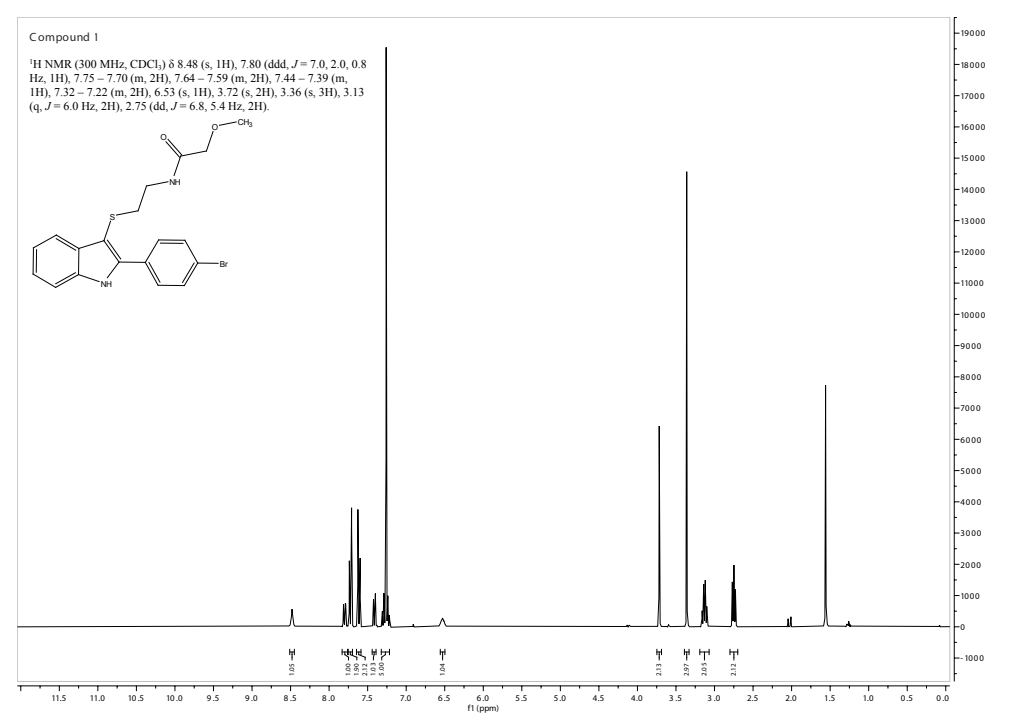
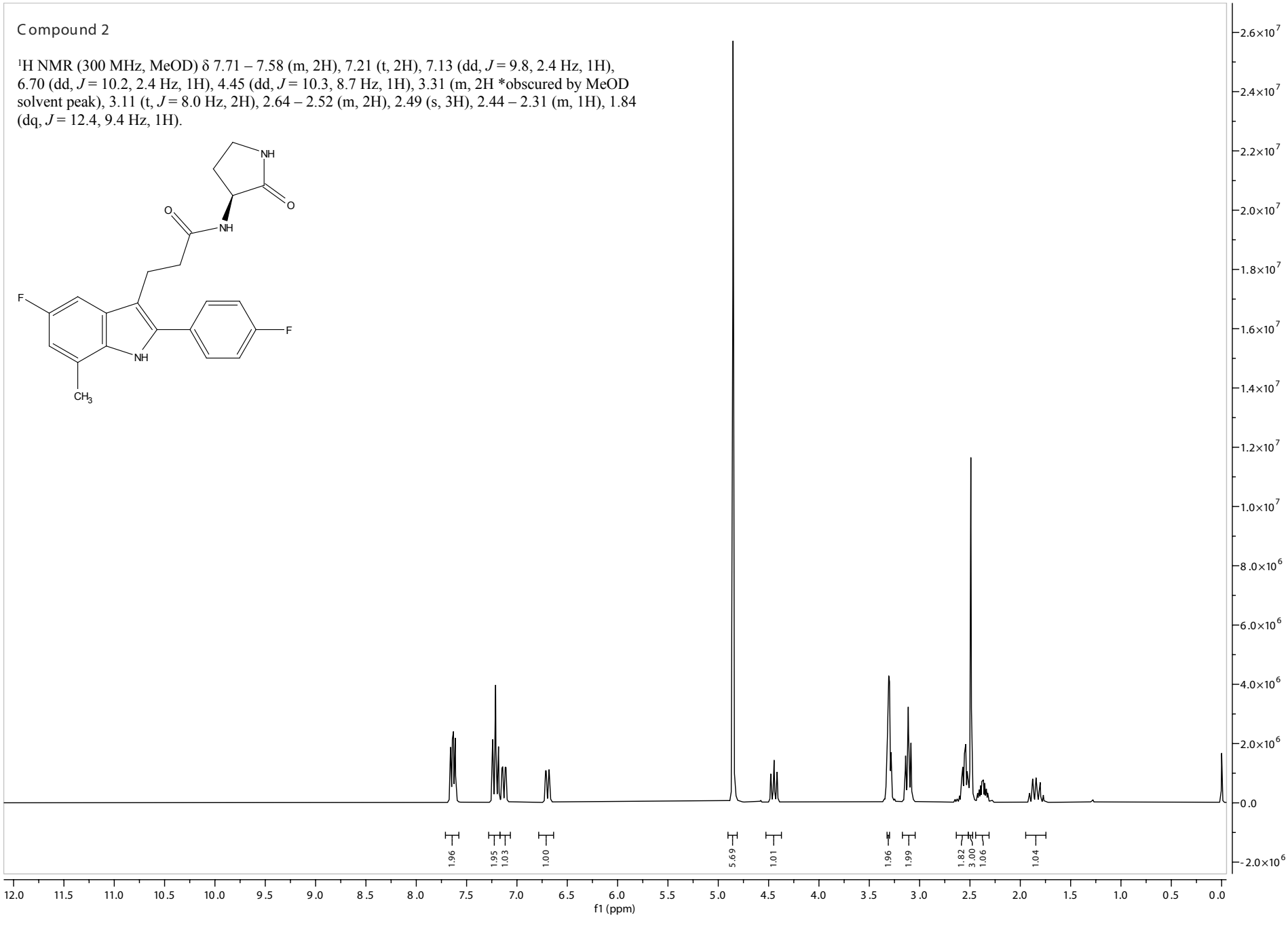
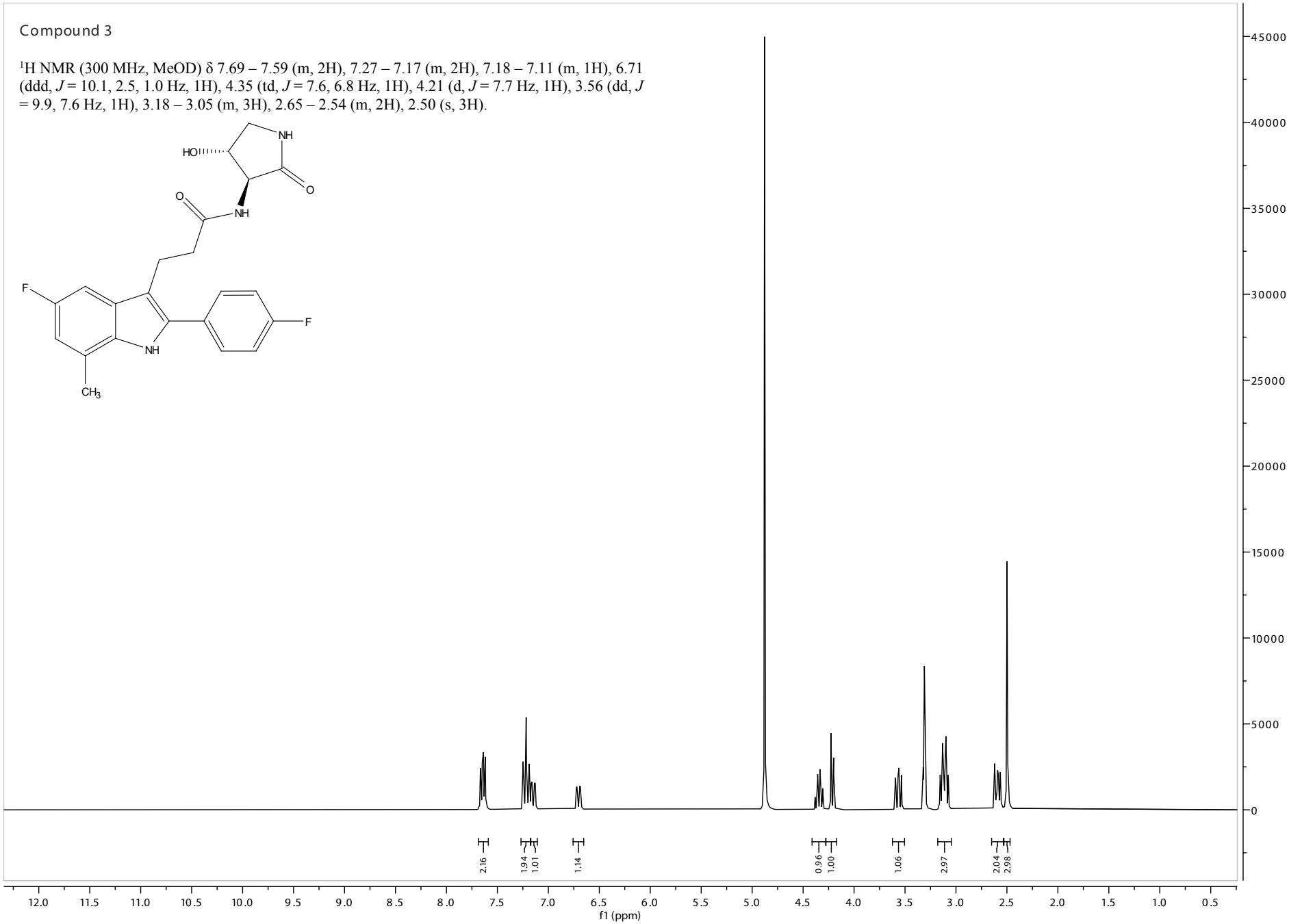


