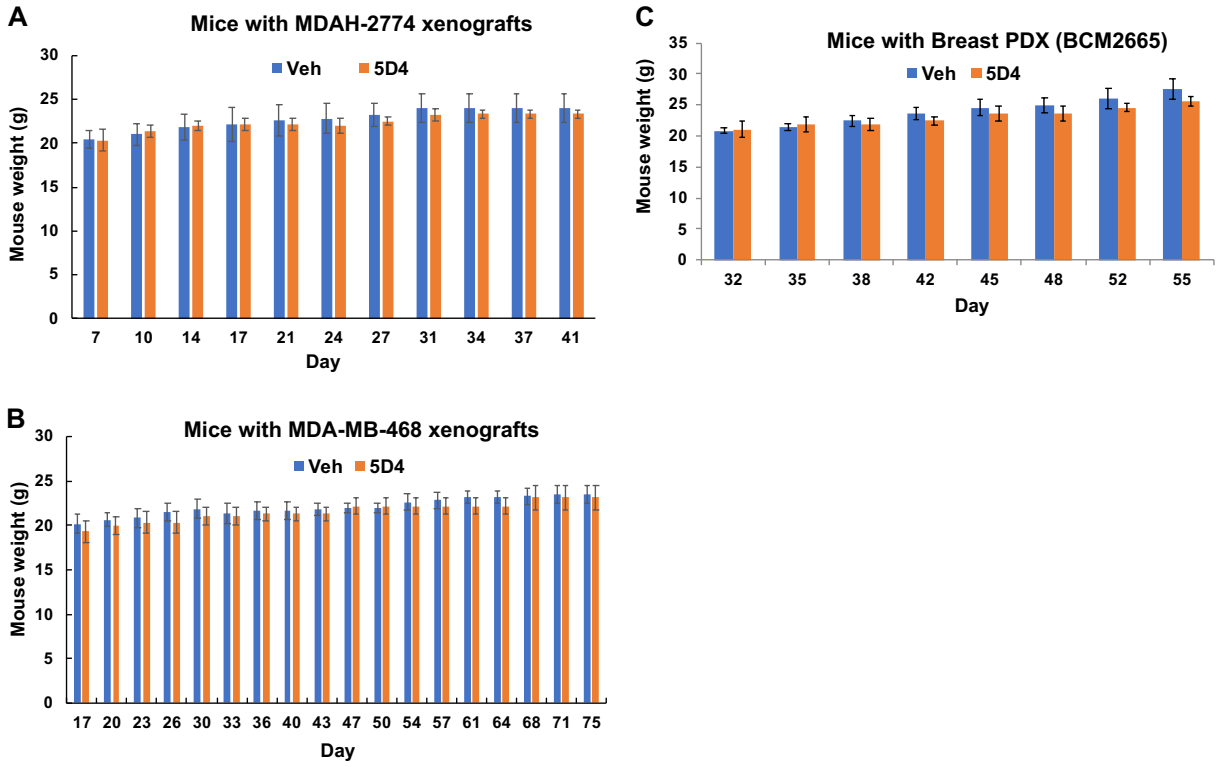
A. Docking scores of 5D4 among different tandem BRCT domains.

B. Cpd 5D4 treatment does not affect the interaction of BRCA1 with BACH1. MDA-MB-468 cells were treated with vehicle DMSO or 2 μM 5D4 for 20 h. Endogenous BRCA1 was immunoprecipitated with an anti-BRCA1 mouse monoclonal antibody or control mouse IgG, followed by immunoblotting using an anti-BACH1 rabbit antibody. The immunoblot was reprobed with an anti-BRCA1 rabbit antibody.



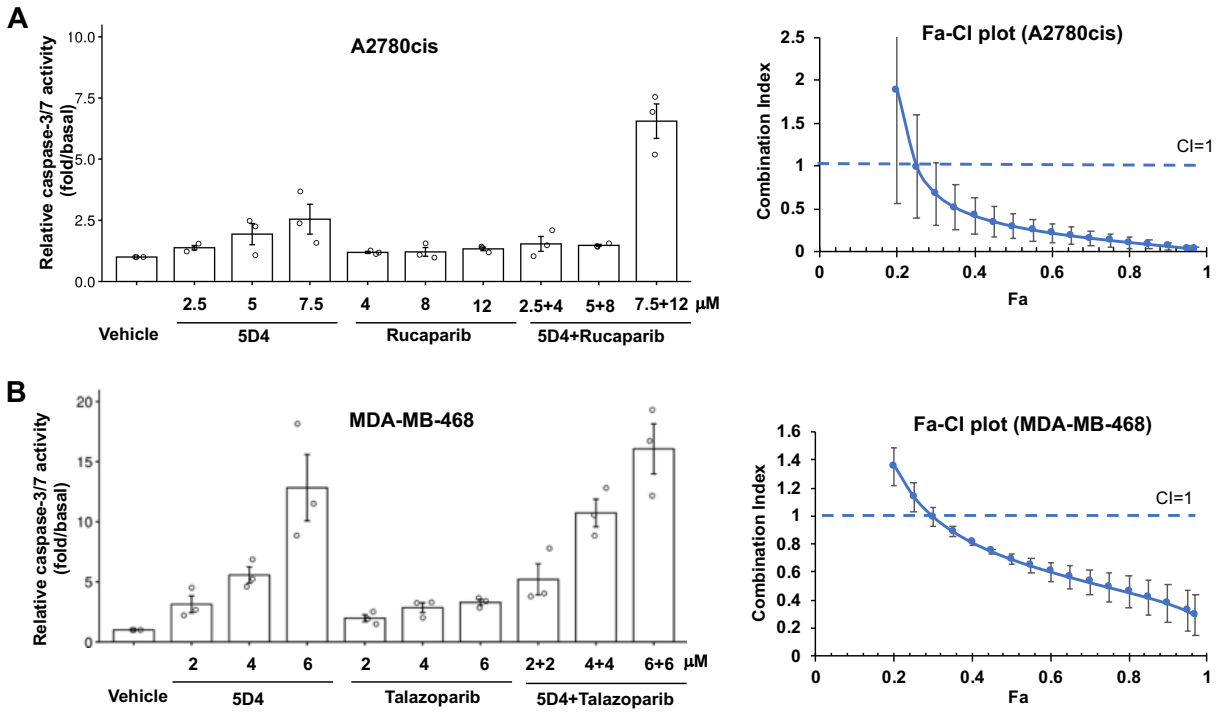
Supplementary Figure S9. TopBP1 depletion and treatment with CalAM or 5D4 share significant similarity in changes of gene expression in MDA-MB-468 cells.

Shown are GSEA from RNA-seq data of MDA-MB-468 cells treated with CalAM, 5D4 or depleted of TopBP1. NES: normalized enrichment score; FDR: false discovery rate.



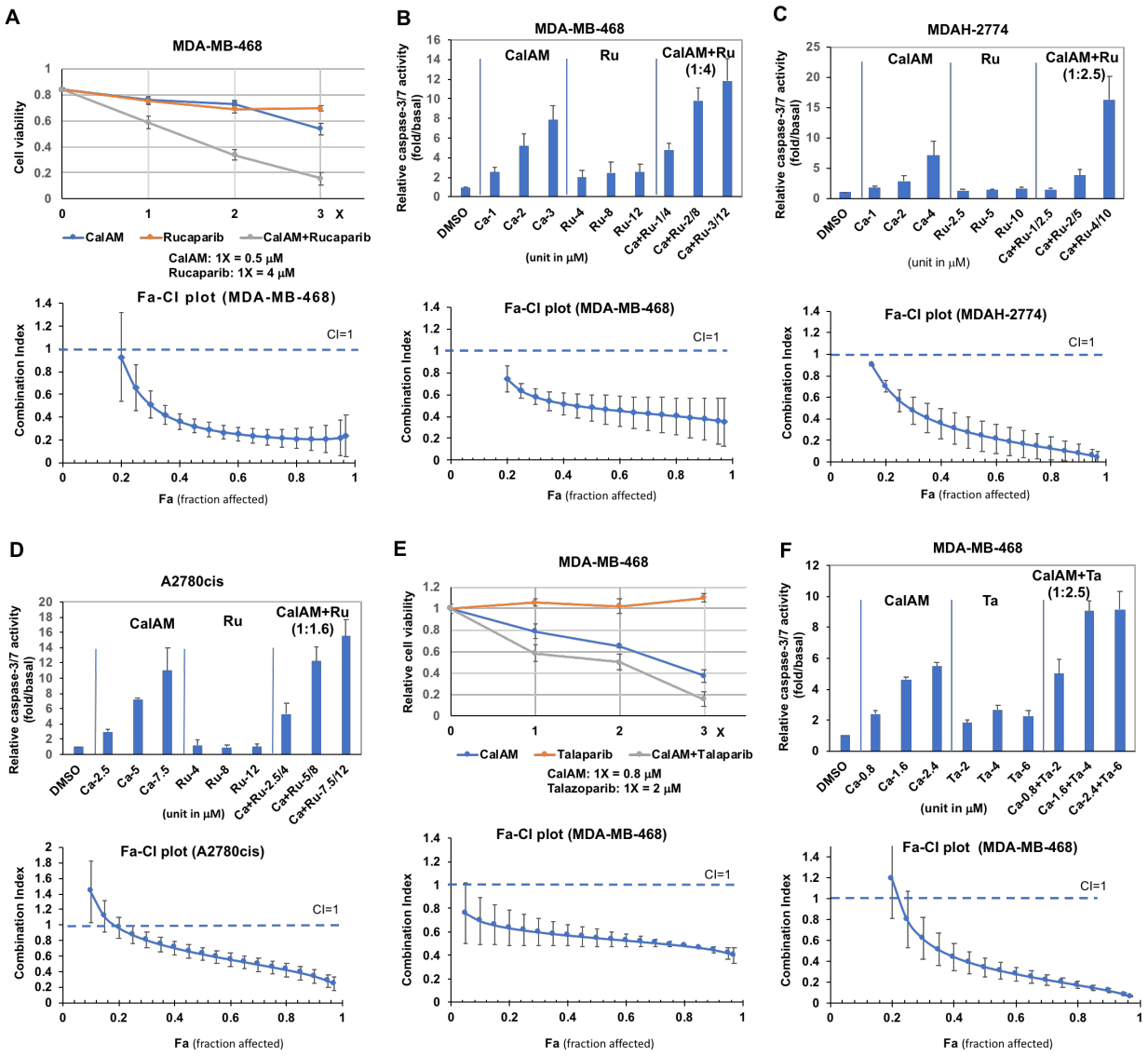
Supplementary Figure S10. Cpd 5D4 treatment does not significantly affect mouse body weight.

Shown are the mean mouse body weights ± SD of the xenograft-bearing mice treated with vehicle or 5D4 as described in Fig. 7A-C. The x-axis represents the day after injection of cells or implantation of PDX.



Supplementary Figure S11. Cpd 5D4 and PARP1/2 inhibitors synergistically inhibit cell viability in ovarian and breast cancer cells.

