Supplemental Materials and Methods

Zhang, G.T. *et al.*

Chemicals and General Procedure

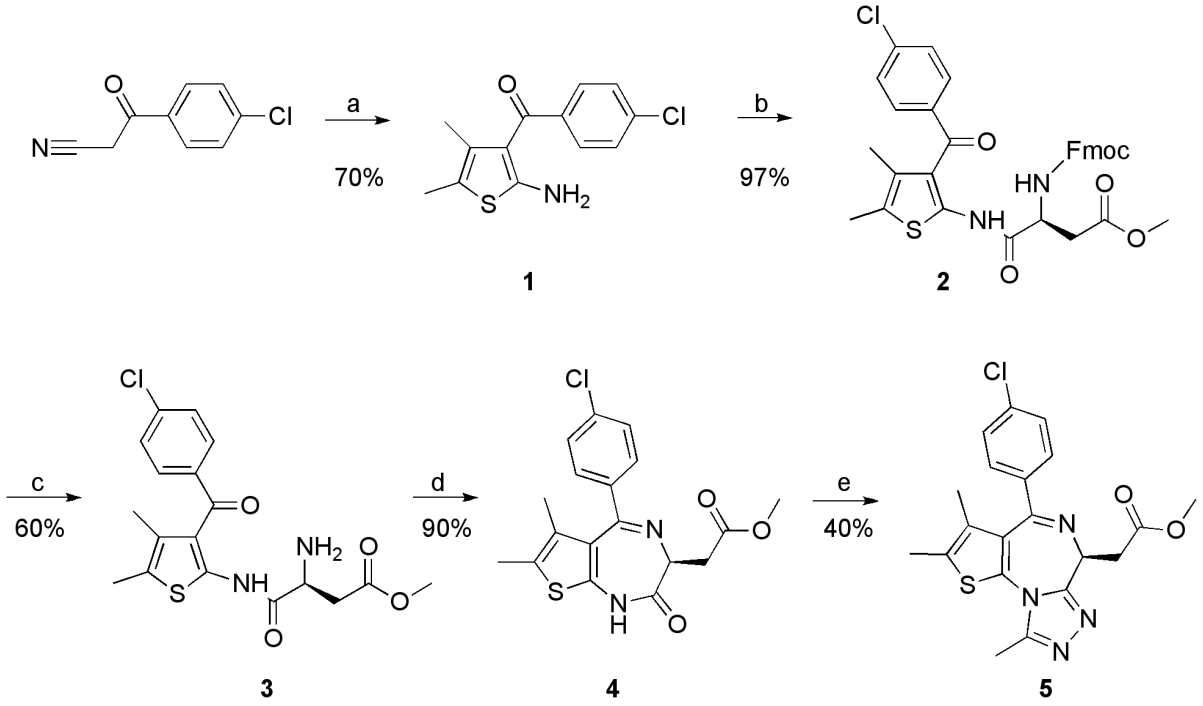
Unless otherwise stated, all commercially available materials were obtained from Acros Organics, Aldrich, Alfa Aesar, EMD, J. T. Baker, Novabiochem, or TCI, and were used without any further purification. When necessary, solvents and reagents were dried prior to use, using standard protocols. All non-aqueous reactions were carried out in oven-dried glassware under an atmosphere of argon. Automatic chromatography was performed on a Biotage Isolera system equipped with a variable wavelength detector and a fraction collector, using Biotage SNAP cartridge KP-Sil 50 g. Analytical thin layer chromatography (TLC) was performed employing Sigma-Aldrich 250 μm 60F-254 silica plates. The plates were visualized either by exposure to UV light, staining with iodine impregnated silica gel, or by staining with ceric ammonium molybdate (CAM). Preparative TLC was performed employing Silicycle 1000 μm Silia*Plate* Prep silica plates. LC/MS analysis was conducted on an Agilent Technologies high-resolution ESI-TOF mass spectrometer attached to an Agilent Technologies 1200 HPLC system. Samples were ionized by electrospray ionization (ESI) in positive mode. Chromatography was performed on a 2.1 × 150 mm Zorbax 300SB-C18 5 μm column with water containing 0.1% formic as solvent A and acetonitrile containing 0.1% formic acid as solvent B at a flow rate of 0.4 mL/min. The gradient program was as follows: 1% B (0-1 min), 1-99% B (1-4 min), 99% B (4-7 min). The temperature of the column was held at 50 °C for the entire analysis. HPLC purification was performed on a 9.4 mm × 250 mm Eclipse XDB-C18 5 μm column coupled with an Agilent Technologies 1200 preparative HPLC system. The gradient program was as follows: 10% B (0-4 min), 10-90% B (4-14 min), 90% B (14-18 min). Chiral purification was performed on a 4.6 mm × 250 mm (R,R)-Whelk-O1 5100 Kromasil 5 μm chiral column from Regis Technologies, Inc., which is coupled with an Agilent Technologies 1200 semi-preparative HPLC system. The samples were eluted with 1:1 methanol/ ethanol at a flow rate of 1.0 mL/min. NMR spectra were acquired on a Bruker DRX-600 spectrometer at 600 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts are expressed in parts per million downfield from tetramethylsilane (TMS), using either TMS or the solvent resonance as an internal standard (TMS, ^{1}H: 0 ppm; chloroform, ^{13}C: 77.0 ppm; DMSO-d6, ^{1}H: 2.50 ppm; ^{13}C: 39.5 ppm; methanol-d4, ^{1}H: 3.31 ppm; ^{13}C: 49.0 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q =quartet, m = multiplet, br = broad), integration, and coupling constant. IR spectra were obtained on a Bruker TENSOR 27 series FT-IR spectrometer equipped with a Diamond ATR. Finally, for optical activity analysis of enantiomers, optical rotations were recorded on a Jasco P-2000 multi-option polarimeter that is equipped with a sodium lamp as radiation source (589 nm).

Chemical Synthesis

The synthesis of stereospecific thioenotriazolodiazepine (**5**), also named as MS417, was carried out by following the reaction steps as outlined in **Scheme 1**.