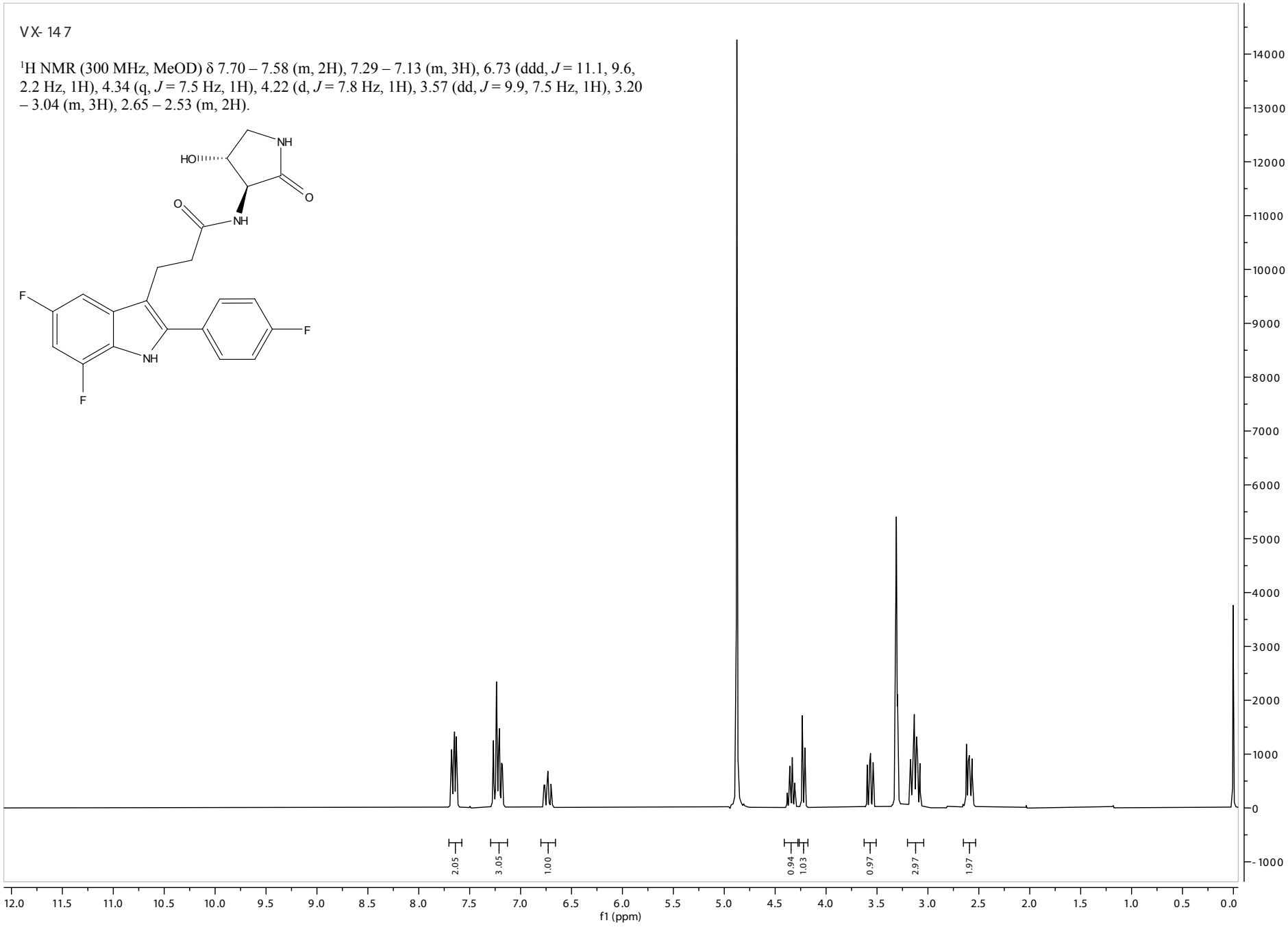
Supplementary Fig. 1. NMR spectra of compound 1