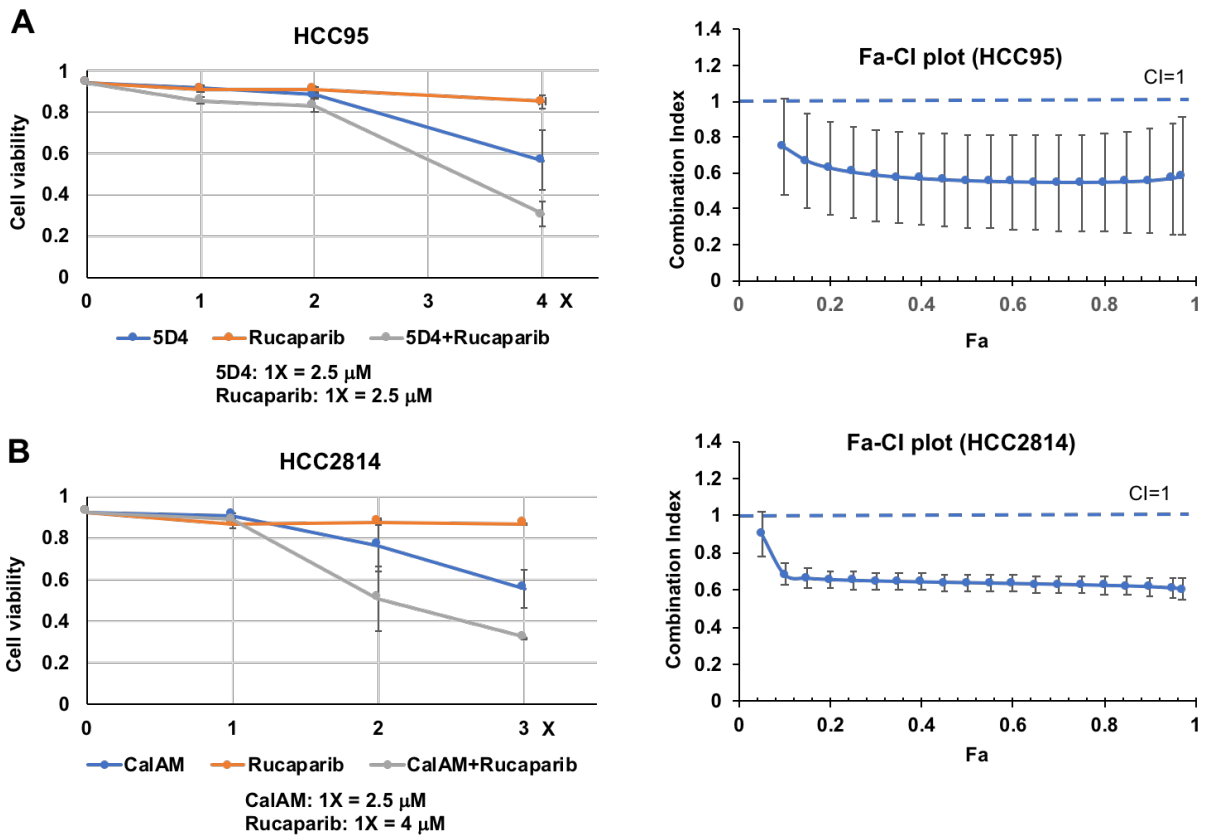
A2780cis (A) or MDA-MB-468 (B) cells were treated with 5D4, rucaparib, talazoparib or a combination of 5D4 with either rucaparib or talazoparib as indicated for 20 h. Active caspase-3/7 was determined by Caspase-Glo® 3/7 Assay and was normalized to protein concentrations. Data shown are the mean ± SEM from three or four independent experiments. Combination index (CI) values and Fa (Fraction affected)-CI plots were generated using CompuSyn software. CI < 1 indicates synergism.



Supplementary Figure S12. Calcein AM and PARP inhibitors synergistically inhibit cell viability in breast and ovarian cancer cells.

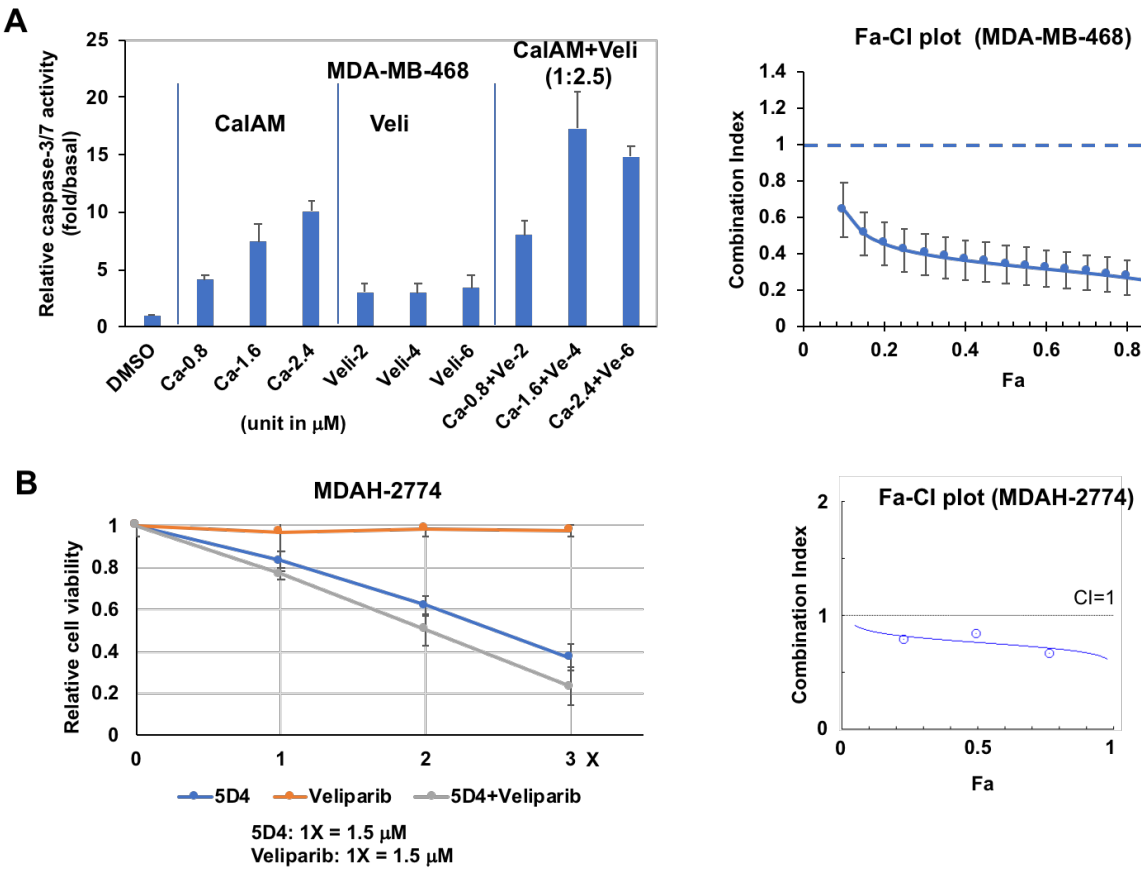
MDA-MB-468, MDAH-2774 or A2780cis cells were treated with vehicle DMSO, CalAM, rucaparib (Ru), talazoparib (Ta) or a combination of CalAM (Ca) with either rucaparib or talazoparib as indicated for 45 h. Cell viability was determined by CCK-8 assay (**A, E**). Data shown are the mean ± SD done in triplicates. Active caspase-3/7 was determined by Caspase-Glo®

3/7 Assay and was normalized to protein concentrations (**B**, **C**, **D**, **F**). Data shown are the mean ± SEM from three or four independent experiments. Lower panels are corresponding Fa-CI plots.



Supplementary Figure S13. TopBP1i and rucaparib synergistically inhibit cell viability in lung cancer cells.

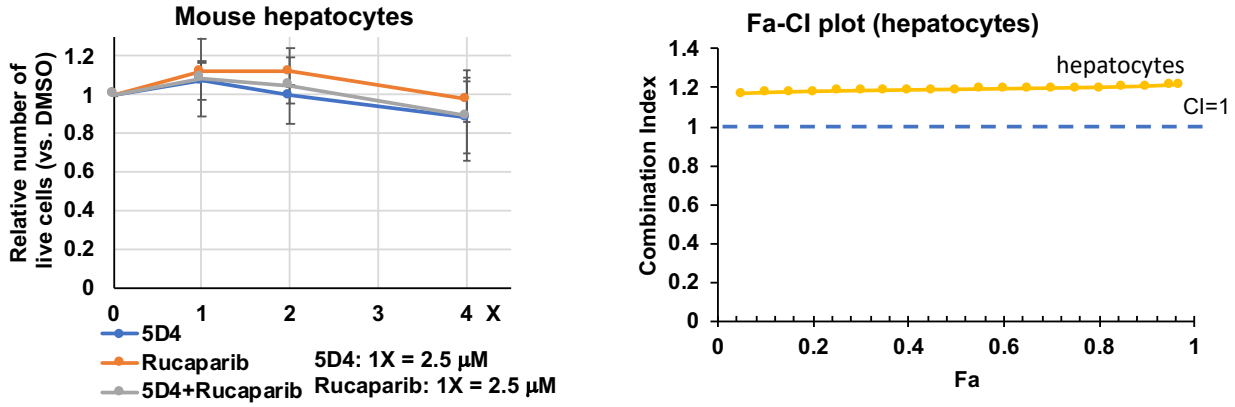
HCC95 (A) or HCC2814 (B) lung cancer cells were treated with TopBP1i (CalAM or 5D4), rucaparib or both as indicated for 45 h, followed by trypan blue exclusion assay to determine the cell viability. Data shown are the mean ± SD done in quadruplicates. Right panels are corresponding Fa-CI plots.



Supplementary Figure S14. TopBP1i and veliparib synergistically inhibit cell viability in breast and ovarian cancer cells.

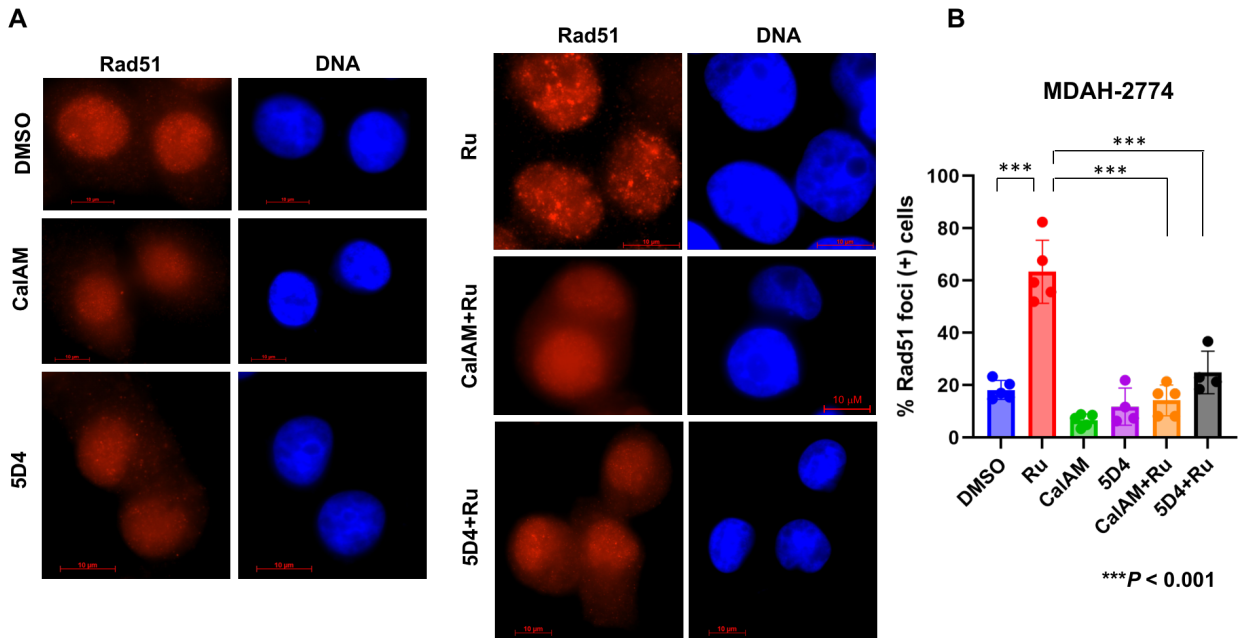
A. MDA-MB-468 cells were treated with CalAM, veliparib or both as indicated for 20 h. Active caspase-3/7 was determined by Caspase-Glo® 3/7 Assay and was normalized to protein concentrations. Data shown are the mean ± SEM from three independent experiments.

B. MDAH-2774 cells were treated with 5D4, veliparib or both as indicated for 45 h. Cell viability was determined by CCK-8 assay. Data shown are the mean ± SD done in quadruplicates. Right panels are corresponding Fa-CI plots.



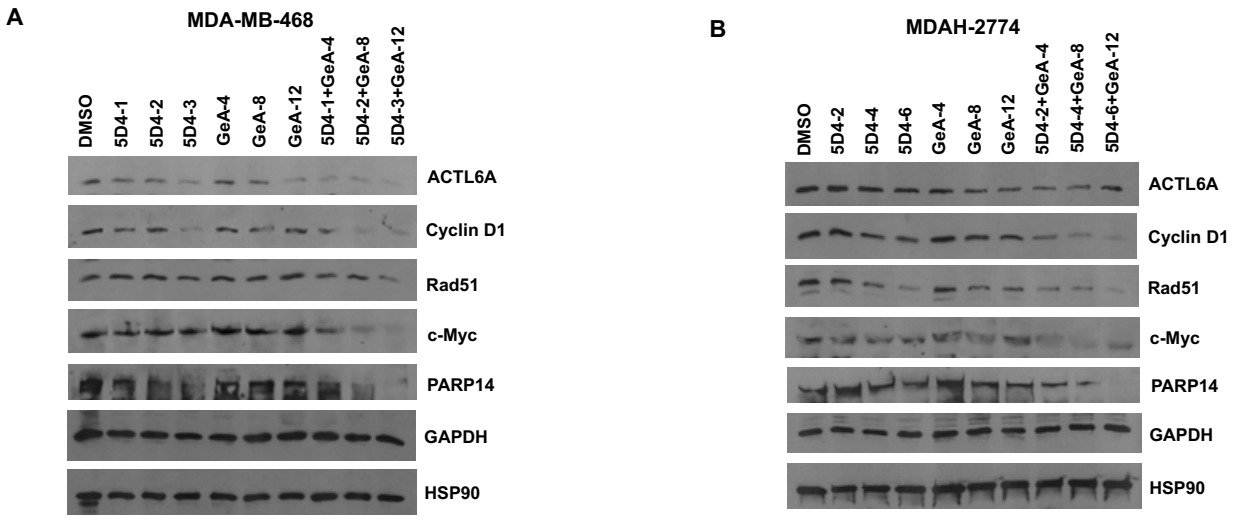
Supplementary Figure S15. Cpd 5D4 or a combination of 5D4 with rucaparib does not affect the viability of untransformed mouse hepatocytes.