(2-amino-4,5-dimethylthiophen-3-yl)(4-chlorophenyl)methanone (1). A 100 mL pressure vessel was charged with 4-chlorobenzoylacetonitrile (2.88 g, 15.71 mmol), 2-butanone (1.14 g, 15.71 mmol, 1.41 mL), and sulfur (0.51 g, 15.71 mmol) sequentially. This mixture was dissolved in absolute ethanol (35.0 mL) and allowed to stir vigorously. To this solution was added piperidine (0.90 g, 15.71 mmol, 1.04 mL) dropwise. The solution was heated up to 110˚C for 10 h under argon in an oil bath. The organic solvent was removed in vacuo. Purification by automatic chromatography (4:1 hexane in ethyl acetate, R_f = 0.36) provided the title product as a beautiful yellow crystal (2.65 g, 63%). ^{1}H NMR ($CDCl_3$) δ 7.47 (d, J = 7.4, 2H), 7.39 (d, J =

7.4, 2H), 6.53 (br s, 2H), 2.14 (s, 3H), 1.56 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 191.5, 163.1, 140.1, 136.7, 129.4, 128.4, 117.1, 15.5, 12.5. FTIR (neat, cm^{-1}) 3344 (s, NH_2), 3242 (s, NH_2) 1085 (s, C-N), 692 (s, C-S). MS calculated for $C_{13}H_{12}ClNOS$ $[M+H]^+$ 266.03, found 266.05. Purity >99%, t_R = 6.3 min.



Scheme 1. Synthesis of Stereospecific Thioenotriazolodiazepine **5**. Conditions: **(a)** 4-chlorobenzoyl-acetonitrile (1.0 equiv), 2-butanone (1.0 equiv), sulfur (1.0 equiv), piperidine (1.0 equiv), EtOH, 110°C, 10 h; **(b)** Fmoc-L-Asp(OMe) (1.0 equiv), PyBOP (0.95 equiv), DIPEA (2.75 eq.), DMF, room temperature, 4 h; **(c)** 20% piperidine, room temperature, 1h; **(d)** silica gel, toluene, 90°C, overnight; **(e)** i) KO*t*Bu (1.1 eq.), THF, -78 °C → -10°C , 30 min; ii) $PO(OEt)_2Cl$ (1.2 eq), -78°C → -10°C, 45 min; iii) acetic hydrazide (3.0 eq.), BuOH, 90 °C, 1h.

(S)-methyl 3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-((3-(4-chlorobenzoyl)-4,5-dimethylthiophen-2-yl)amino)-4-oxobutanoate (2). A 4.0 mL vial was charged with Fmoc-L-aspartic acid methyl ester (369.4 mg, 1.0 mmol) and 1.0 mL DMF, PyBOP (494.4 mg, 0.95 mmol) and DIPEA (355.4 mg, 2.75 mmol) sequentially. After stirring at room temperature for 10 min, **1** (265.8 mg, 1.0 mmol) was added as solid all at once and was allowed to stir vigorously at ambient temperature for 4 h. The crude sample was concentrated in vacuo to a minimal possible volume. Purification by automatic chromatography (4:1 hexane in ethyl acetate, R_f = 0.18) provided the title compound as a bright yellow powder (4.53 g, 97%). ^{1}H NMR ($CDCl_3$) δ 11.61 (s, 1H), 7.57 (d, J = 8.4, 2H), 7.43 (d, J = 8.4, 2H), 5.81 (d, 1H), 4.75 (m, 1H), 3.70 (s, 3H), 3.23 (m, 1H), 2.83 (m, 1H), 2.27 (s, 3H), 1.69 (s, 3H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 193.0, 172.0, 168.8, 155.4, 145.3, 138.5, 138.2, 130.2, 128.6, 127.6, 125.3, 123.1, 113.2, 81.1, 52.8, 52.2, 35.4, 28.3, 15.0, 14.2. FTIR (neat, cm^{-1}) 3105 (s, NH), 2975 (s, N-H), 1731 (s, C=O), 1214 (s, C-O), 669 (s, C-S). MS calculated for $C_{23}H_{27}ClN_2NaO_6S$ $[M+Na]^+$ 517.12, found 517.13. Purity >99%, t_R = 6.8 min.

(S)-methyl 3-amino-4-((3-(4-chlorobenzoyl)-4,5-dimethylthiophen-2-yl)amino)-4-oxobutanoate (3). A 20 mL scintillation vial was charged with **2** (810 mg, 1.31 mmol) and 20% piperidine in DMF (6.0 mL). This mixture was allowed to stir vigorously at room temperature for

1h. The reaction was quenched by adding saturated NaCl (aq., 40 mL). The aqueous layer was extracted three times by ethyl acetate (40 mL × 3). The combined organic layer was washed with brine and dried on anhydrous Na_2SO_4. The volatiles were removed in vacuo. The title compound appeared as yellow oil (300 mg, 58%). ^{1}H NMR ($CDCl_3$) δ 12.18 (br s, 1H), 7.60 (d, J = 8.4, 2H), 7.43 (d, J = 8.4, 2H), 3.91 (t, J = 3.9, 1H), 3.72 (s, 3H), 3.01 (m, 1H), 2.83 (m, 1H), 2.28 (s, 3H), 1.92 (br s, 2H), 1.73 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 193.4, 171.3, 170.8, 165.4, 142.1, 138.9, 137.5, 130.4, 128.7, 128.1, 127.0, 125.0, 52.9, 50.1, 34.8, 14.6, 14.0. FTIR (neat, cm^{-1}) 1290 (s, C-O), 1210 (d, C-O). MS calculated for $C_{18}H_{19}ClN_2O_4S$ $[M+H]^+$ 395.08, found 395.08. Purity >99%, t_R = 5.8 min.