Supplementary Fig. 2. NMR spectra of compound 2

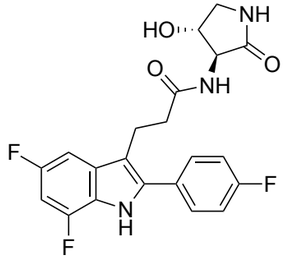


Supplementary Fig. 3. NMR spectra of compound 3

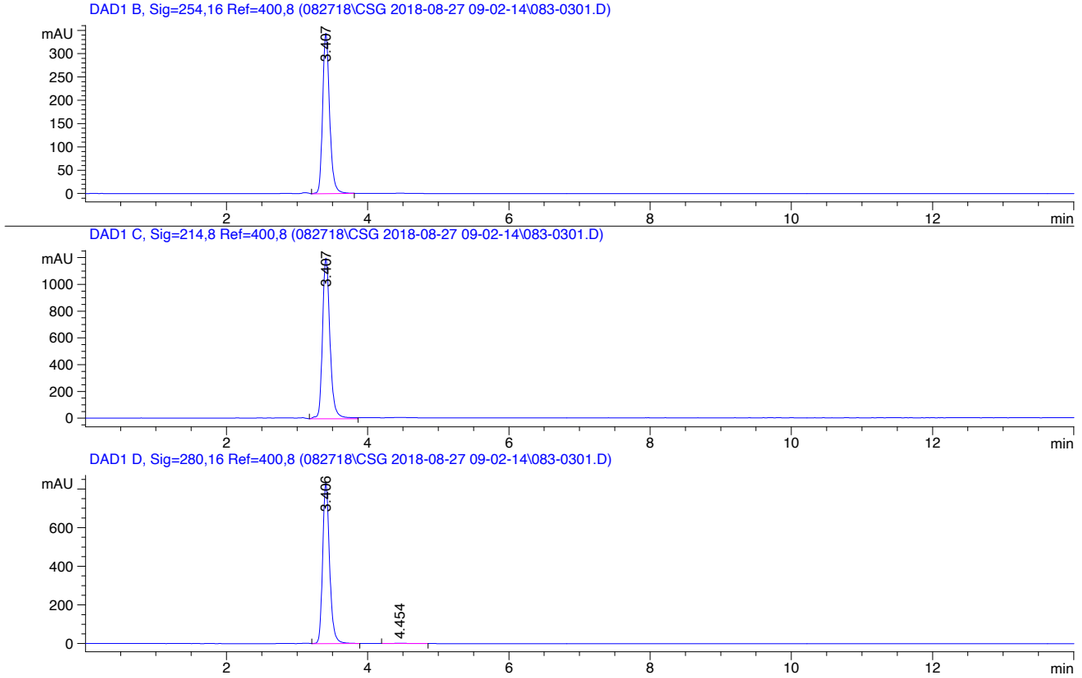


Supplementary Fig. 4. NMR spectra of VX-147

A



B



C

Signal 2: DAD1 B, Sig=254,16 Ref=400,8

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.407	VB	0.1039	2358.86890	344.46649	100.0000
Totals :				2358.86890	344.46649	

Signal 3: DAD1 C, Sig=214,8 Ref=400,8

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.407	VB	0.1119	8827.65039	1199.55701	100.0000
Totals :				8827.65039	1199.55701	

Signal 4: DAD1 D, Sig=280,16 Ref=400,8

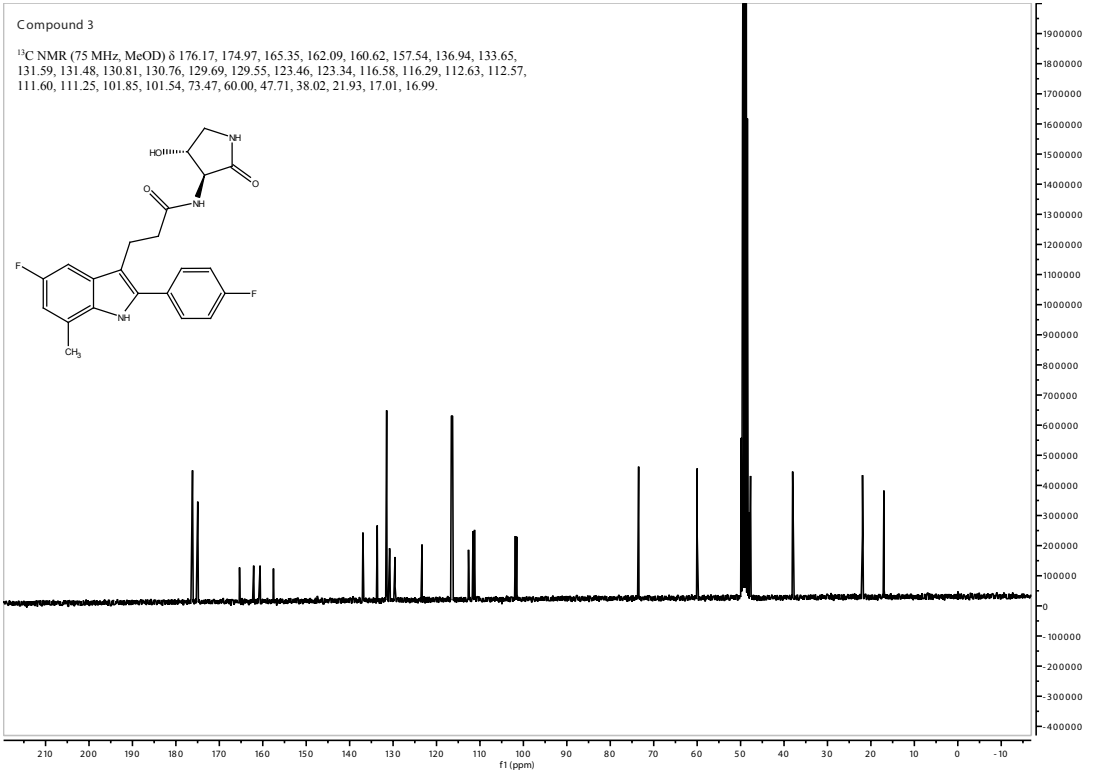
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.406	VB	0.1034	5657.46436	831.20184	99.6068
2	4.454	BB	0.2465	22.33398	1.15061	0.3932
Totals :				5679.79834	832.35246	

Supplementary Fig. 5. HPLC trace at Multiple Wavelengths of VX-147

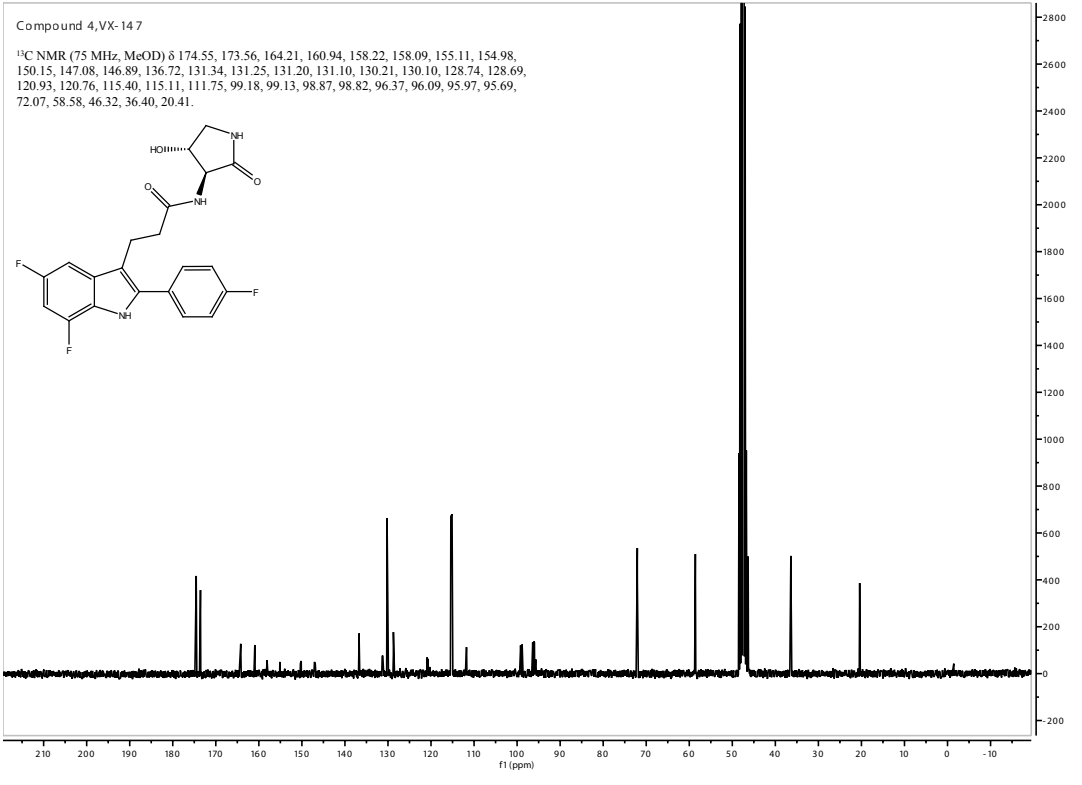
(A) VX-147, 3-[5,7-difluoro-2-(4-fluorophenyl)-1H-indol-3-yl]-N-[(3S,4R)-4-hydroxy-2-oxo-pyrrolidin-3-yl] propenamide.