AML12 mouse hepatocytes were treated with 5D4, rucaparib, or a combination of 5D4 with rucaparib as indicated for 45 h. The viable AML12 mouse hepatocytes after treatment were determined by trypan blue exclusion. The number of live cells was normalized to that of vehicle (DMSO) control. Data shown are the mean ± SD from three independent experiments. Right panel is Fa-CI plot.



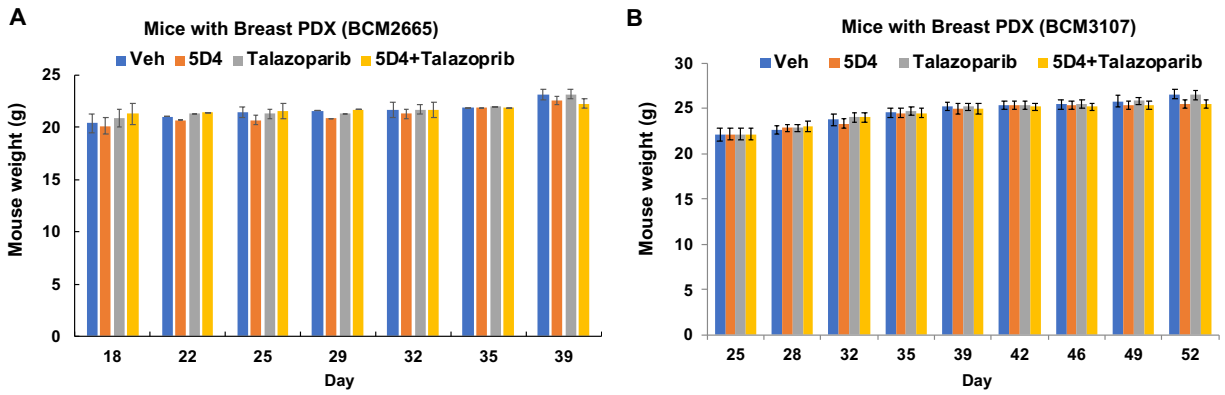
Supplementary Figure S16. TopBP1i attenuates PARPi-induced Rad51 foci formation.

MDAH-2774 cells were treated with 2 μM CalAM, 4 μM 5D4, 10 μM rucaparib or a combination of rucaparib with either CalAM or 5D4 for 18 h. After fixation and permeabilization, cells were subjected to immunostaining using an anti-Rad51 rabbit antibody, followed by Texas Red-X-conjugated anti-rabbit secondary antibody. Nuclei was stained by Hoechst 33258. The images were captured under fluorescence microscope using 100X objective. The number of Rad51 foci (+) cells was counted under 40X objective. At least 60 cells per sample were counted (n = 4-5).



Supplementary Figure S17. Cpd 5D4 and PARP-14 inhibitor GeA-69 synergistically inhibit the expression of MYC and its target genes in breast and ovarian cancer cells.

MDA-MB-468 (A) or MDAH-2774 (B) cells were treated with different concentrations of 5D4, GeA-69 or both for 20 h. Cells were harvested and the whole cell lysates were subjected to immunoblotting using antibodies specific to the indicated proteins, respectively. GAPDH and HSP90 serve as loading controls.



Supplementary Figure S18. Combination of Cpd 5D4 and talazoparib does not significantly affect mouse body weight.

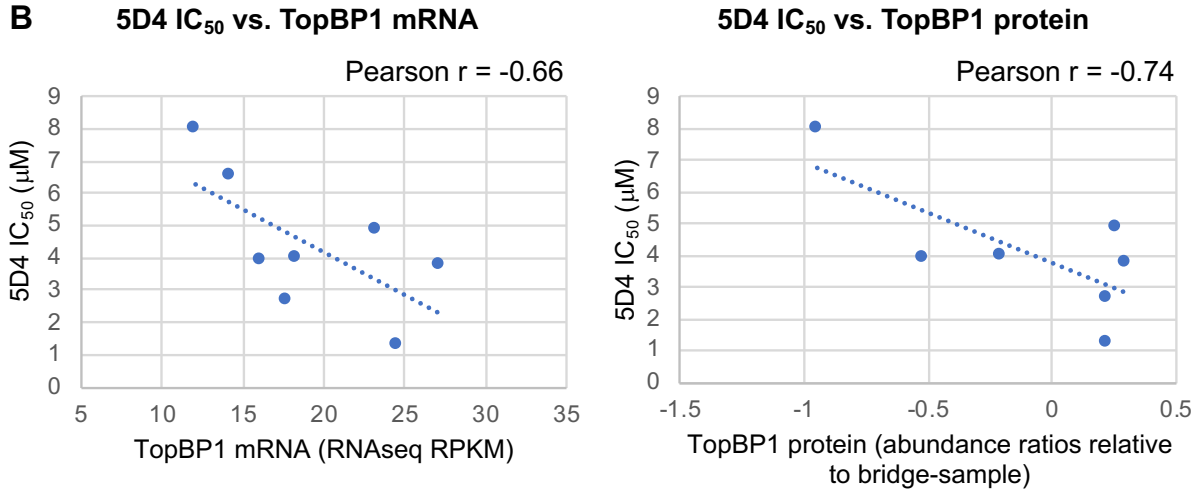
Shown are the mean mouse body weights ± SD of the xenograft-bearing mice treated with vehicle, 5D4, talazoparib or combination as described in Fig. 10A-B. The x-axis represents the day after injection of cells or implantation of PDX.

A

cell line	tissue of origin	TP53 status	MYC Amp	BRCA1	BRCA2	MYC mRNA	TopBP1 mRNA	MYC protein	TopBP1 protein	5D4 IC_{50} (μM)
T47D	breast cancer	L194F	No	WT	WT	20.55	23.28	-0.60	0.27	4.83 ± 0.29 (n=3)
MDA-MB-468	breast cancer	R273H	No	WT	M965I	38.16	17.70	NA	0.22	2.65 ± 0.785 (n=4)
BT549	breast cancer	R249S	Yes	WT	WT	38.56	27.19	-0.17	0.30	3.73 ± 0.23 (n=3)
HCC1937	breast cancer	R306*	Yes	Q1777fs	WT	26.26	24.53	0.29	0.22	1.25 ± 0.07 (n=2)
MDAH-2774	ovarian cancer	R273H	NA*	NA	NA	NA	NA	NA	NA	3.675 ± 0.25 (n=4)
OVCAR8	ovarian cancer	Y126fs	Yes	WT**	WT	45.29	16.14	-0.04	-0.52	3.9
HCC95	lung squamous cell carcinoma	R335fs	No	WT	WT	96.24	12.03	0.60	-0.94	8 ± 0.7 (n=2)
H2170	lung squamous cell carcinoma	R158G	Yes	WT	WT	180.94	15.45	0.66	-0.74	2.33 ± 1.04 (n=3)
HCC2814	lung squamous cell carcinoma	C176W	No	WT	WT	33.16	14.19	NA	NA	6.53 ± 5.75 (n=3)
A549	lung adenocarcinoma	WT	No	WT	WT	41.38	18.27	0.21	-0.21	4 ± 0.82 (n=3)

MYC and TopBP1 mRNA: RNAseq RPKM; MYC and TopBP1 proteins: abundance ratios relative to bridge-sample.
*MDAH-2774 cells harbor a frameshift mutation in FBXW7 , an E3 ubiquitin ligase for MYC.
***BRCA1*-methylated
NA: not available

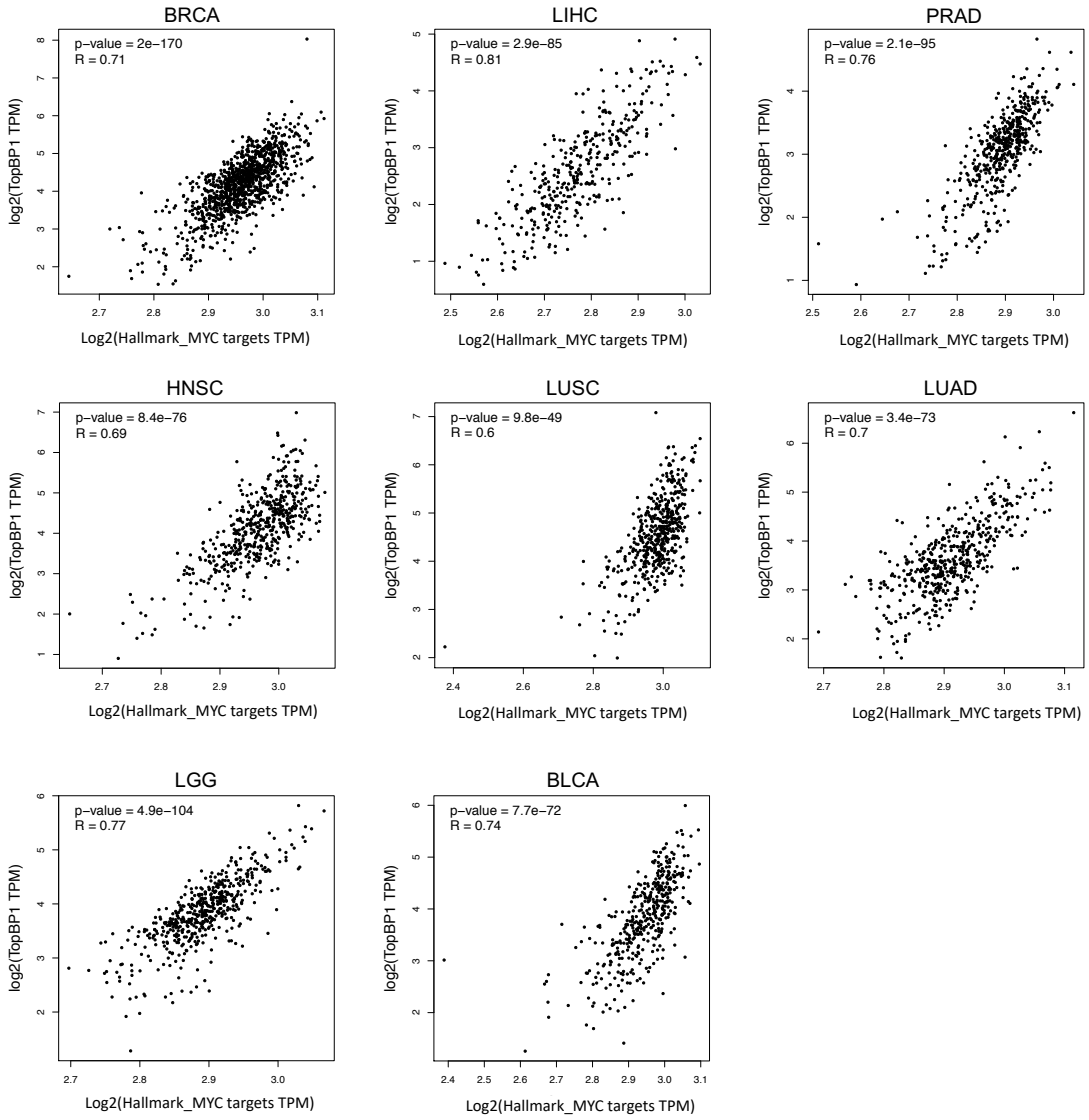
B



Supplementary Figure S19. Summary of the cell lines and correlation between TopBP1 expression and the response to 5D4.

A. The genetic status and gene expression of the cell lines are extracted from CCLE database. The IC_{50} of 5D4 for each cell line was determined by CCK-8 assay or trypan blue exclusion assay. Shown are the mean ± SD from multiple independent experiments.