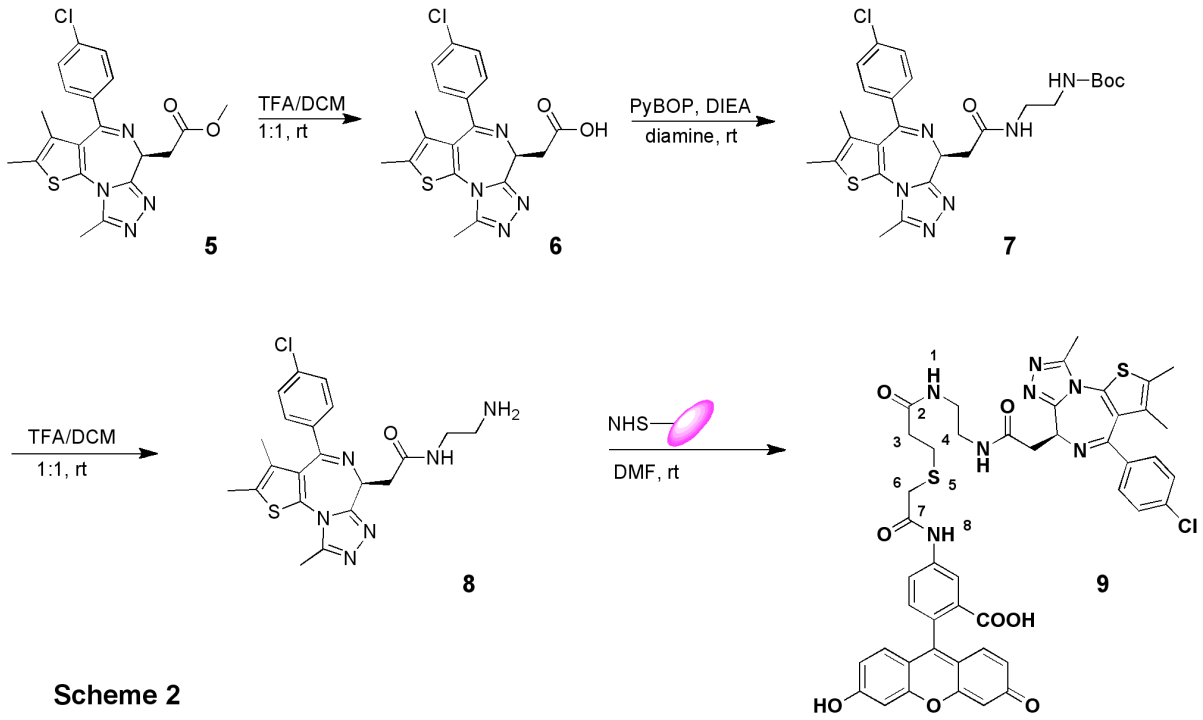
(S)-methyl 2-(5-(4-chlorophenyl)-6,7-dimethyl-2-oxo-2,3-dihydro-1H-thieno[2,3-e][1,4]diazepin-3-yl)acetate (4). A 35 mL sealed tube was charged with compound **3** (300 mg, 0.76 mmol) and toluene (24 mL). The mixture was allowed to heat to 90 °C and refluxed for overnight. The crude sample was first filtered and then concentrated in vacuo. Purification by automatic chromatography (3:2 hexane in ethyl acetate, R_f = 0.16) provided the title compound as a bright yellow crystal (265 mg, 90%). ^{1}H NMR ($CDCl_3$) δ 9.49 (s, 1H), 7.41 (d, J = 8.4, 2H), 7.32 (d, J = 8.4, 2H), 4.25 (m, 1H), 3.73 (s, 3H), 3.43 (m, 1H), 3.17 (m, 1H), 2.27 (s, 3H), 1.58 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 172.4, 169.4, 165.3, 141.5, 136.8, 136.3, 130.2, 129.5, 128.5, 127.5, 126.7, 113.3, 61.2, 51.8, 36.3, 14.4, 12.9. FTIR (neat, cm^{-1}) 1290 (s, C-O), 1210 (d, C-O). MS calculated for $C_{18}H_{17}ClN_2O_3S$ $[M+H]^+$ 377.06, found 377.07. Purity >99%, t_R = 6.4 min.

Methyl 2-((6S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4] triazolo[4,3-a][1,4]diazepin-6-yl)acetate (5). A 50 mL round bottom flask was charged with compound **4** (125 mg, 0.3 mmol) and THF (1.6 mL). The solution was cooled to -78 °C and potassium tert-butoxide (37.0 mg, 0.33 mmol, 1.1 eq.). This mixture was warmed up to -10 °C and sustained for 30 min. The mixture was cooled again to -78 °C and diethyl chlorophosphate (62.1 mg, 0.36 mmol) was added drop wise. The solution was warmed to -10 °C and sustained for 45 min. After reaction was complete, n-butanol (1.8 mL) and acetic hydrazide (66.7 mg, 900 mmol) were added sequentially. This mixture was stirred at room temperature for 1 h and heated up to 90 °C for 1 h. The crude sample was filtered and the residual concentrated in vacuo. Purification by automatic chromatography (0-100% ethyl acetate in hexane, R_f = 0.3) provided the title compound. The pure product appeared as a pale yellow crystal (55.0 mg, 40%). ^{1}H NMR ($CDCl_3$) δ 7.42 (d, J = 7.8, 2H), 7.35 (d, J = 7.8, 2H), 4.62 (m, 1H), 3.79 (s, 3H), 3.65 (m, 2H), 2.69 (s, 3H), 2.43 (s, 3H), 1.70 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 172.1, 163.9, 155.3, 150.0, 136.8, 136.6, 132.3, 130.9, 130.8, 130.4, 129.9, 129.8, 128.7, 116.8, 53.8, 51.9, 36.7, 14.4, 13.1, 11.9. FTIR (neat, cm^{-1}) 1290 (s, C-O), 1210 (d, C-O). MS calculated for $C_{20}H_{19}ClN_4O_2S$ $[M+H]^+$ 415.09, found 415.09. Purity >99%, t_R = 6.6 min. Chiral resolution: t_{6R} = 8.2 min, t_{6S} = 11.5 min (See **Figure S6**). Specific rotation activities (α_D^{20}) of **5** (also named as MS417) and its enantiomer (i.e. MS566) were measured to be 19.9 and -20.1, respectively (see **Table S4**).

Synthesis of FITC-labeled chemical probe. A fluorescein-conjugated thioenotriazolodiazepine compound (**9**), also named as MS574, was synthesized by using the synthetic scheme as described in **Scheme 2**.

2-((6S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic acid (6). A 4 mL vial was charged with compound **5** (2.6 mg, 6.3 μmol) and THF (0.4 mL). The solution was stirred vigorously at room temperature for 5 min. To this solution was added lithium hydroxide monohydrate (2.7 mg, 63 μmol) and deionized water (0.1 mL). The mixture was stirred at room temperature for 2 h and the reaction was quenched. The organic content was removed *in vacuo* and the residual material was dissolved in H_2O. The solution was

adjusted to pH 14 with 1.0 M NaOH (aq.) and the aqueous layer was washed with diethyl ether. The pH was then carefully adjusted to pH 1 with 1.0 M HCl (aq.), and the solution was extracted by diethyl ether. The organic phase was dried over $MgSO_4$ and concentrated to provide the title compound. The pure product appeared as a pale yellow oil (2.6 mg, ~100%). ^{1}H NMR ($CDCl_3$) δ 7.42 (d, J = 7.8, 2H), 7.35 (d, J = 7.8, 2H), 4.62 (m, 1H), 3.65 (m, 2H), 2.69 (s, 3H), 2.43 (s, 3H), 1.70 (s, 3H). MS calculated for $C_{19}H_{17}ClN_4O_2S$ $[M+H]^+$ 401.08, found 401.08. Purity >99%, t_R = 5.2 min.