(B) HPLC traces of VX-147 run in with a linear gradient of 10% to 90% acetonitrile (ACN) in water over a 12 min run with an initial 2 min at 10% ACN in water. Flow rate is 1 mL/min. UV detection at λ = 254 nm, 218 nm, and 280 nm.

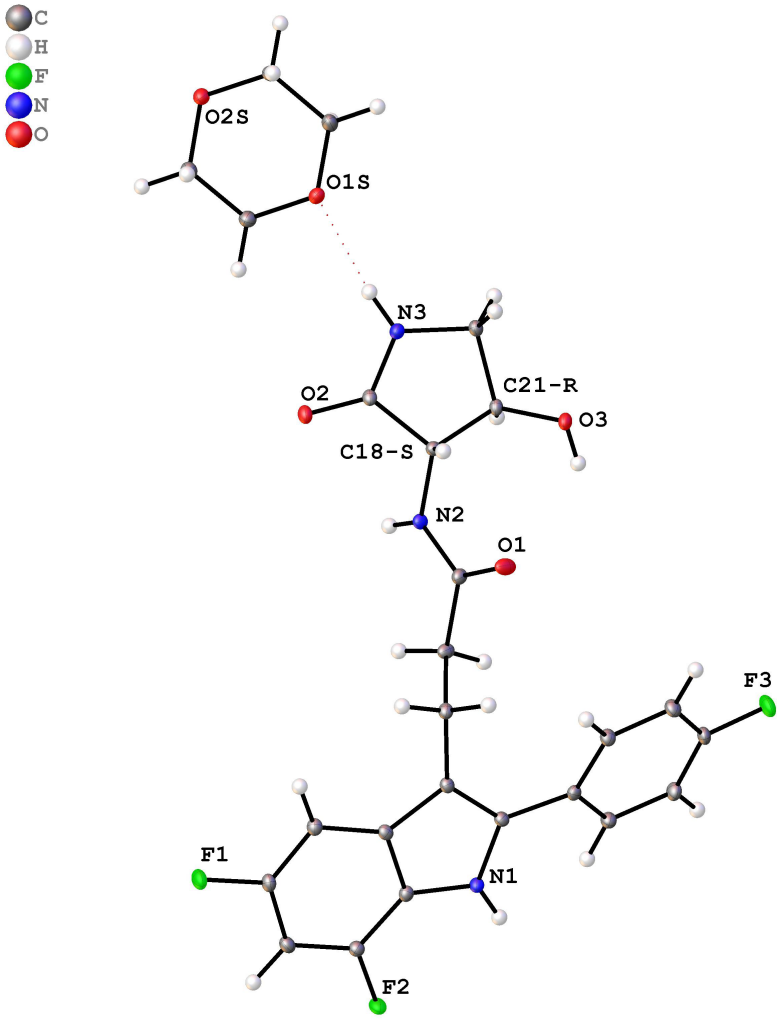
(C) Signal intensity and AUC for peaks identified in (B).



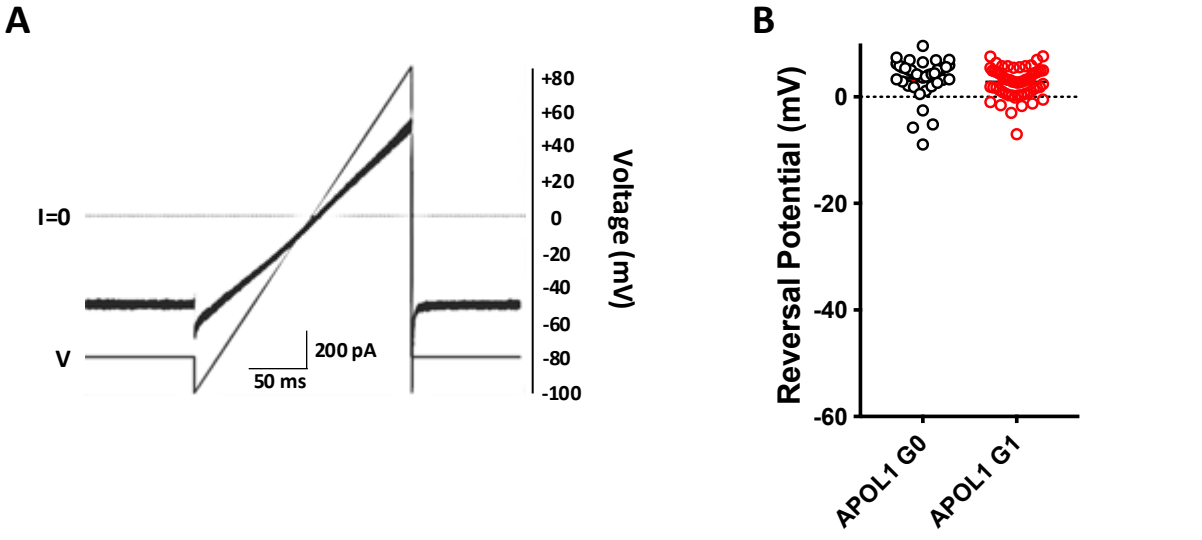
Supplementary Fig. 6. 13C NMR trace for Compound 3.



Supplementary Fig. 7. 13C NMR trace for VX-147.