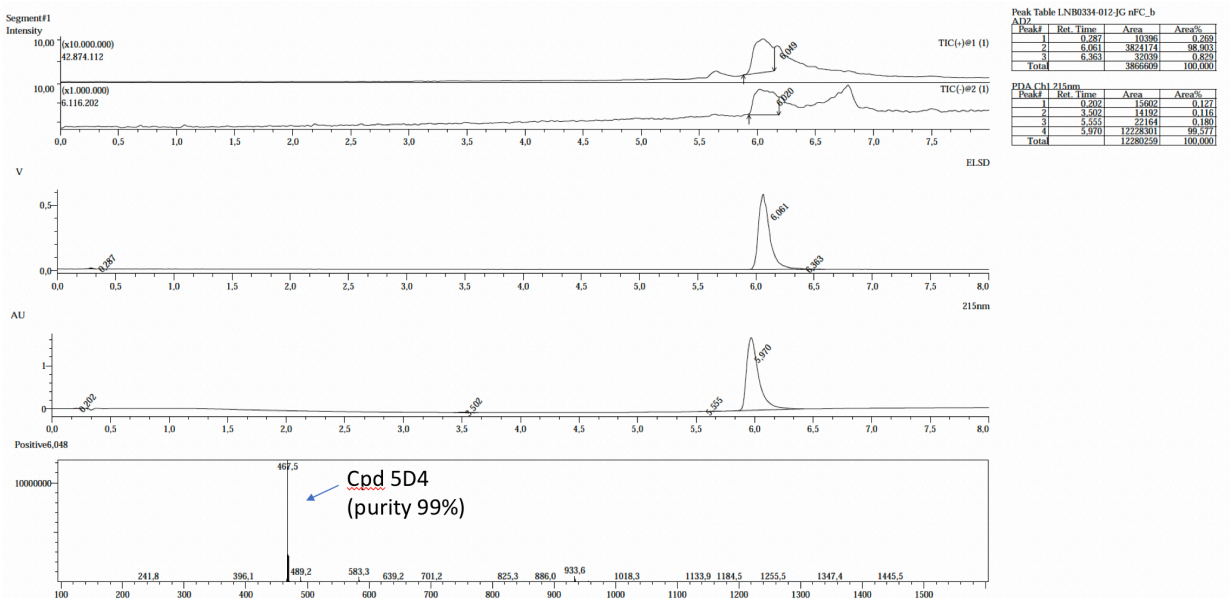
B. The IC_{50} of 5D4 for each cell line is plotted against TopBP1 mRNA or protein levels.



Supplementary Figure S20. Correlation between TopBP1 expression and MYC target gene signature in multiple types of cancer in TCGA database.

The correlation between the gene expression of 200 MYC target genes in gene set Hallmark_MYC_targets_v1 and TopBP1 expression in TCGA database was evaluated. R value represents Pearson correlation coefficient. BRCA, breast invasive cancer; LIHC, liver hepatocellular carcinoma; PRAD, prostate adenocarcinoma; HNSC, head and neck squamous cell carcinoma; LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; LGG, low grade glioma; BLCA, bladder urothelial carcinoma.

LCMS



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AD2

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3	6.363	32039	0.829
Total		3866609	100.000

PDA Ch1 215nm

Peak#	Ret. Time	Area	Area%
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2	3.502	14192	0.116
3	5.555	22164	0.180
4	5.970	12228301	99.577
Total		12280259	100.000

Supplementary Figure S21. LCMS analysis of Cpd 5D4.

Supplementary Figure S22. 1D and 2D NMR analysis of Cpd 5D4.

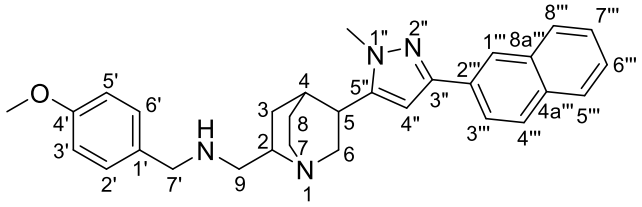
(See next page.)

Supplementary Figure S23. HRESIMS analysis of Cpd 5D4.

(See last page of Supplementary Information.)

Supplementary Figure S22. 1D and 2D NMR and MS data of Cpd 5D4

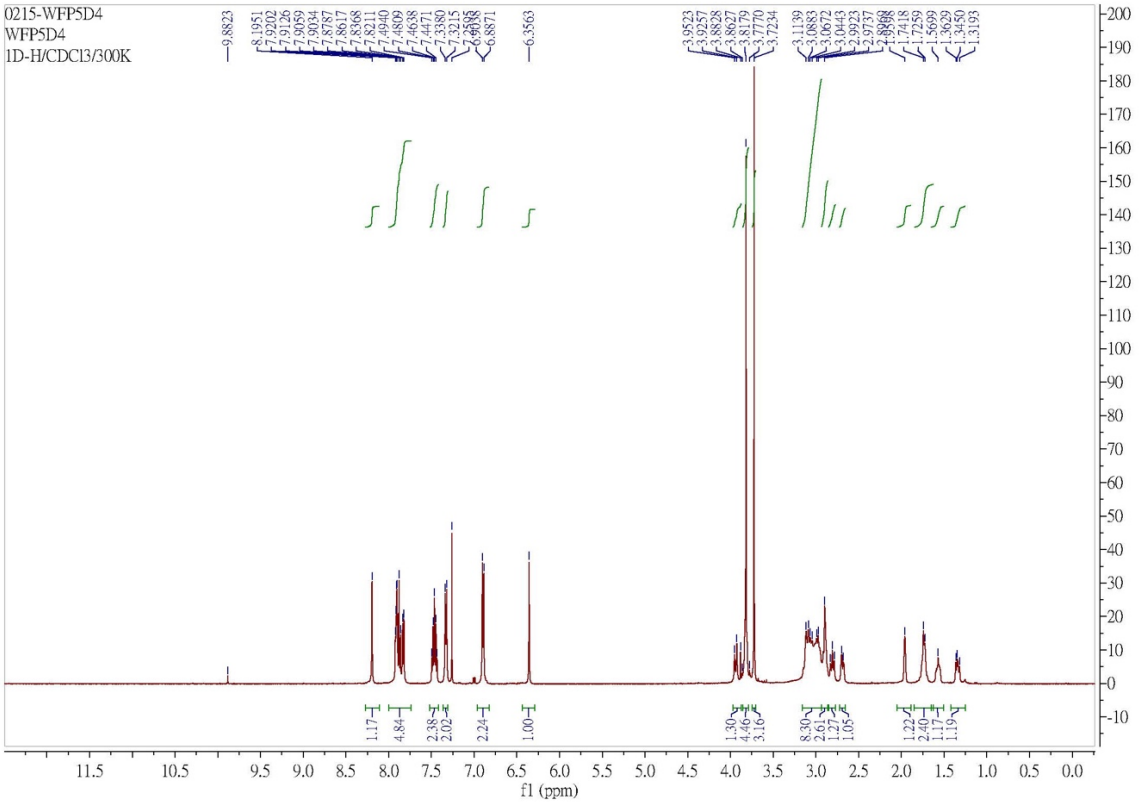
NMR spectra were recorded with Bruker AV-500 MHz spectrometers and chemical shifts were measured in δ (ppm) with $CDCl_3$ as an internal standard. High-resolution mass spectra (HRMS) was measured with an AB SCIE X (QSTAR® XL) High Resolution Electrospray (ESI) Mass Spectrometry. TLC was carried out on silica gel 60 F254 (Merck) plate. Spot was detected by UV and Dragendorff's spray reagent.

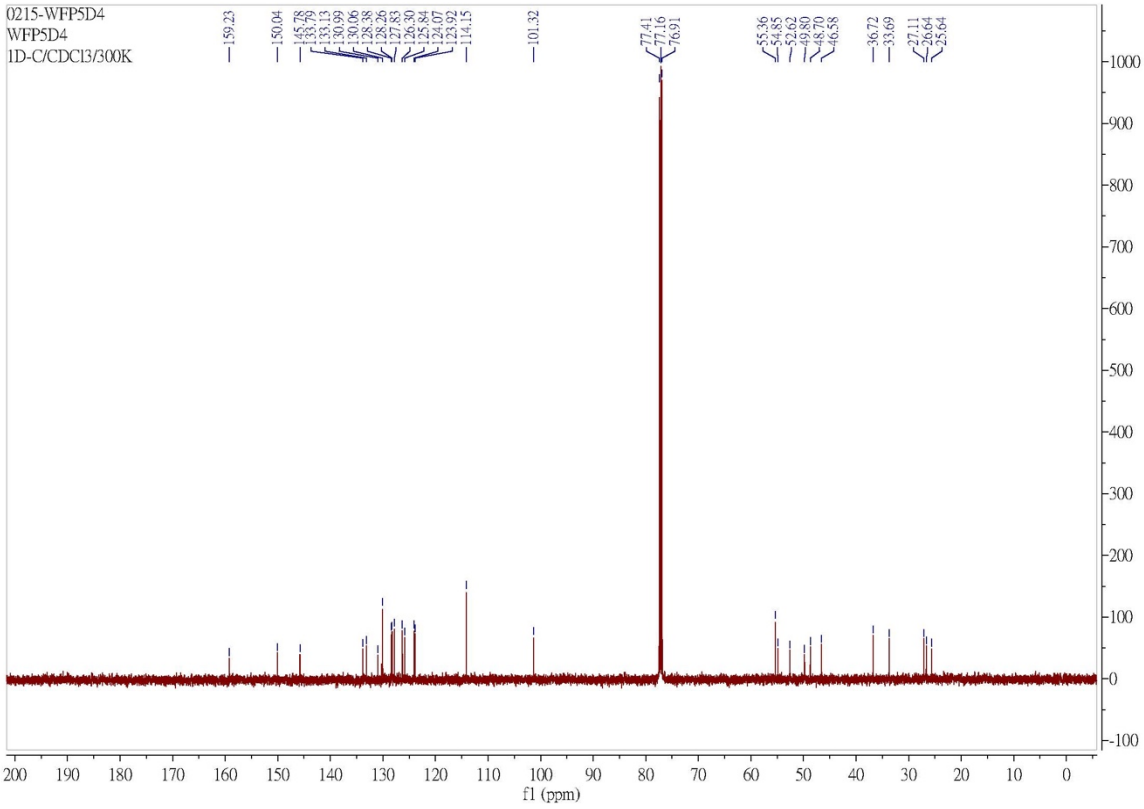


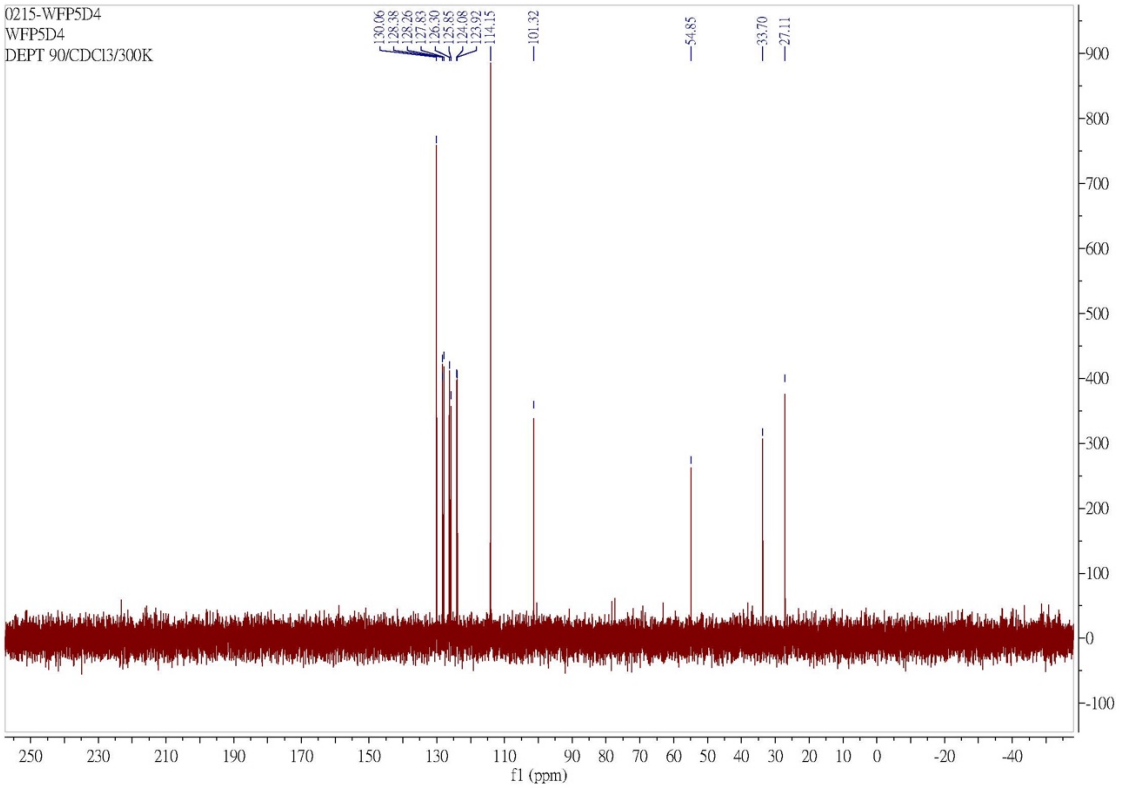
[(4-Methoxyphenyl)methyl]({[(2*R*,4*S*,5*R*)-5-[1-methyl-3-(naphthalen-2-yl)-1*H*-pyrazol-5-yl]-1-azabicyclo[2.2.2]octan-2-yl]methyl})amine