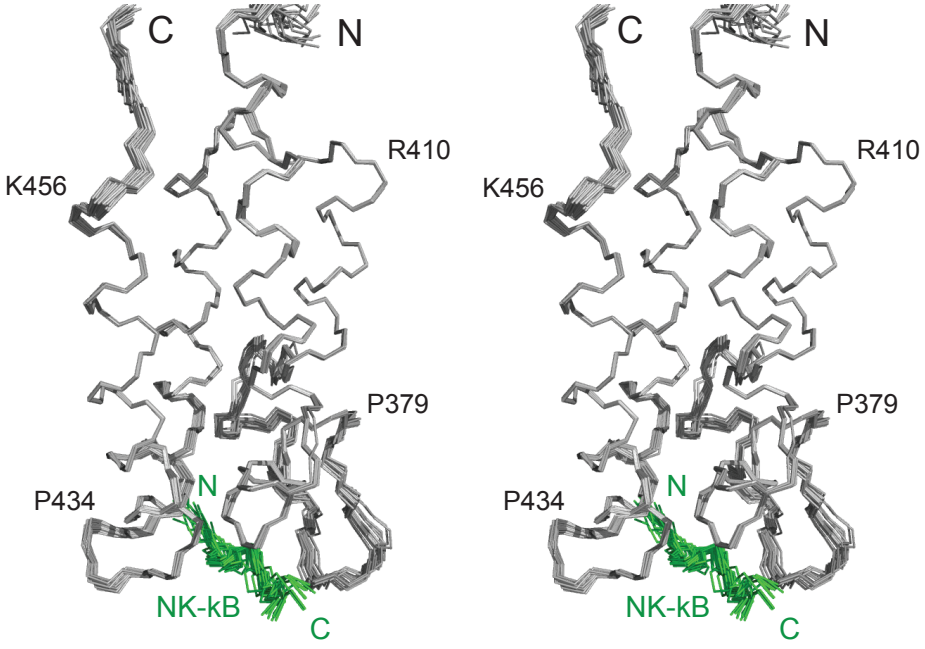
Scheme 2

***tert*-butyl (2-(2-((6*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4] triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamido)ethyl)carbamate (7)**. A 4 mL vial was charged with compound **6** (20.0 mg, 49.9 μmol) and DMF (0.5 mL). To this solution was added PyBOP (24.7 mg, 47.4 μmol) and DIPEA (32.2 mg, 250 μmol). The mixture was stirred at room temperature for 10 min before N-Boc-ethylenediamine (8.0 mg, 50.0 μmol) was added. The reaction was allowed for overnight. The reaction was quenched with 1:1 (v/v) ratio of ethyl acetate and brine. The aqueous layer was extracted with ethyl acetate (2×1 mL). The combined organic layer was washed with brine and dried over sodium sulfate. The organic solvents were removed under reduced pressure and purification by automatic chromatography (0-10% methanol in dichloromethane, R_f = 0.1) provided the title compound. The pure product appeared as a colorless oil (5.2 mg, 20%). ^{1}H NMR ($CDCl_3$) δ 7.41 (d, J = 8.4, 2H), 7.33 (d, J = 8.4, 2H), 6.98 (br s, 1H), 5.19 (br s, 1H), 4.64 (t, J = 6.6, 1H), 3.44-3.34 (m, 2H), 3.33-3.20 (m, 2H), 2.67 (s, 3H), 2.40 (s, 3H), 1.68 (s, 3H), 1.43 (s, 9H). MS calculated for $C_{26}H_{31}ClN_6O_3S$ $[M+H]^+$ 543.19, found 543.21. Purity > 95%, t_R = 5.2 min.

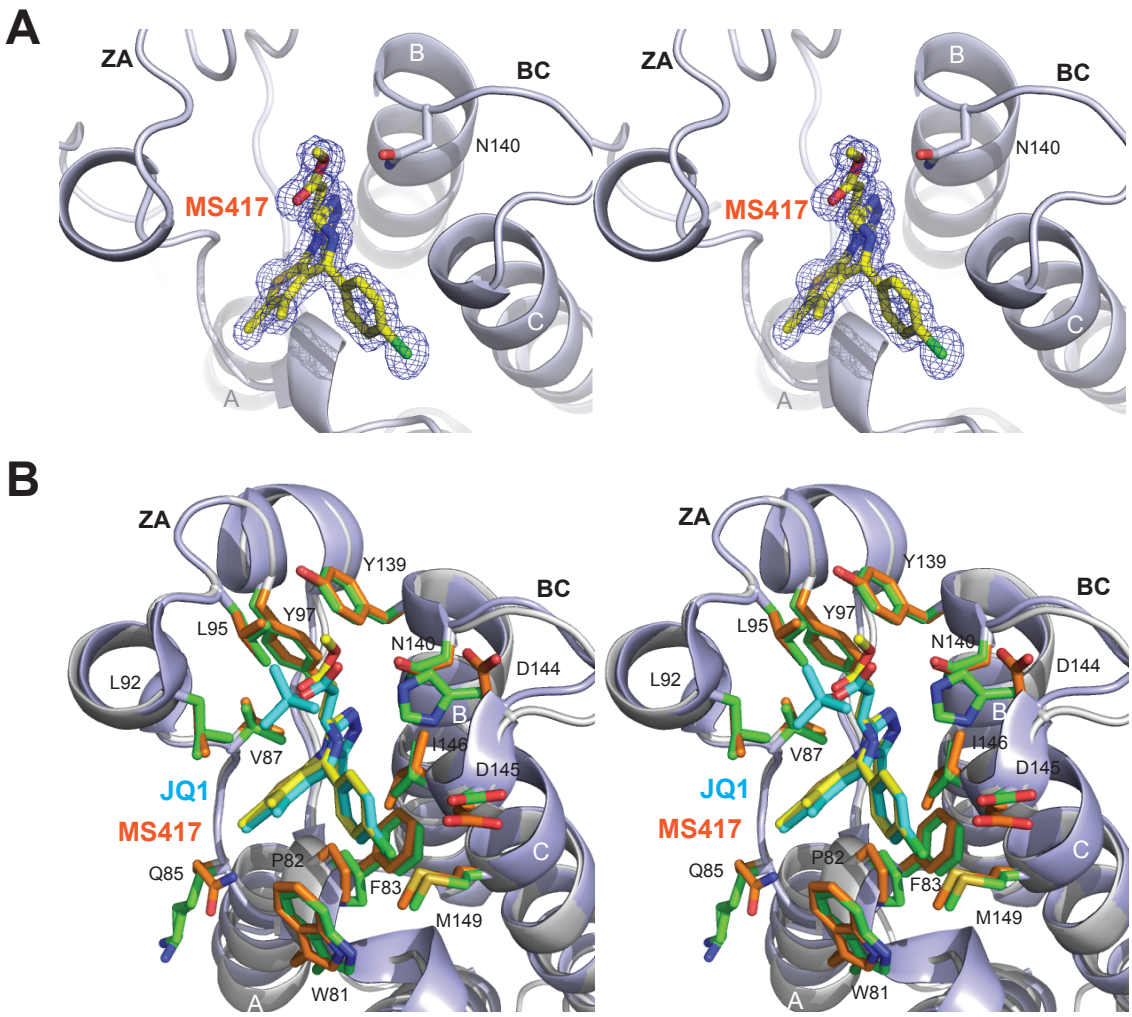
N-(2-aminoethyl)-2-((6S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo [4,3-a][1,4]diazepin-6-yl)acetamide (8). A 4 mL vial was charged with compound **7** (4.0 mg, 7.4 μmol). To this solution was added 1:1 (v/v) ratio of DCM and TFA. The mixture was stirred at room temperature for 2 h until completion as indicated by TLC. The organic content was