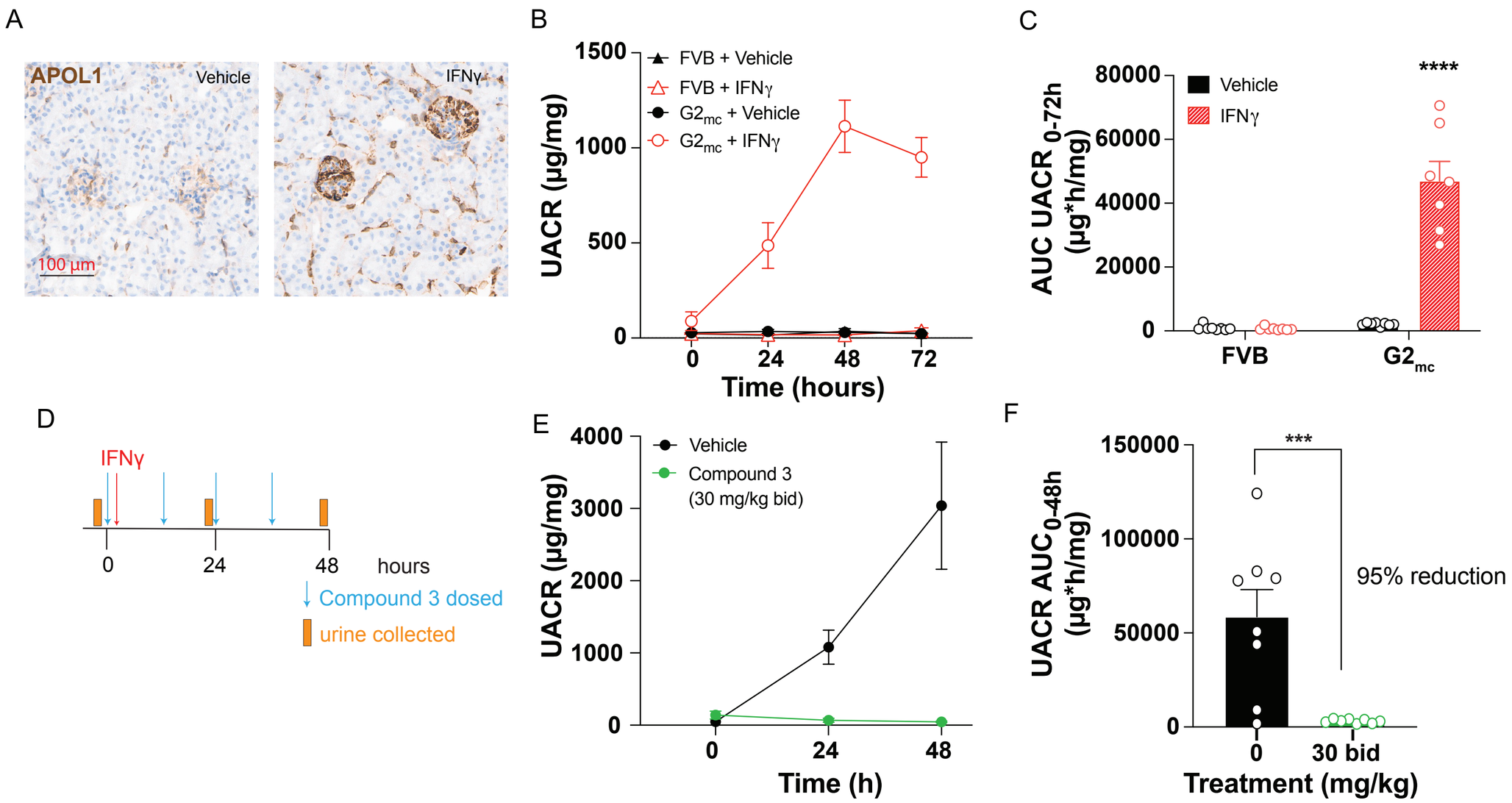
Supplementary Fig. 8. Thermal ellipsoid plot showing the asymmetric unit of VX-147 dioxane solvate.
Ellipsoids are at 50% probability.



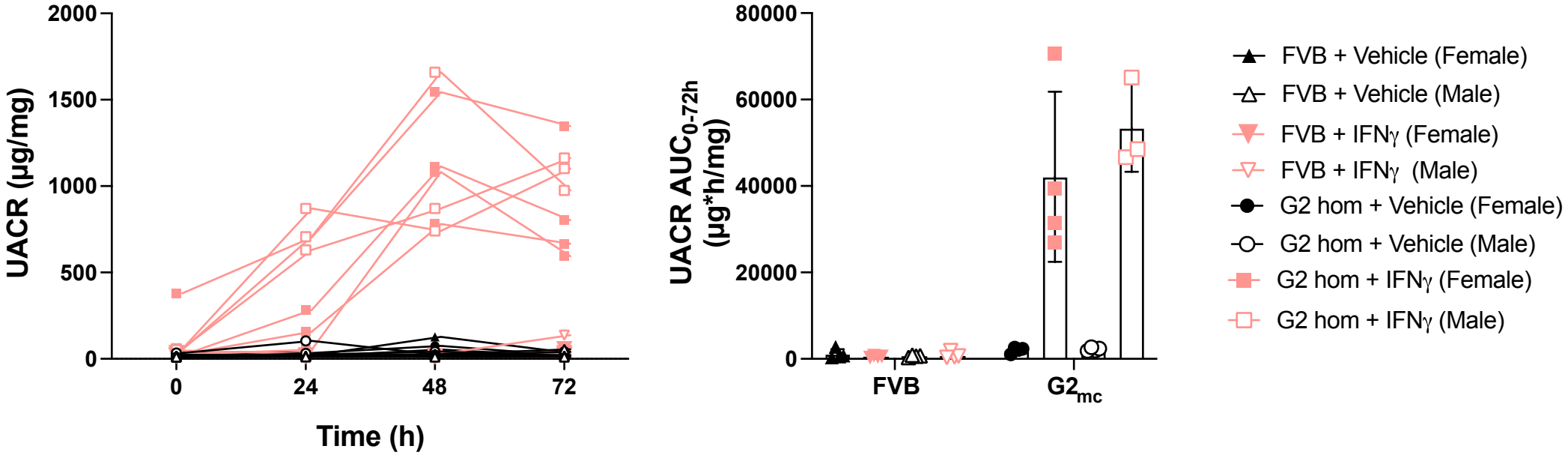
Supplementary Fig. 9: APOL1-mediated currents recorded with NaCl extracellularly and CsF intracellularly.

(A) Representative voltage ramp-current response in APOL1 G1-expressing HEK293 cells with NaCl extracellularly and CsF intracellularly.

(B) Average reversal potential of APOL1-G0, -G1, -mediated currents in these conditions.