R_f 0.69 (CH_2Cl_2:CH_3OH = 100:1); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.35 (1H, m, H-3), 1.57 (1H, m, H-3), 1.73 (2H, m, H-8), 1.96 (1H, s, H-4); 2.68 (1H, m, H-9), 2.81 (1H, t, *J* = 11.0 Hz, H-9), 2.89-2.93 (2H, m, H-5,7), 3.04-3.11 (4H, m, H-2,6,7), 3.72 (3H, s, 4′-OCH_3), 3.82 (3H, s, NCH_3), 3.88 (1H, m, H-7′), 3.93 (1H, m, H-7′), 6.36 (1H, s, H-4″), 6.89 (2H, d, *J* = 8.30 Hz, H-3′,5′), 7.32 (2H, d, *J* = 8.30 Hz, H-2′,6′), 7.43-7.49 (2H, m, H-4‴,7‴), 7.82-7.92 (4H, m, H-3‴,5‴,6‴,8‴), 8.20 (1H, s, H-1‴); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 159.2 (C-4′), 150.0 (C-3″), 145.8 (C-5″), 133.8 (C-4a‴), 133.1 (C-8a‴), 131.0 (C-2‴), 130.3 (C-1′), 130.1 (C-2′, C-6′), 128.4 (C-6‴), 128.3 (C-3‴), 127.8 (C-5‴), 126.3 (C-7‴), 125.8 (C-4‴), 124.1 (C-8‴), 123.9 (C-1‴), 114.2 (C-3′, C-5′), 101.3 (C-4″), 55.4 (O*C*H_3), 54.9 (C-2), 52.6 (C-7′), 49.8 (C-9), 48.7 (C-6), 46.6 (C-7), 36.7 (N-*C*H_3), 33.7 (C-5), 27.1 (C-4), 26.6 (C-8), 25.6 (C-3); HRESIMS *m*/*z* 467.2811 $[M + H]^+$ (calcd for $C_{30}H_{35}N_4O$, 467.2811).

1D and 2D NMR of Cpd 5D4



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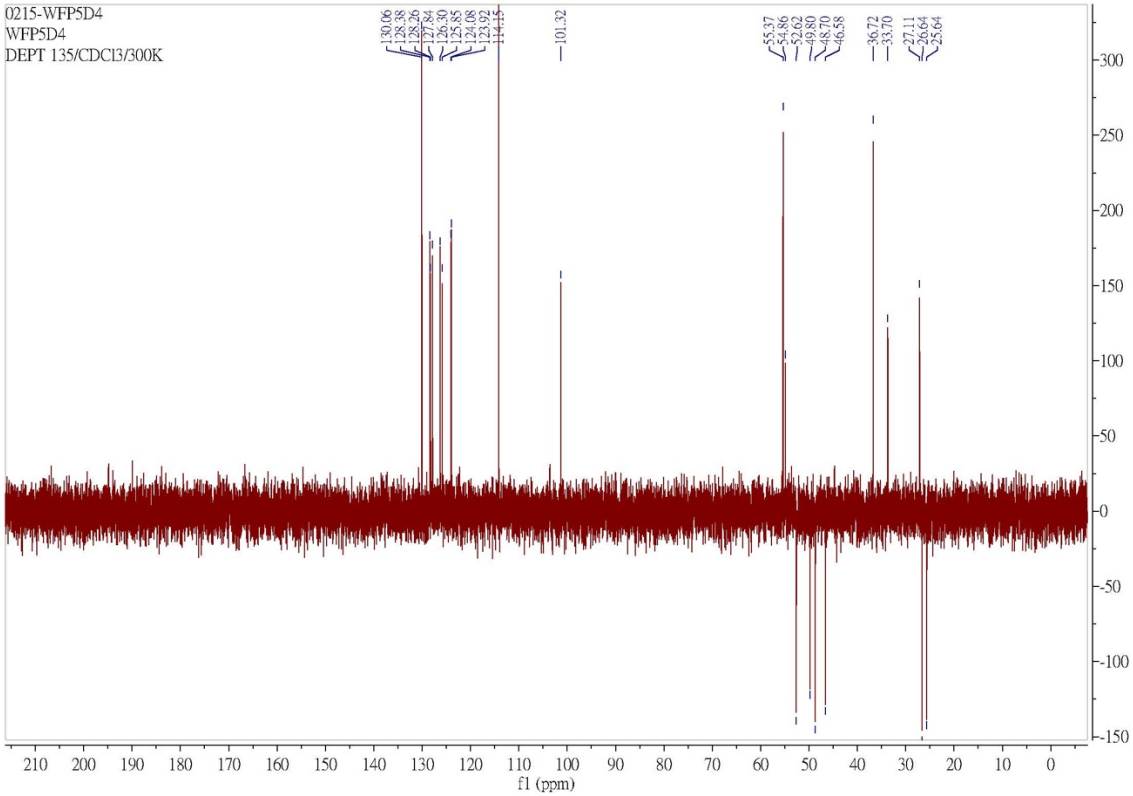
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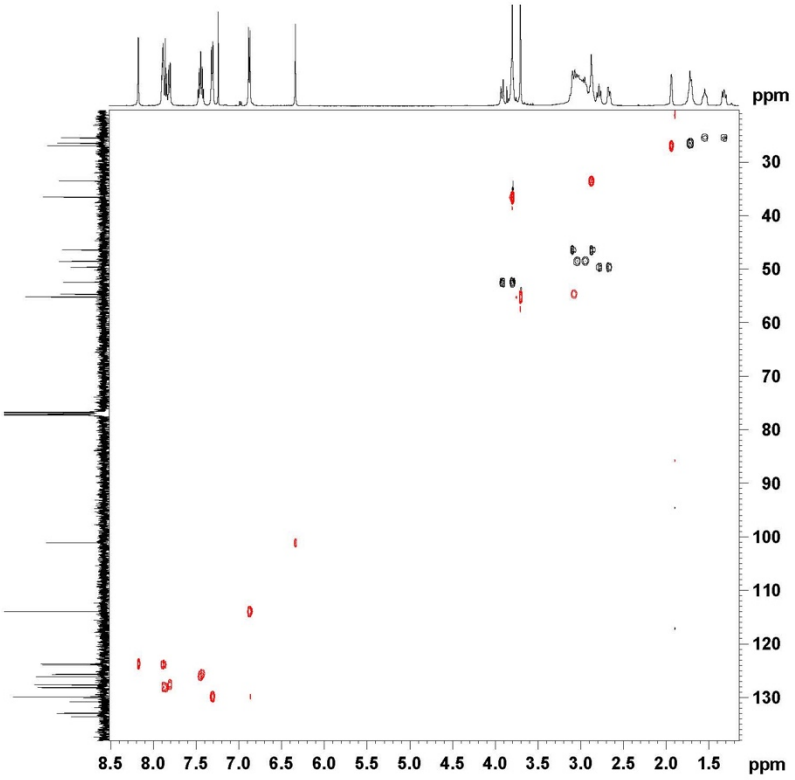

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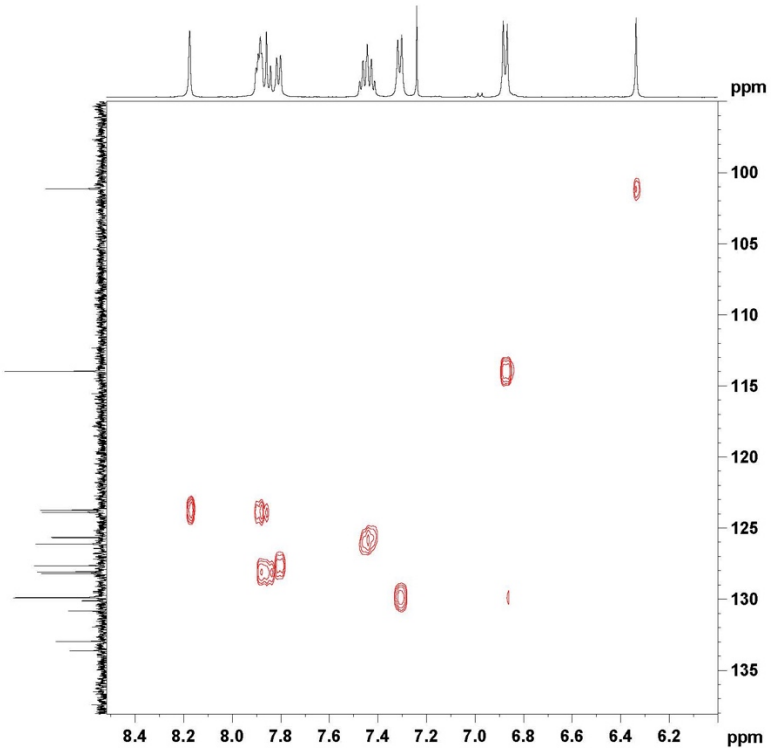

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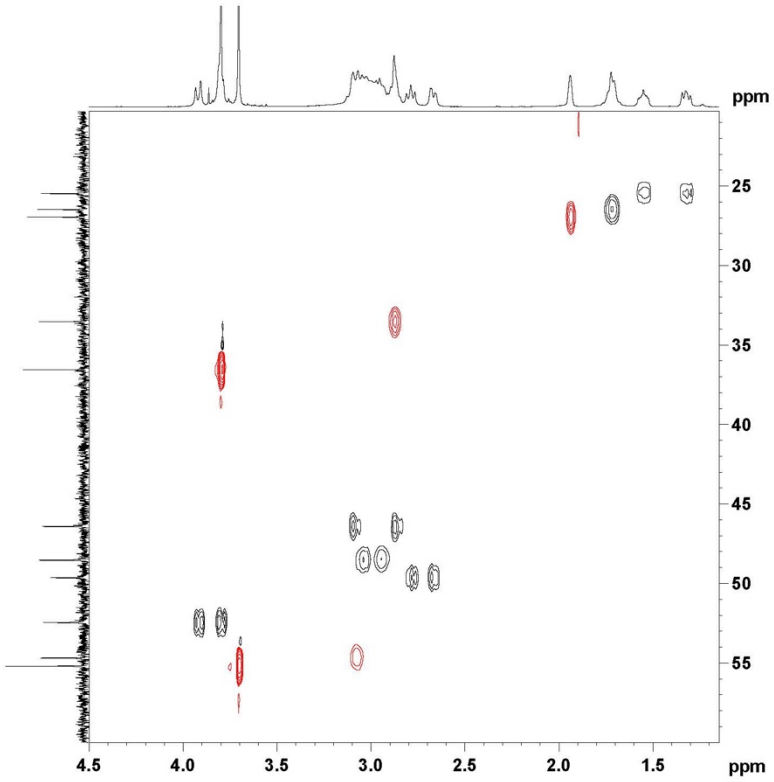


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P24                    2000.00 usec
PCPD2                     80.00 usec
PL0                      120.00 dB
PL2                       -4.00 dB
PL12                       8.20 dB
SFO2               125.7691072 MHz
SP3                        1.30 dB
SP7                        1.30 dB
SPNAM3    Crp60,0.5,20.1
SPNAM7     Crp60comp.4
SPOAL3                   0.500
SPOAL7                   0.500
SPOFF3                   0.00 Hz
SPOFF7                   0.00 Hz