removed in vacuo and the residual was put on high vacuum for overnight. The product appeared as pale yellow oil and was used for the next step without further purification. MS calculated for $C_{21}H_{23}ClN_6OS$ $[M+H]^+$ 443.14, found 443.15. Purity > 99%, t_R = 4.8 min.

2-((6S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic amide - a Fluorescein-5-EX conjugate (9) (also named as **MS574**). A 4 mL vial was charged with compound 8 (3.3 mg, 7.4 µmol). DMF (300 µL), Fluorescein-5-EX (4.4 mg, 7.4 µmol), and DIPEA (9.5 mg, 73.6 µmol) were added sequentially. The mixture was allowed to stir at room temperature for overnight. At the time, the reaction was stopped and the solvent removed by air stream. Purification by HPLC provided the title compound (10-90 % ACN in water, t_R = 12.8 min. The pure product appeared as a bright yellow powder (2.9 mg, 45%). MS calculated for $C_{46}H_{40}ClN_7O_8S_2$ $[M+H]^+$ 918.21, found 918.22. Purity > 99%, t_R = 5.2 min.

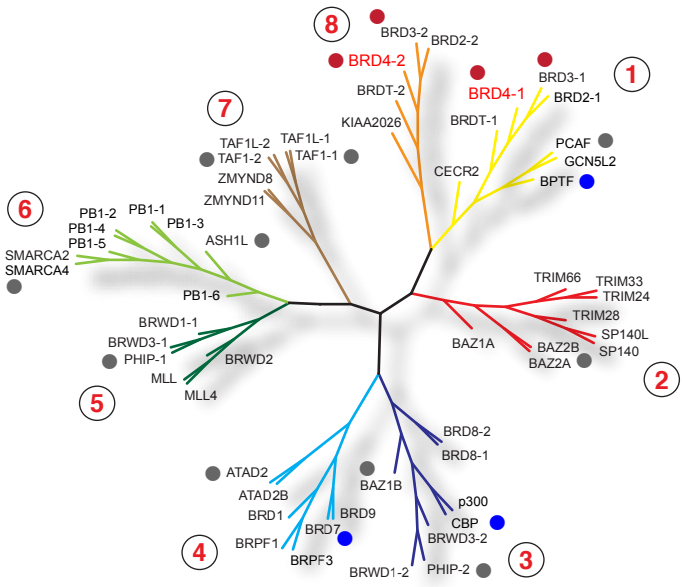


Supplementary Figure 1. The NMR solution structure of the BRD4-BD2 bound to an NF-κB-K310ac peptide.
Superimposition of the backbone atoms (N, Cα and C') of the final 20 NMR structures of the bromodomain in complex with the NF-kB-K310ac peptide (green).



Supplemental Figure 2. (**A**) Stereoview of the crystal structure highlighting the electron density of MS417 bound in the BRD4-BD1. (**B**) Comparison of the 3D crystal structures of the BRD4-BD1 bound to MS417 (light blue) and the BRD2-BD2 bound to JQ1 (grey) (PDB ID 3ONI). Side chains of the key protein residues located at the ligand binding sites in each BrD are shown, color-coded by atom type. Carbon atoms are shown in orange in BRD4-BD1/MS417 complex, and green in the BRD2-BD2/JQ1 complex.

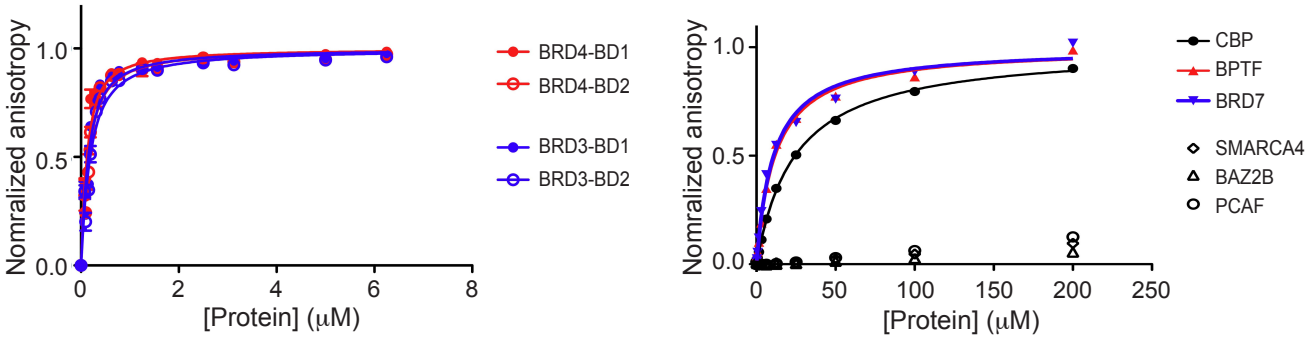
A



C

BrD	K_d (μM)
BRD4-BD1	0.085 ± 0.009
BRD4-BD2	0.113 ± 0.010
BRD3-BD1	0.110 ± 0.010
BRD3-BD2	0.147 ± 0.012
CBP	18.0 ± 0.9
BRD7	10.8 ± 1.0
BPTF	11.8 ± 0.7
BAZ1B	No binding
BAZ2B	No binding
ATAD2	No binding
ASH1L	No binding
SMARCA4	No binding
PCAF	No binding
PHIP-1	No binding
TAF1L	No binding
TAF1	No binding