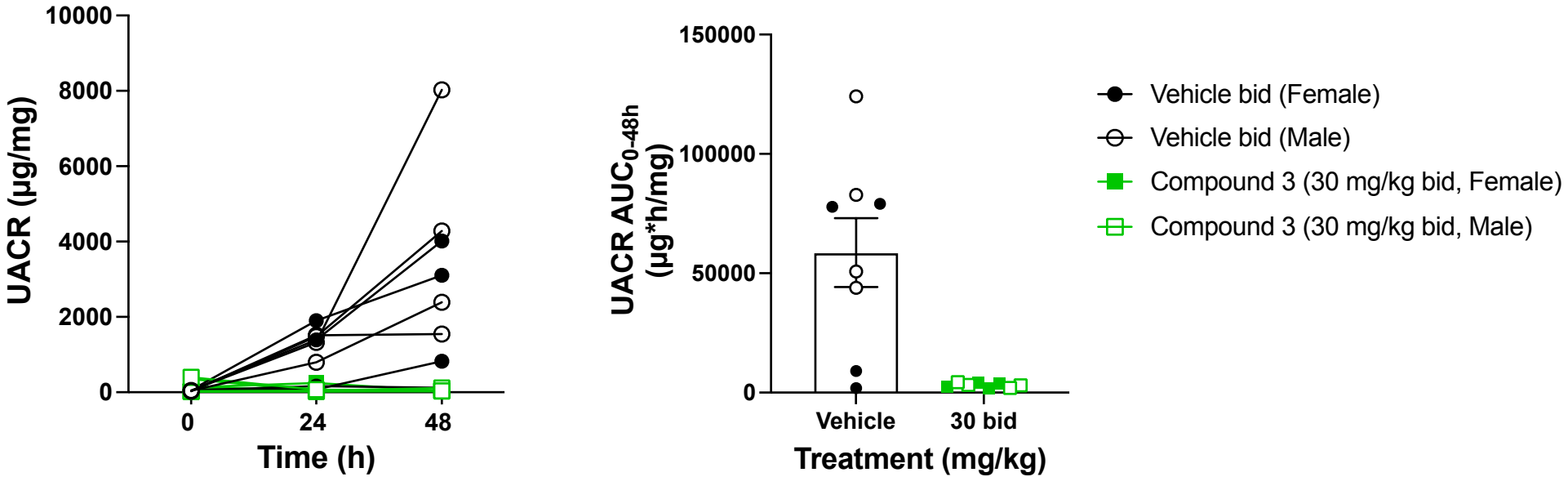


Supplementary Fig. 10: Interferon-induced APOL1-mediated proteinuria in APOL1 G2 multicopy mice is prevented by an analog of VX-147, Compound 3. **(A)** Assessment of APOL1 induction in APOL1 G2 multicopy transgenic mice in response to IFNγ injection compared to saline. Brown chromogen is DAB (3,3'Diaminobenzidine) staining with hematoxylin counterstain. The primary antibody used to detect for APOL1 is a rabbit anti-APOL1 monoclonal antibody, (Abcam; Cat # ab252218) with 1:250 dilution. **(B)** Assessment of urinary albumin-to-creatinine ratio (UACR) in response to IFNγ in FVB mice or APOL1 G2 multicopy transgenic mice was measured across 72 hours. n=7-8/group, all groups had n=4 females (F), and 4 males (M) except G2mc + IFNγ group had 4F/3M. **(C)** UACR area under the curve (AUC) was calculated from panel **(B). (D)** Experimental dosing paradigm for prophylactic assessment of compound 3 efficacy highlighting times of compound 3 administration, IFNγ injection, and urine collection. n=8/group (4F /4M). **(E)** Oral administration of Compound 3 twice daily at a dose of 30 mg/kg reduces IFNγ-induced proteinuria. UACR was measured across 48 hours. **(F)** UACR AUC was calculated from panel **(E)** to assess the magnitude of the effect of compound 3 reduction of proteinuria. Percent reduction in UACR AUC with compound 3 treatment was calculated. Log-transformed AUC data in panel **(C)** and **(F)** were analyzed with a two tailed t-test. Statistical significance was set at $p \leq 0.05$. ***$p<0.001$; ****$p<0.0001$. $p<0.0001$ for panel C and $p=0.0003$ for panel F. All results are presented as arithmetic mean ± SEM with each symbol representing an individual mouse.



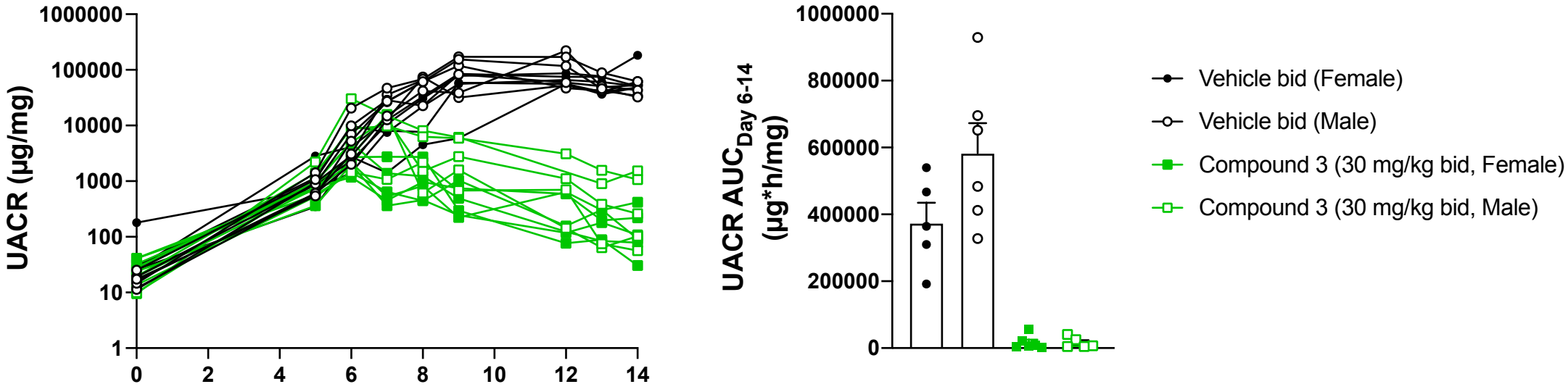
Supplementary Fig. 11

Assessment of urinary albumin-to-creatinine ratio (UACR) and UACR AUC in response to IFNγ in FVB mice or APOL1 G2 multicopy transgenic mice over the course of 72 hours divided by sex. All groups had n=4 females (F), and 4 males (M) except G2mc + IFNγ group had 4F/3M. Results are presented as each symbol as an individual mouse and additionally for AUC UACR the bar and error bars are the arithmetic mean ± SEM.