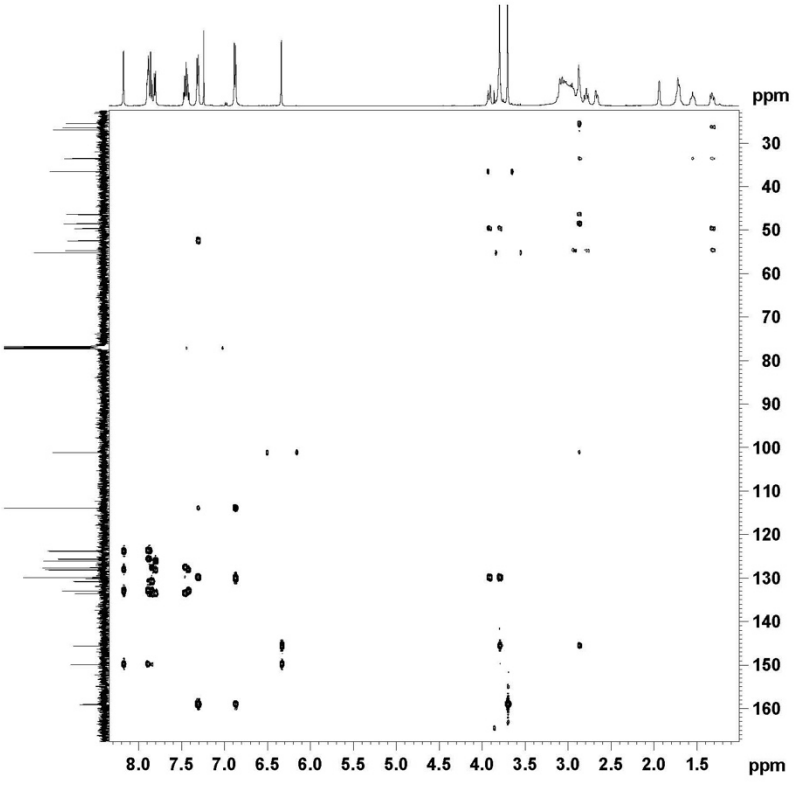
====== GRADIENT CHANNEL =====
GPNAM1              SINE.100
GPNAM2              SINE.100
GPNAM3              SINE.100
GPNAM4              SINE.100
GPZ1                   80.00 %
GPZ2                   20.10 %
GPZ3                   11.00 %
GPZ4                   -5.00 %
P16                 1000.00 usec
P19                  600.00 usec

F1 - Acquisition parameters
NDO                            2
TD                           256
SFO1               125.7691 MHz
FIDRES              78.596581 Hz
SW                    159.981 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                          1024
SF                 500.1300232 MHz
WDW                       SINE
SSB                            2
LB                          0.00 Hz
GB                             0
PC                          1.40

F1 - Processing parameters
SI                          1024
MC2        echo-antiecho
SF                 125.7577923 MHz
WDW                       SINE
SSB                            2
LB                          0.00 Hz
GB                             0
```

WFP5D4
HMBC/CDCl3/300K




```
Current Data Parameters
NAME                  0215-WFP5D4
EXPNO                          13
PROCNO                          1

F2 - Acquisition Parameters
Date_               20190215
Time_                   17.51
INSTRUM                 spect
PROBHD    5 mm TXI 13C Z
PULPROG    hmbcgplpndqf
TD                        2048
SOLVENT                 CDCl3
NS                             2
DS                            16
SWH                   5482.456 Hz
FIDRES                2.676980 Hz
AQ                    0.1868276 sec
RG                         16384
DW                       91.200 usec
DE                         6.50 usec
TE                        300.0 K
CNST2               145.0000000
CNST13                8.0000000
d0                    0.00000300 sec
D1                    1.50000000 sec
d2                    0.00344828 sec
d6                    0.06250000 sec
D16                   0.00020000 sec
IN0                   0.00002485 sec

======== CHANNEL f1 ========
NUC1                         1H
P1                         9.00 usec
p2                        18.00 usec
PL1                        -4.60 dB
SFO1               500.1327507 MHz

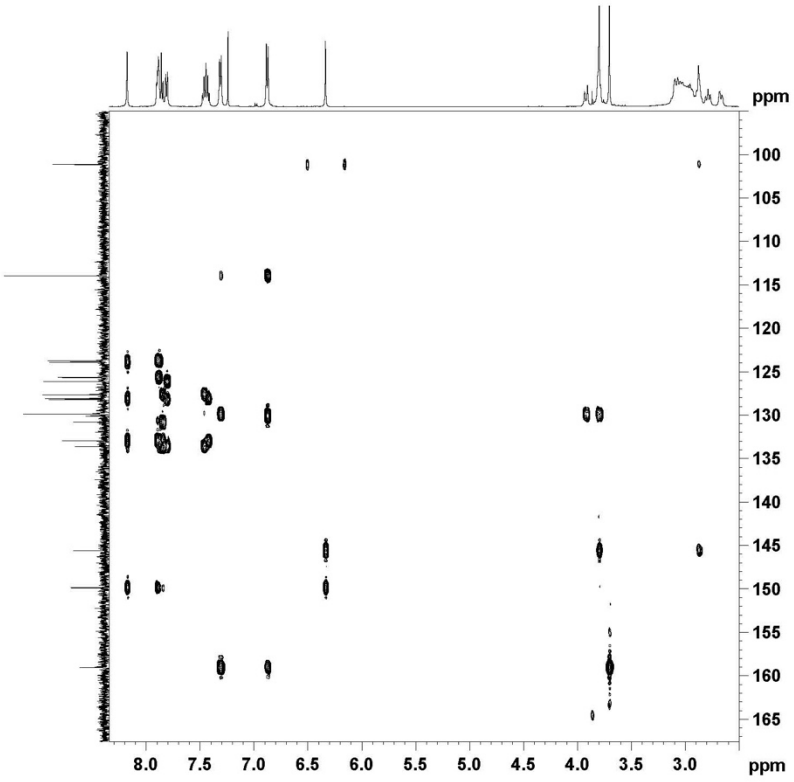
======== CHANNEL f2 ========
NUC2                       13C
P3                        15.70 usec
PL2                        -4.00 dB
SFO2               125.7691072 MHz

====== GRADIENT CHANNEL =====
GPNAM1              SINE.100
GPNAM2              SINE.100
GPNAM3              SINE.100
GPZ1                   50.00 %
GPZ2                   30.00 %
GPZ3                   40.10 %
P16                 1000.00 usec

F1 - Acquisition parameters
NDO                            2
TD                           256
SFO1               125.7691 MHz
FIDRES              78.596581 Hz
SW                    159.981 ppm
FnMODE                       QF

F2 - Processing parameters
SI                          1024
SF                 500.1300232 MHz
WDW                       SINE
SSB                            0
LB                          0.00 Hz
GB                             0
PC                          1.40

F1 - Processing parameters
SI                          1024
MC2                          QF
SF                 125.7577923 MHz
WDW                       SINE
SSB                            0
LB                          0.00 Hz
GB                             0
```



WFP5D4
HMBC/CDC13/300K

```
Current Data Parameters
NAME                 0215-WFP5D4
EXPNO                         13
PROCNO                         1

F2 - Acquisition Parameters
Date_               20190215
Time                   17.51
INSTRUM                 spect
PROBHD    5 mm TXI 13C Z
PULPROG      hmbcgplpndqf
TD                     2048
SOLVENT              CDCl3
NS                        2
DS                       16
SWH                5482.456 Hz
FIDRES             2.676980 Hz
AQ                 0.1868276 sec
RG                     16384
DW                   91.200 usec
DE                    6.50 usec
TE                   300.0 K
CNST2              145.0000000
CNST13               8.0000000
d0                 0.00000300 sec
D1                 1.50000000 sec
d2                 0.00344828 sec
d6                 0.06250000 sec
D16                0.00020000 sec
IN0                0.00002485 sec

======== CHANNEL f1 ========
NUC1                      1H
P1                      9.00 usec
p2                     18.00 usec
PL1                    -4.60 dB
SFO1            500.1327507 MHz

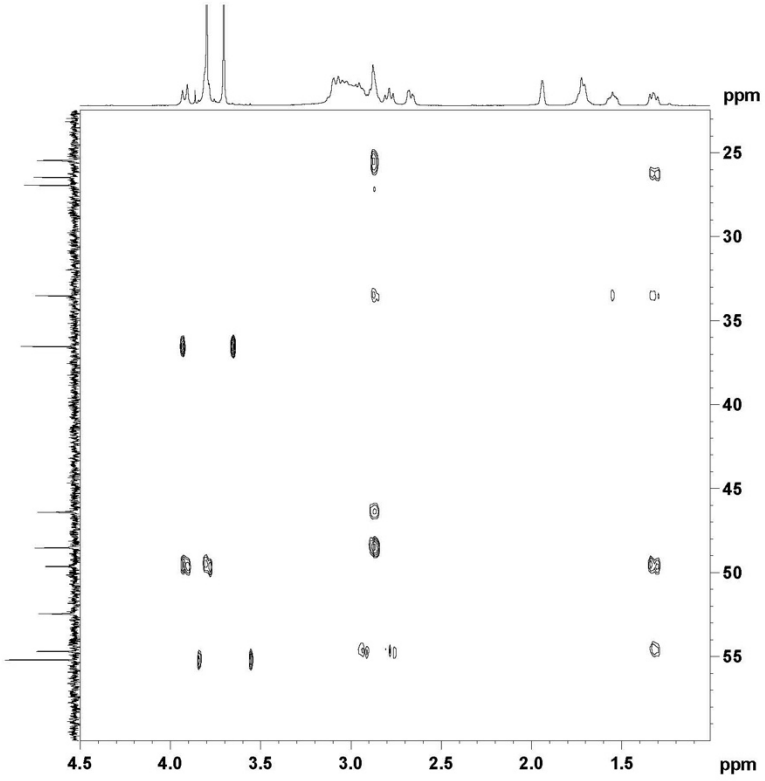
======== CHANNEL f2 ========
NUC2                      13C
P3                     15.70 usec
PL2                    -4.00 dB
SFO2            125.7691072 MHz

====== GRADIENT CHANNEL =====
GPNAM1               SINE.100
GPNAM2               SINE.100
GPNAM3               SINE.100
GPZ1                    50.00 %
GPZ2                    30.00 %
GPZ3                    40.10 %
P16                   1000.00 usec

F1 - Acquisition parameters
ND0                           2
TD                          256
SFO1             125.7691 MHz
FIDRES            78.596581 Hz
SW                  159.981 ppm
FnMODE                     QF

F2 - Processing parameters
SI                         1024
SF               500.1300232 MHz
WDW                      SINE
SSB                         0
LB                       0.00 Hz
GB                          0
PC                       1.40

F1 - Processing parameters
SI                         1024
MC2                        QF
SF               125.7577923 MHz
WDW                      SINE
SSB                         0
LB                       0.00 Hz
GB                          0
```



WFP5D4
HMBC/CDC13/300K

```
Current Data Parameters
NAME                 0215-WFP5D4
EXPNO                         13
PROCNO                         1

F2 - Acquisition Parameters
Date_               20190215
Time                   17.51
INSTRUM                 spect
PROBHD    5 mm TXI 13C Z
PULPROG      hmbcgplpndqf
TD                     2048
SOLVENT              CDCl3
NS                        2
DS                       16
SWH                5482.456 Hz
FIDRES             2.676980 Hz
AQ                 0.1868276 sec
RG                     16384
DW                   91.200 usec
DE                    6.50 usec
TE                   300.0 K
CNST2              145.0000000
CNST13               8.0000000
d0                 0.00000300 sec
D1                 1.50000000 sec
d2                 0.00344828 sec
d6                 0.06250000 sec
D16                0.00020000 sec
IN0                0.00002485 sec

======== CHANNEL f1 ========
NUC1                      1H
P1                      9.00 usec
p2                     18.00 usec
PL1                    -4.60 dB
SFO1            500.1327507 MHz

======== CHANNEL f2 ========
NUC2                      13C
P3                     15.70 usec
PL2                    -4.00 dB
SFO2            125.7691072 MHz