B

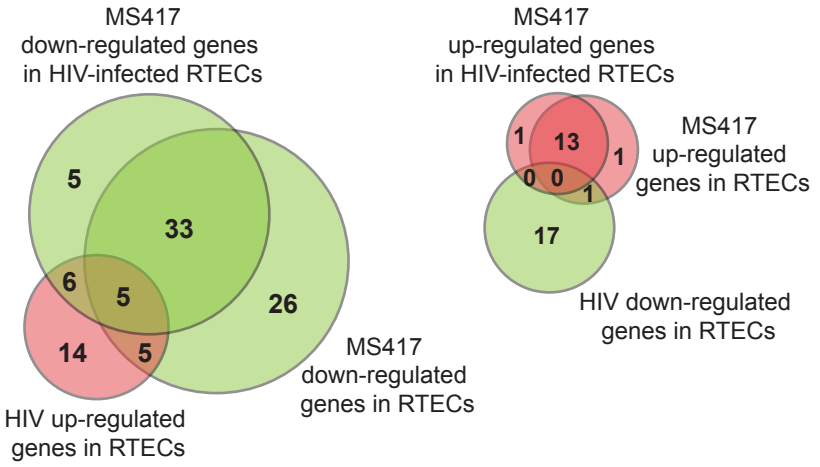


Supplemental Figure 3. Binding affinity of FITC-labeled MS417 (i.e. MS574) to different human BrDs.

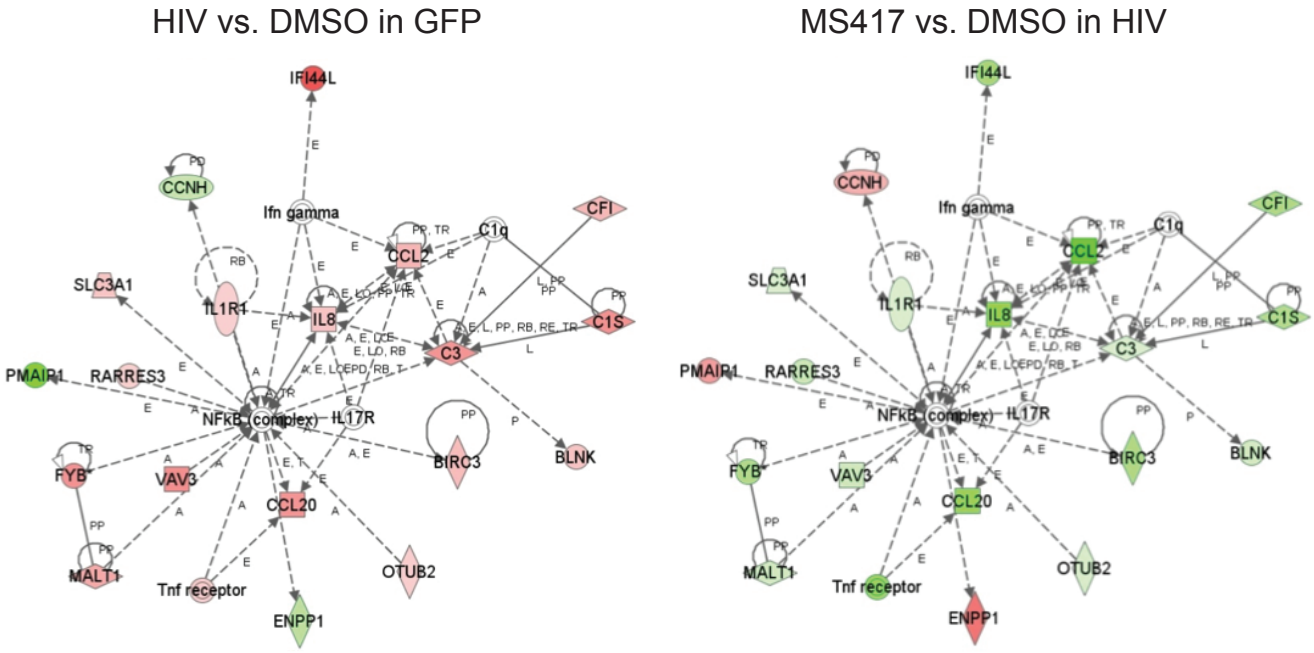
(**A**) Phyologenetic tree depicting a family of human bromodomains. The BrDs that were evaluated in binding to FITC-labeled MS417 (also named as MS574) are indicated by a dot. These dots are color-coded for high affinity (red), modest affinity (blue), or no binding (gray) as shown in ***C***. (**B**) Representative fluoescence anisotropy binding curves of FITC-MS417 to different BrDs as a function of protein concentration. (**C**) Table listing the affinity (Kd) of the FITC-MS417 binding to the BrDs that represent different subgroups of the human BrD family as illustrated in the phyologenetic tree.