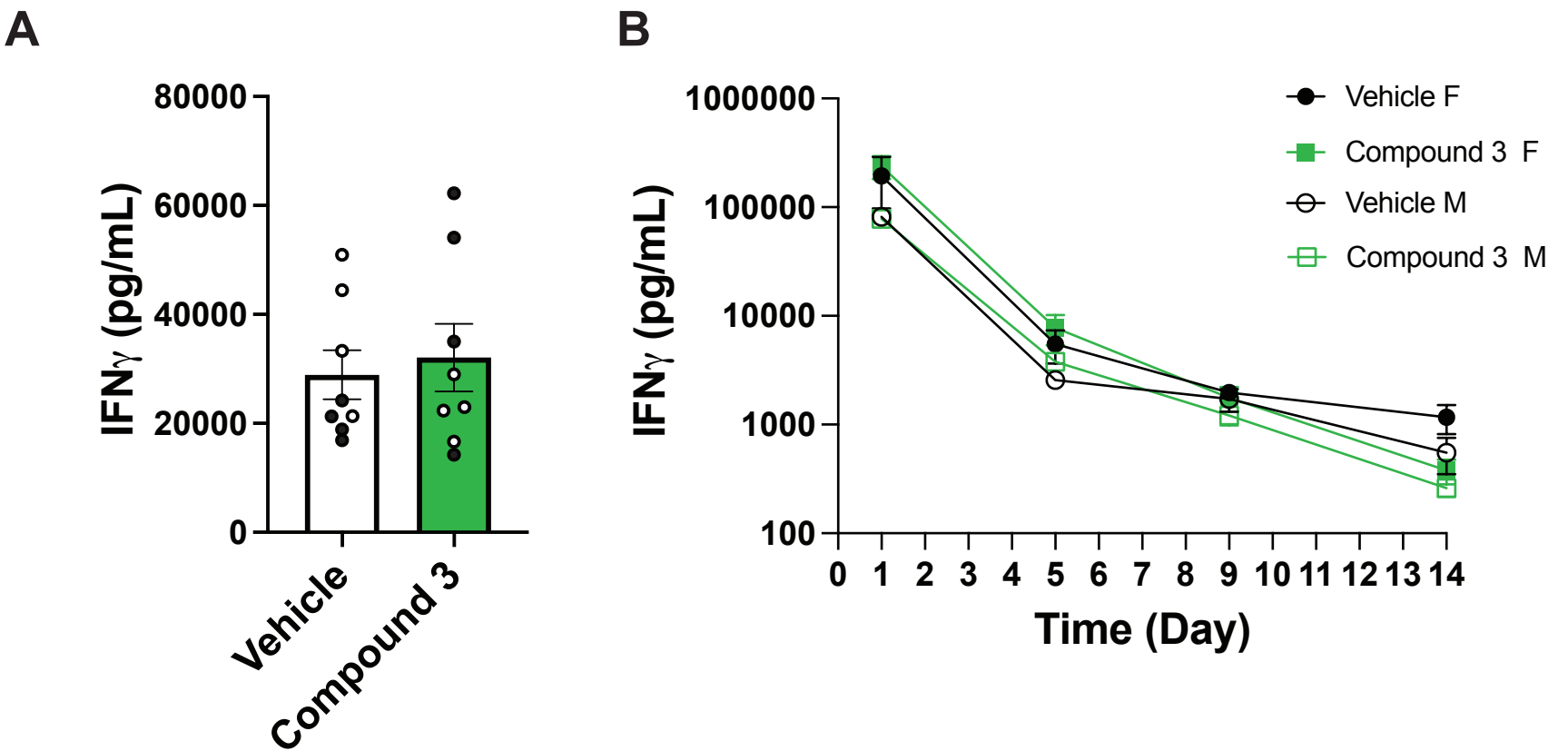
Supplementary Fig. 12

Assessment of urinary albumin-to-creatinine ratio (UACR) and UACR AUC in response to IFNγ and the impact of Compound 3 oral administration twice day at a dose of 30 mg/kg. Both treatment conditions have a total of 8 mice, with 4F and 4M. Results are presented as each symbol as an individual mouse and additionally for AUC UACR the bar and error bars are the arithmetic mean ± SEM.