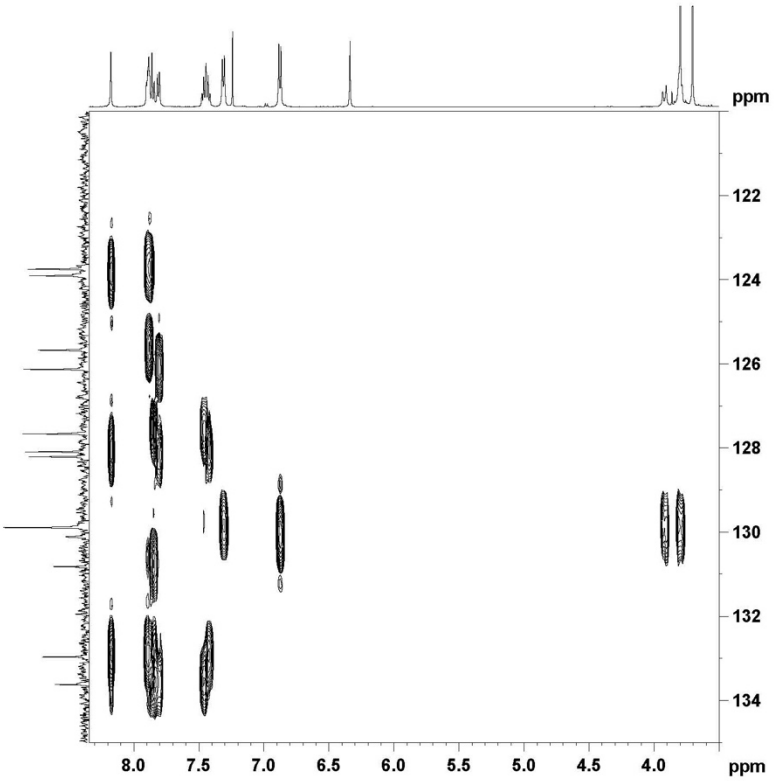
====== GRADIENT CHANNEL =====
GPNAM1               SINE.100
GPNAM2               SINE.100
GPNAM3               SINE.100
GPZ1                    50.00 %
GPZ2                    30.00 %
GPZ3                    40.10 %
P16                   1000.00 usec

F1 - Acquisition parameters
ND0                           2
TD                          256
SFO1             125.7691 MHz
FIDRES            78.596581 Hz
SW                  159.981 ppm
FnMODE                     QF

F2 - Processing parameters
SI                         1024
SF               500.1300232 MHz
WDW                      SINE
SSB                         0
LB                       0.00 Hz
GB                          0
PC                       1.40

F1 - Processing parameters
SI                         1024
MC2                        QF
SF               125.7577923 MHz
WDW                      SINE
SSB                         0
LB                       0.00 Hz
GB                          0
```

WFP5D4
HMBC/CDCl3/300K




```
Current Data Parameters
NAME                 0215-WFP5D4
EXPNO                         13
PROCNO                         1

F2 - Acquisition Parameters
Date_               20190215
Time                   17.51
INSTRUM                spect
PROBHD    5 mm TXI 13C Z
PULPROG    hmbcgplpndqf
TD                       2048
SOLVENT                 CDCl3
NS                          2
DS                         16
SWH                  5482.456 Hz
FIDRES               2.676980 Hz
AQ                   0.1868276 sec
RG                       16384
DW                     91.200 usec
DE                      6.50 usec
TE                     300.0 K
CNST2               145.0000000
CNST13                8.0000000
d0                   0.00000300 sec
D1                   1.50000000 sec
d2                   0.00344828 sec
d6                   0.06250000 sec
D16                  0.00020000 sec
IN0                  0.00002485 sec

======== CHANNEL f1 ========
NUC1                       1H
P1                       9.00 usec
p2                      18.00 usec
PL1                     -4.60 dB
SFO1               500.1327507 MHz

======== CHANNEL f2 ========
NUC2                      13C
P3                      15.70 usec
PL2                     -4.00 dB
SFO2               125.7691072 MHz

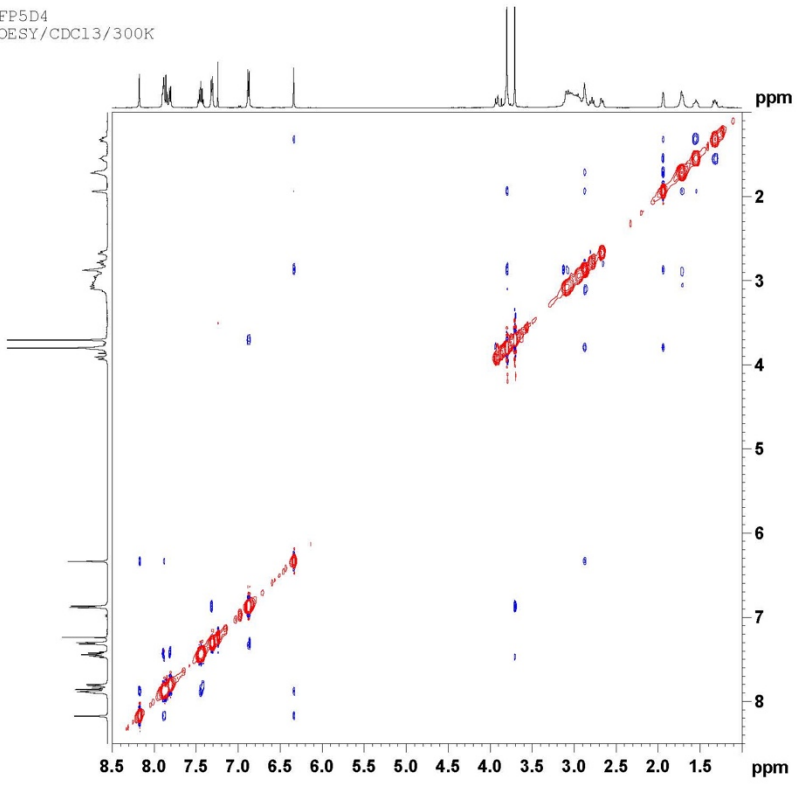
====== GRADIENT CHANNEL =====
GPNAM1               SINE.100
GPNAM2               SINE.100
GPNAM3               SINE.100
GPZ1                    50.00 %
GPZ2                    30.00 %
GPZ3                    40.10 %
P16                   1000.00 usec

F1 - Acquisition parameters
ND0                          2
TD                         256
SFO1               125.7691 MHz
FIDRES              78.596581 Hz
SW                   159.981 ppm
FnMODE                     QF

F2 - Processing parameters
SI                        1024
SF                 500.1300232 MHz
WDW                       SINE
SSB                          0
LB                        0.00 Hz
GB                           0
PC                        1.40

F1 - Processing parameters
SI                        1024
MC2                         QF
SF                 125.7577923 MHz
WDW                       SINE
SSB                          0
LB                        0.00 Hz
GB                           0
```

WFP5D4
NOESY/CDCl3/300K




```
Current Data Parameters
NAME                 0215-WFP5D4
EXPNO                         12
PROCNO                         1

F2 - Acquisition Parameters
Date_               20190215
Time                   17.28
INSTRUM                spect
PROBHD    5 mm TXI 13C Z
PULPROG          noesygpph
TD                       2048
SOLVENT                 CDCl3
NS                          2
DS                         16
SWH                  5482.456 Hz
FIDRES               2.676980 Hz
AQ                   0.1868276 sec
RG                       228.1
DW                     91.200 usec
DE                      6.50 usec
TE                     300.0 K
d0                   0.00007942 sec
D1                   2.00000000 sec
D8                   0.40000001 sec
D16                  0.00020000 sec
IN0                  0.00018175 sec
ST1CNT                      128
TAU                  0.19880000 sec

======== CHANNEL f1 ========
NUC1                       1H
P1                       9.00 usec
p2                      18.00 usec
PL1                     -4.60 dB
SFO1               500.1327507 MHz

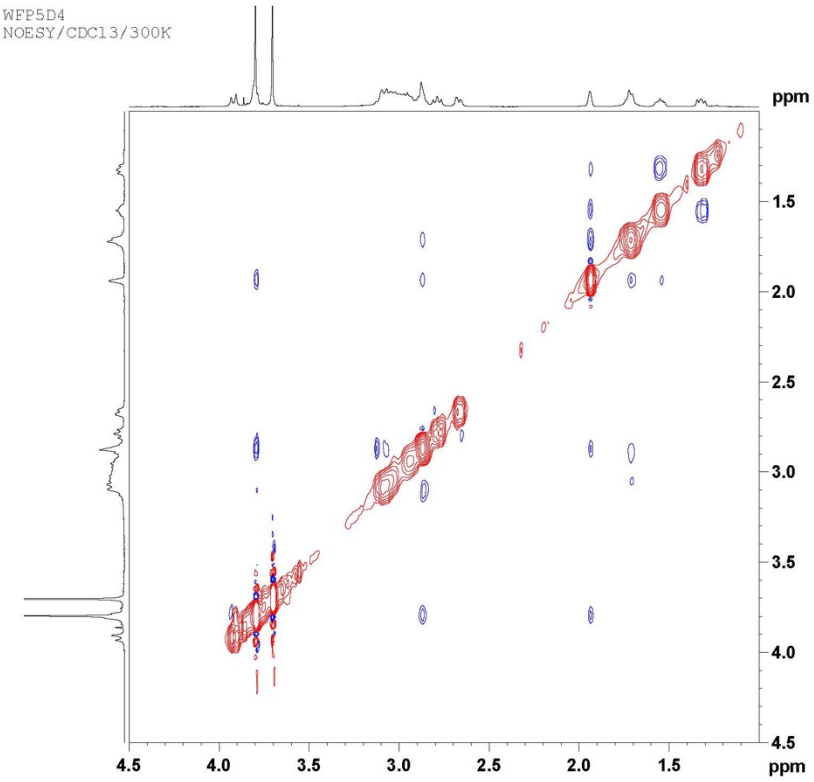
====== GRADIENT CHANNEL =====
GPNAM1               SINE.100
GPNAM2               SINE.100
GPZ1                    40.00 %
GPZ2                   -40.00 %
P16                   1000.00 usec

F1 - Acquisition parameters
ND0                          1
TD                         256
SFO1               500.1328 MHz
FIDRES              21.492435 Hz
SW                    11.001 ppm
FnMODE          States-TPPI

F2 - Processing parameters
SI                        1024
SF                 500.1300232 MHz
WDW                      QSINE
SSB                          2
LB                        0.00 Hz
GB                           0
PC                        1.40

F1 - Processing parameters
SI                        1024
MC2             States-TPPI
SF                 500.1300232 MHz
WDW                      QSINE
SSB                          2
LB                        0.00 Hz
GB                           0
```

WFP5D4
NOESY/CDCl3/300K




```
Current Data Parameters
NAME          0215-WFP5D4
EXPNO                  12
PROCNO                  1

F2 - Acquisition Parameters
Date_            20190215
Time                17.28
INSTRUM             spect
PROBHD    5 mm TXI 13C Z
PULPROG         noesygpph
TD                   2048
SOLVENT             CDCl3
NS                      2
DS                     16
SWH             5482.456 Hz
FIDRES          2.676980 Hz
AQ              0.1868276 sec
RG                  228.1
DW                 91.200 usec
DE                  6.50 usec
TE                 300.0 K
d0              0.00007942 sec
D1              2.00000000 sec
D8              0.40000001 sec
D16             0.00020000 sec
IN0             0.00018175 sec
ST1CNT                 128
TAU             0.19880000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  9.00 usec
p2                 18.00 usec
PL1                -4.60 dB
SFO1         500.1327507 MHz

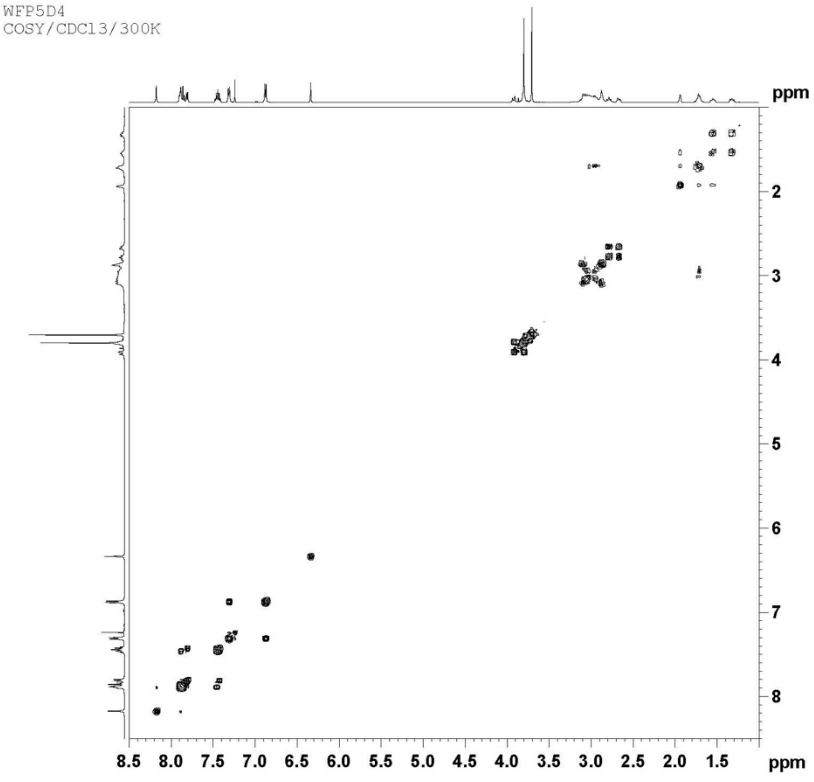
====== GRADIENT CHANNEL =====
GPNAM1          SINE.100
GPNAM2          SINE.100
GPZ1               40.00 %
GPZ2              -40.00 %
P16              1000.00 usec