A

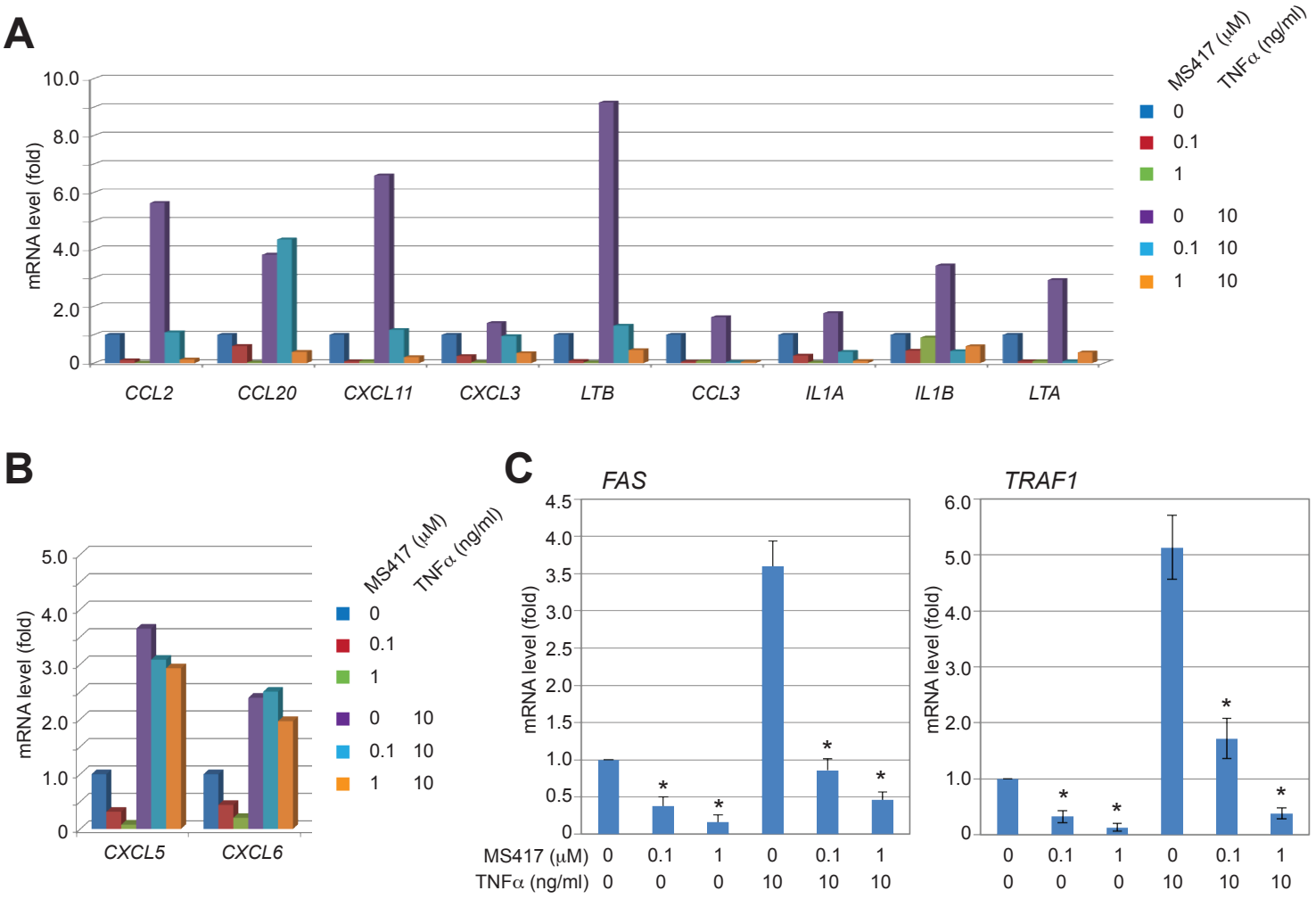
	Gene expression	Total genes	NF-κB targets	Enrichment fold	P value
HIV *vs.* DMSO in GFP	Up-regulation	866	30	1.48	0.028
	Down-regulation	685	18	1.12	0.52
MS417 *vs.* DMSO in HIV	Down-regulation	892	49	2.48	1.4e-7
	Up-regulation	1192	14	0.51	8.3e-3
MS417 *vs.* DMSO in GFP	Down-regulation	1467	69	2.11	1.7e-7
	Up-regulation	1999	15	0.32	5.3e-7



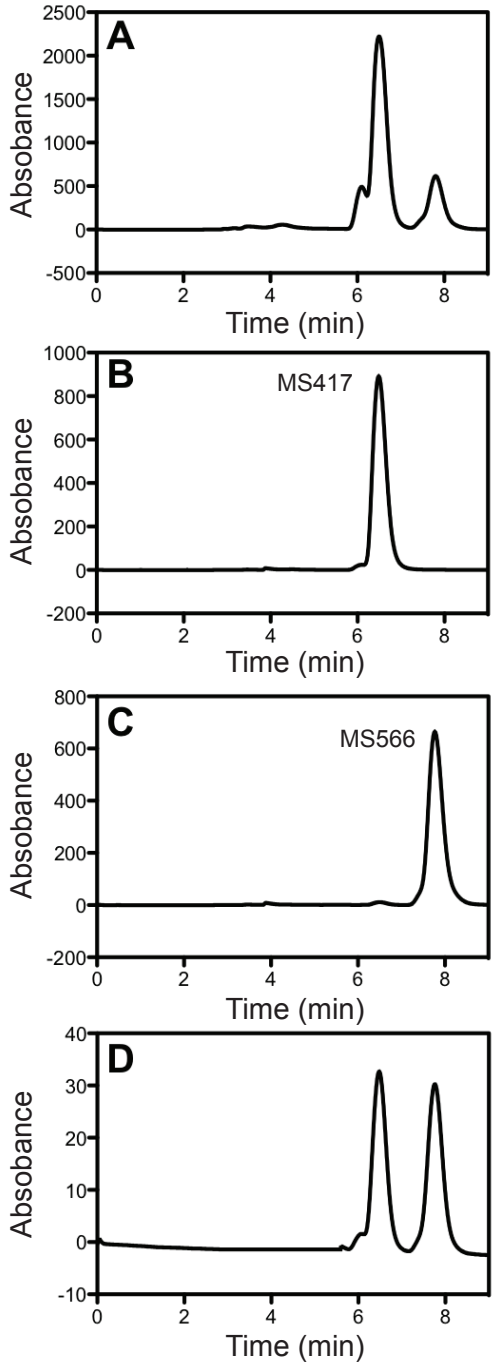
B



Supplemental Figure 4. Microarray analysis of MS417 effects on gene transcription in human primary renal tubular epithelial cells (RTECs).
(**A**) Venn diagram of the NF-κB targets with transcriptional expression affected by both MS417 and HIV infection. These genes were identified by Significance Analysis of Microarray program using q value of 0.05. Upper, a table lists enrichment statistics of these NF-κB targets affected by HIV infection and/or MS417. The enrichment fold is the ratio of the percentage of NF-κB targets in differentially expressed genes to that of NF-κB targets in the whole gene list. The p value was calculated using Fisher's exact test. **(B)** Regulation of the NF-κB network derived from Ingenuity Knowledge database. The genes are color-coded in red or green indicating their up- or down-regulation under the specified conditions.