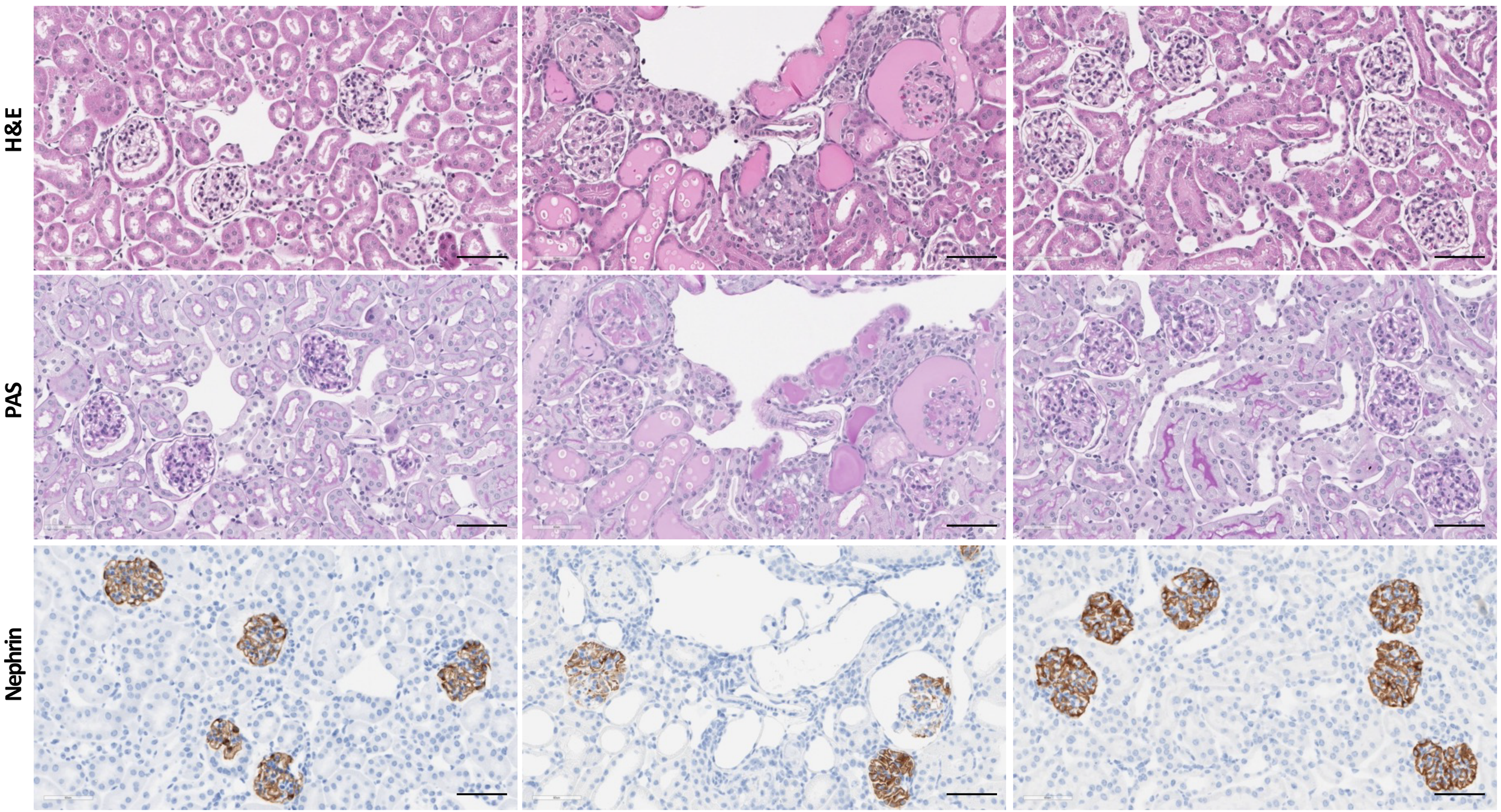


Supplementary Fig. 13

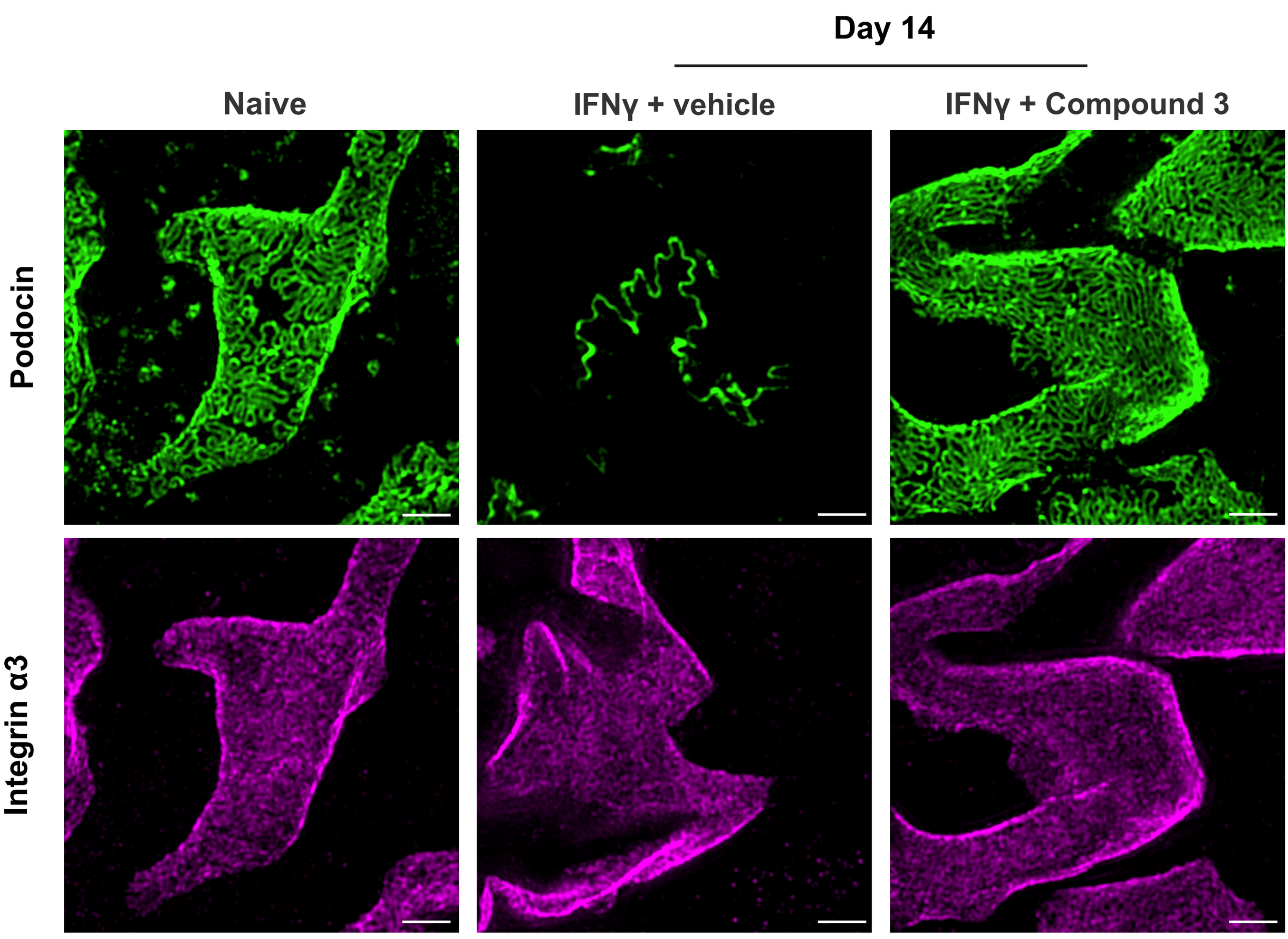
Assessment of urinary albumin-to-creatinine ratio (UACR) and UACR AUC in response to hydrodynamic injection of IFNγ plasmid in APOL1 G2 transgenic mice over 14 days. n=11-12/group. Vehicle group had 5F/7M; Compound 3 group had 6F/5M. Results are presented as each symbol as an individual mouse and additionally for AUC UACR the bar and error bars are the arithmetic mean ± SEM.



Supplementary Fig. 14. Detection of serum IFNγ levels in the mouse model experiments.
(A) Serum IFNγ levels from mice in supplemental figure 10E n=8/group with 4F/4M. Results are presented as arithmetic mean ± SEM. Female mice are represented by closed circles and male mice by open circles. **(B)** Serum IFNγ levels from mice in figure 4. n=11-12/group. Vehicle group had 5F/7M and compound 3 group had 6F/6M. Results are presented as arithmetic mean ± SEM. On day 5, animals were assigned to treatment groups (day 5) with similar mean UACR levels. Treatment groups on day 5 also had similar mean serum IFNγ levels.



Supplementary Fig. 15
Mouse kidney sections from Fig. 5 to provide additional images of glomeruli. Scale bar is 60 µm.



Supplementary Fig. 16

Podocin and integrin α3 staining for the representative of images from Fig. 5K-M. Scale bar is 1 μm.

Supplementary tables

Identification code	VX-147	
Empirical formula	C25 H26 F3 N3 O5	
Formula weight	505.49	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 4.7928(2) Å	α= 90°.
	b = 18.3138(6) Å	β= 98.056(2)°.
	c = 13.0474(5) Å	γ = 90°.
Volume	1133.93(7) $Å^3$	
Z	2	
Density (calculated)	1.480 Mg/m^3	
Absorption coefficient	1.023 mm^{-1}	
F(000)	528	
Crystal size	0.380 x 0.128 x 0.050 mm^3	
Theta range for data collection	3.421 to 72.162°.	
Index ranges	-5<=h<=5, -22<=k<=22, -16<=l<=16	
Reflections collected	60490	
Independent reflections	4443 [R(int) = 0.0402]	
Completeness to theta = 67.679°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.951 and 0.803	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4443 / 5 / 337	
Goodness-of-fit on F^2	1.055	
Final R indices [I>2sigma(I)]	R1 = 0.0276, wR2 = 0.0682	
R indices (all data)	R1 = 0.0285, wR2 = 0.0690	
Absolute structure parameter	-0.06(4)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.185 and -0.157 $e.Å^{-3}$	

Supplementary Table 1. Crystal data and structure refinement for VX--147.

Assay Name	% Inh @ 10 μM
Phosphodiesterase PDE3	-8
Phosphodiesterase PDE4D2	17
Adenosine A_1	4
Adenosine A_{2A}	15
Adrenergic α_{1A}	12.5
Adrenergic $\alpha 2_A$	26
Adrenergic β_1	-1.5
Adrenergic β_2	-2
Calcium Channel L-Type, Benzothiazepine	-15
Cannabinoid CB_1	-2
Dopamine D_1	11
Dopamine D_{2S}	-9
Endothelin ET_A	-19
$GABA_A$, Chloride Channel, TBOB	-5
$GABA_A$, Flunitrazepam, Central	-6
$GABA_B$, Non-Selective	1
Glutamate, AMPA	-3
Glutamate, NMDA, Glycine	3
Histamine H_1	17
Muscarinic M_1	14
Muscarinic M_2	-4
Muscarinic M_3	0
Neuropeptide Y Y_1	1
Nicotinic Acetylcholine α3β4	-4
Opiate δ_1 (OP1, DOP)	1
Opiate κ(OP2, KOP)	9
Opiate μ(OP3, MOP)	5
Serotonin (5-Hydroxytryptamine) 5-HT_{1A}	-8
Serotonin (5-Hydroxytryptamine) 5-HT_{2B}	10
Sodium Channel, Site 2	-1
Tachykinin NK_1	25
Transporter, Dopamine (DAT)	17
Transporter, Norepinephrine (NET)	21
Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	8

Supplementary Table 2: Compound 3 off-targeting profiling in Eurofins Off-target Activity Screen

34 targets were evaluated for Compound 3-mediated inhibition at 10 μM. A more extensive off-target panel is available for VX-147/inaxaplin in a previous publication [25].

Mouse	$AUC_{0\text{-}12h}$, day 6 (h*µg/mL)	$AUC_{0\text{-}12h}$, day 13 (h*µg/mL)	C_{max} (µg/mL)	C_{min} (µg/mL)
1	42.9	38.2	9.69	0.477
2	29.6	33.1	8.49	0.31
3	35.6	18.9	6.75	0.344
4	34.1	28.3	9.03	0.416
5	40.1	33.3	10.3	0.281
6	30.2	34.9	8.4	0.411
7	26.4	40	9.05	0.388
8	27.5	36.8	6.79	0.403
9	31.6	28.8	8.37	0.455
10	22.6	17.8	3.86	0.301
11	29.7	27.8	7.61	0.218

Supplementary Table 3: Compound 3 exposure data from the mice in the treatment group from Figure 4.