F1 - Acquisition parameters
ND0                     1
TD                    256
SFO1            500.1328 MHz
FIDRES         21.492435 Hz
SW                 11.001 ppm
FnMODE       States-TPPI

F2 - Processing parameters
SI                   1024
SF            500.1300232 MHz
WDW                 QSINE
SSB                     2
LB                   0.00 Hz
GB                      0
PC                   1.40

F1 - Processing parameters
SI                   1024
MC2          States-TPPI
SF            500.1300232 MHz
WDW                 QSINE
SSB                     2
LB                   0.00 Hz
GB                      0
```

WFP5D4
COSY/CDCl3/300K




```
Current Data Parameters
NAME          0215-WFP5D4
EXPNO                  11
PROCNO                  1

F2 - Acquisition Parameters
Date_            20190215
Time                17.18
INSTRUM             spect
PROBHD    5 mm TXI 13C Z
PULPROG          cosygpqf
TD                   2048
SOLVENT             CDCl3
NS                      1
DS                     16
SWH             5482.456 Hz
FIDRES          2.676980 Hz
AQ              0.1868276 sec
RG                  143.7
DW                 91.200 usec
DE                  6.50 usec
TE                 300.0 K
d0              0.00000300 sec
D1              2.00000000 sec
d13             0.00000400 sec
D16             0.00020000 sec
IN0             0.00018175 sec

======== CHANNEL f1 ========
NUC1                   1H
P0                  9.00 usec
P1                  9.00 usec
PL1                -4.60 dB
SFO1         500.1327507 MHz

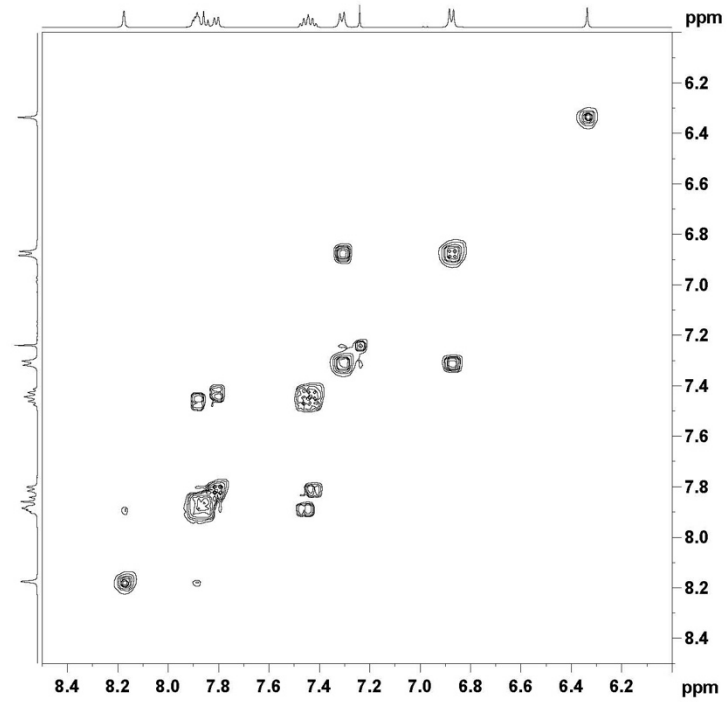
====== GRADIENT CHANNEL =====
GPNAM1          SINE.100
GPZ1               10.00 %
P16              1000.00 usec

F1 - Acquisition parameters
ND0                     1
TD                    256
SFO1            500.1328 MHz
FIDRES         21.492435 Hz
SW                 11.001 ppm
FnMODE                 QF

F2 - Processing parameters
SI                   1024
SF            500.1300232 MHz
WDW                 SINE
SSB                    0
LB                   0.00 Hz
GB                      0
PC                   1.40

F1 - Processing parameters
SI                   1024
MC2                    QF
SF            500.1300232 MHz
WDW                 SINE
SSB                    0
LB                   0.00 Hz
GB                      0
```

WFP5D4
COSY/CDCl3/300K




```
Current Data Parameters
NAME         0215-WFP5D4
EXPNO                 11
PROCNO                 1

F2 - Acquisition Parameters
Date_           20190215
Time              17.18
INSTRUM           spect
PROBHD   5 mm TXI 13C Z
PULPROG        cosygpqf
TD                 2048
SOLVENT           CDCl3
NS                    1
DS                   16
SWH            5482.456 Hz
FIDRES         2.676980 Hz
AQ             0.1868276 sec
RG                143.7
DW              91.200 usec
DE               6.50 usec
TE              300.0 K
d0           0.00000300 sec
D1           2.00000000 sec
d13          0.00000400 sec
D16          0.00020000 sec
IN0          0.00018175 sec

======== CHANNEL f1 ========
NUC1                  1H
P0                 9.00 usec
P1                 9.00 usec
PL1               -4.60 dB
SFO1        500.1327507 MHz

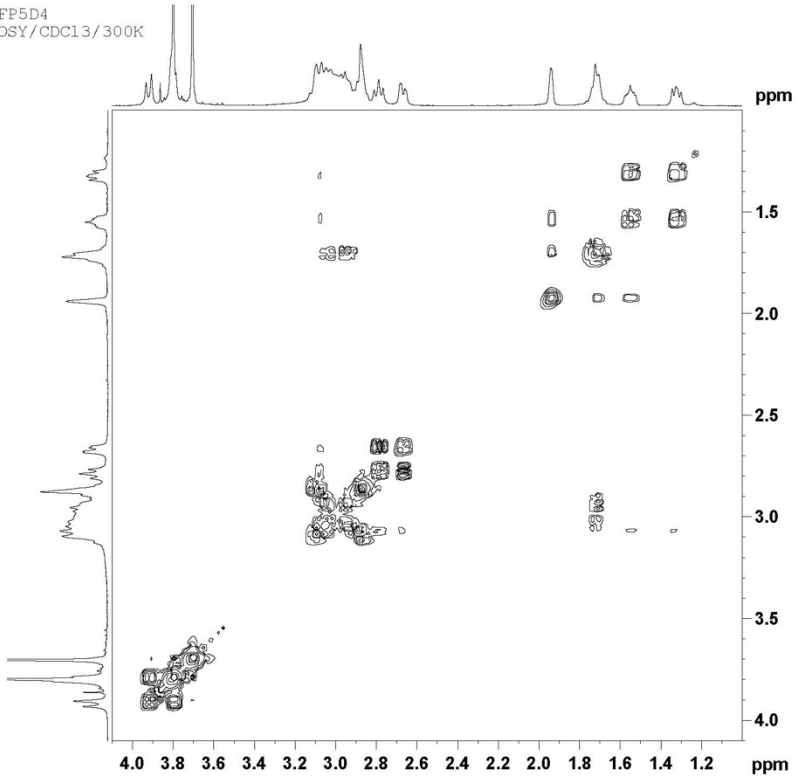
====== GRADIENT CHANNEL =====
GPNAM1          SINE.100
GPZ1               10.00 %
P16              1000.00 usec

F1 - Acquisition parameters
ND0                   1
TD                  256
SFO1          500.1328 MHz
FIDRES       21.492435 Hz
SW              11.001 ppm
FnMODE               QF

F2 - Processing parameters
SI                 1024
SF          500.1300232 MHz
WDW                SINE
SSB                   0
LB                 0.00 Hz
GB                    0
PC                 1.40

F1 - Processing parameters
SI                 1024
MC2                  QF
SF          500.1300232 MHz
WDW                SINE
SSB                   0
LB                 0.00 Hz
GB                    0
```

WFP5D4
COSY/CDCl3/300K




```
Current Data Parameters
NAME         0215-WFP5D4
EXPNO                 11
PROCNO                 1

F2 - Acquisition Parameters
Date_           20190215
Time              17.18
INSTRUM           spect
PROBHD   5 mm TXI 13C Z
PULPROG        cosygpqf
TD                 2048
SOLVENT           CDCl3
NS                    1
DS                   16
SWH            5482.456 Hz
FIDRES         2.676980 Hz
AQ             0.1868276 sec
RG                143.7
DW              91.200 usec
DE               6.50 usec
TE              300.0 K
d0           0.00000300 sec
D1           2.00000000 sec
d13          0.00000400 sec
D16          0.00020000 sec
IN0          0.00018175 sec

======== CHANNEL f1 ========
NUC1                  1H
P0                 9.00 usec
P1                 9.00 usec
PL1               -4.60 dB
SFO1        500.1327507 MHz

====== GRADIENT CHANNEL =====
GPNAM1          SINE.100
GPZ1               10.00 %
P16              1000.00 usec

F1 - Acquisition parameters
ND0                   1
TD                  256
SFO1          500.1328 MHz
FIDRES       21.492435 Hz
SW              11.001 ppm
FnMODE               QF

F2 - Processing parameters
SI                 1024
SF          500.1300232 MHz
WDW                SINE
SSB                   0
LB                 0.00 Hz
GB                    0
PC                 1.40

F1 - Processing parameters
SI                 1024
MC2                  QF
SF          500.1300232 MHz
WDW                SINE
SSB                   0
LB                 0.00 Hz
GB                    0
```

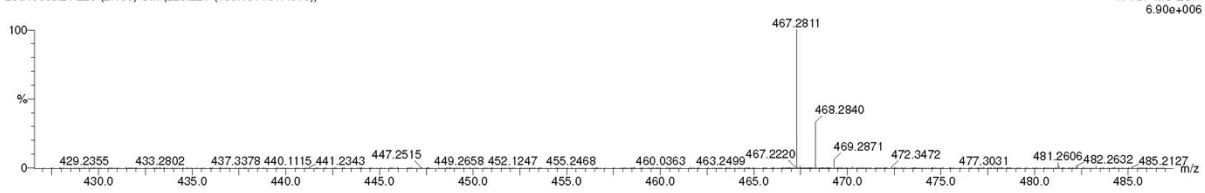
Supplementary Figure S23. HRESIMS analysis of Cpd 5D4.

Elemental Composition Report

Single Mass Analysis
Tolerance = 200.0 PPM / DBE: min = -10.0, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
611 formula(e) evaluated with 99 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 1-80 H: 1-100 N: 1-10 O: 1-10
5D4
200408esi21 220 (2.160) Cm (220:221-(180:181+317:318))

1: TOF MS ES+
6.90e+006



Minimum:				-10.0	
Maximum:		5.0	200.0	100.0	
Mass	Calc. Mass	mDa	PPM	DBE	Formula
467.2811	467.2811	0.0	0.0	15.5	C30 H35 N4 O
	467.2829	-1.8	-3.9	2.5	C18 H39 N6 O8
	467.2789	2.2	4.7	-1.5	C13 H39 N8 O10
	467.2843	-3.2	-6.8	7.5	C19 H35 N10 O4
	467.2771	4.0	8.6	11.5	C25 H35 N6 O3
	467.2757	5.4	11.6	6.5	C24 H39 N2 O7
	467.2870	-5.9	-12.6	6.5	C23 H39 N4 O6
	467.2883	-7.2	-15.4	11.5	C24 H35 N8 O2
	467.2730	8.1	17.3	7.5	C20 H35 N8 O5
	467.2901	-9.0	-19.3	-1.5	C12 H39 N10 O9
	467.2717	9.4	20.1	2.5	C19 H39 N4 O9
	467.2910	-9.9	-21.2	10.5	C28 H39 N2 O4
	467.2699	11.2	24.0	15.5	C31 H35 N2 O2
	467.2690	12.1	25.9	3.5	C15 H35 N10 O7
	467.2942	-13.1	-28.0	2.5	C17 H39 N8 O7
	467.2658	15.3	32.7	11.5	C26 H35 N4 O4
	467.2969	-15.8	-33.8	1.5	C21 H43 N2 O9
	467.2982	-17.1	-36.6	6.5	C22 H39 N6 O5
	467.2631	18.0	38.5	12.5	C22 H31 N10 O2
	467.2995	-18.4	-39.4	11.5	C23 H35 N10 O