Supplemental Figure 5. Modulation of transcription of NF-κB target and non-target genes by MS417. (**A**) MS417inhibits TNFα-induced NF-κB target gene expression in RTEC. The RTEC cells were pre-incubated with or without MS417 at the indicated doses for 30 minutes in serum-free medium and then stimulated with or without TNFα for additional 6 hours. The cells were harvested for cytokine and chemokine PCR arrays. Representative data from three independent experiments are shown. (**B**) Some of TNFα-induced NF-κB target genes were not inhibited by MS417. Renal tubular epithelial cells were pre-incubated with or without MS417 at the indicated doses for 30 minutes in serum-free medium and then stimulated with or without TNFa for additional 6 hours. The cells were then harvested for cytokine and chemokine PCR arrays. Representative data from three independent experiments are shown. (**C**) MS417 inhibits the expression of TNFα-induced apoptosis related genes FAS and TRAF1. The RTEC cells were pre-incubated with or without MS417 at the indicated doses for 30 minutes in serum-free medium and then stimulated with or without TNFα for additional 6 hours. Cells were then harvested for real-time PCR analysis.



Supplemental Figure 6. The chiral purification chromatograms of MS417.
(**A**) HPLC chromatogram of MS417 after stereospecific synthesis. The ratio of MS417 over its enantiomer (MS566) is 85:15; (**B**) HPLC chromatogram of MS417 after chiral purification with purity of >99%; (**C**) HPLC chromatogram of an enantiomer (MS566) of MS417 after chiral purification with purity of >99%; (**D**) HPLC chromatogram of MS417 racemic mixture. The ratio of MS417 to MS566 is 50:50.

Supplemental Table 1. NMR restraints and statistics of the final 20 structures of the BRD4-BD2 in complex with a NF-κB-K310ac peptide

NMR distance and dihedral constraints	
Distance constraints	
Total NOE	2,857
Intra-residue	856
Inter-residue	2,001
Sequential (|i - j| = 1)	609
Medium-range (|i - j| < 4)	725
Long-range (|i – j| > 5)	667
Inter-molecular	20
Hydrogen bonds	58
Total dihedral angle restraints	
Φ angle	79
Ψ angle	79
Ramachandran Map Analysis (%)	
Most favored regions	97.8
Additional allowed regions	2.2
Generously allowed regions	0.0
Disallowed regions	0.0
Structure statistics	
Violations (mean +/- s.d.)	
Distance constraints (A)	0.11 ± 0.0085
Dihedral angle constraints (º)	1.07 ± 0.11
Max. dihedral angle violation (º)	1.33
Max. distance constraint violation (A)	0.12
Deviations from idealized geometry	
Bond lengths (A)	0.0011 ± 0.00017
Bond angles (º)	1.1 ± 0.015
Impropers (º)	2.3 ± 0.079
Average pairwise r.m.s. Deviation ** (A)	
Heavy	0.58 ± 0.093
Backbone	0.28 ± 0.093

**: The residue number ranges used in full molecule RMSD calculations are 352-455.
Pairwise r.m.s. deviation was calculated among 20 refined structures
%: Procheck residue numbers are 349-369, 400-409, 415-430 and 436-456.

Supplemental Table 2. Crystallography data and refinement statistics.

	BRD4-BD1/MS417
Data collection	
Space group	$P2_12_12_1$
Cell dimensions	
a, *b*, *c* (Å)	36.84, 44.66, 78.24
α, β, γ (°)	90.0, 90.0, 90.0
Resolution (Å) (highest resolution shell)	39.12-1.40 (1.43-1.4)
Measured reflections	152,108
Unique reflections	25,982
R_{merge}	5.5 (33.5)
I/σ	43.7 (4.8)
Completeness (%)	99.3 (98.3)
Redundancy	5.8 (5.5)
Refinement	
Resolution (Å)	39.10-1.40
No. reflections	24,656
R_{work}/ R_{free} (%)	13.6/18.3
No. atoms	
Protein	1,076
MS417	28
Water	187
B-factors (Å^2)	
Protein	16.8
MS417	11.7
Water	28.3
RMSD	
Bond lengths (Å)	0.025
Bond angles (°)	2.40
Ramachandran plot % residues	
Favored	99.2
Additional allowed	0.8
Generously allowed	0
Disallowed	0

Supplemental Table 3. Summary of body weight, renal function and histology changes

	Body weight	BUN (mg/dl)	AST & ALT	GS index	Podocyte Hypertrophy	Tubular casts/cysts
WT + DMSO	22.4 ± 1.3	23.5 ± 3.4	93 ± 11 & 47 ± 4	0	0	0
Tg26 + DMSO	21.8 ± 1.5	39.4 ± 4.6	95 ± 10 & 45 ± 3	16.3 ± 7.1	1.5 ± 0.6	10.4 ± 4.8
WT + MS417	22.8 ± 1.7	24.2 ± 2.2	95 ± 11 & 47 ± 2	0	0	0
Tg26 + MS417	21.2 ± 1.6	26.8 ± 3.5*	96 ± 10 & 47 ± 3	3.1 ± 0.9*	0.5 ± 0.5*	2.4 ± 1.0*

Note: HIV-1 transgenic (Tg26) mice and the wild-type littermates were treated with vehicle (DMSO) or MS417 at 0.08 mg/kg from age of 4 weeks and the mice were sacrificed at the age of 8 weeks. Body weight of these mice was recorded and blood urea nitrogen (BUN) was measured as described. The liver function test (AST & ALT) was measured by the Charles River Research Animal Diagnosis Service (Wilmington, MA). The kidney sections from these mice were stained for PAS and the glomerular and tubular injuries were scored as described. N=6, *$p<0.05$ as compared to Tg26 mice treated with DMSO (0.1%).

Supplemental Table 4. Optical Rotation Measurements

Compound	α_D^{20}	Concentration	Solvent	Path length	No. of Measurement
Fmoc-L-Asp(OMe)-OH	-25.8	0.01	DMF	10 mm	4
2	18.5	0.01	$CHCl_3$	10 mm	4
3	28.7	0.01	$CHCl_3$	10 mm	4
4	21.1	0.01	$CHCl_3$	10 mm	4
MS417 (**5**)	19.9	0.01	$CHCl_3$	10 mm	4
MS566	-20.1	0.01	$CHCl_3$	10 mm	4