Supporting Information

A Covalent Chemical Probe for *Chikungunya* nsP2 Cysteine Protease with Antialphaviral Activity and Proteome-wide Selectivity

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Table of Contents

Table S1. Inhibitory effects of **3** on human and viral cysteine proteases

Enzyme	Substrate	Control	% Inhibition			
			3 (10 μM)	**Reference** (0.1 x IC_{50}) [a]	**Reference** (1 x IC_{50}) [a]	**Reference** (10 x IC_{50}) [a]
Cathepsin B	5 μM Z-Leu-Arg-AMC	E-64	10	13	57	97
Cathepsin F	10 μM Z-Phe-Arg-AMC	Cystatin C	10	12	50	88
Cathepsin K	70 μM Cathepsin K Substrate	E-64	3	12	46	99
Cathepsin L	5 μM Z-Leu-Arg-AMC	E-64	12	7	51	97
Cathepsin S	5 μM Z-Leu-Arg-AMC	E-64	14	14	55	91
Cathepsin V	5 μM Z-Leu-Arg-AMC	E-64	7	7	50	99
MALT1	20 μM Ac-LRSR-AMC	Z-VRPR-FMK	7	11	54	89
A20	100 nM Ub-AMC	Ub-Aldehyde	0	3	35	83
Ataxin-3	100 nM Ub-AMC	Ub-Aldehyde	7	8	47	90
OTUD6B	100 nM Ub-AMC	Ub-Aldehyde	1	7	44	94
UCHL1	100 nM Ub-AMC	Ub-Aldehyde	3	2	30	97
UCHL3	100 nM Ub-AMC	Ub-Aldehyde	8	8	44	99
USP1	100 nM Ub-AMC	Ub-Aldehyde	1	19	73	96
USP2	100 nM Ub-AMC	Ub-Aldehyde	0	13	51	90
USP5	100 nM Ub-AMC	Ub-Aldehyde	1	13	55	97
USP7	100 nM Ub-AMC	Ub-Aldehyde	5	14	41	94
USP8	100 nM Ub-AMC	Ub-Aldehyde	5	18	53	89
USP10	100 nM Ub-AMC	Ub-Aldehyde	1	18	40	83
USP14	100 nM Ub-AMC	Ub-Aldehyde	8	11	46	95
USP20	100 nM Ub-AMC	Ub-Aldehyde	1	28	64	96
3CL Pro (MERS-CoV)	10 μM 3CL MERS-Cov Substrate	Protease Cocktail	1	6	42	83
Papain-like Pro (SARS-CoV-2)	25 μM PLPro Substrate	GRL0617	0	8	45	82
3CL Pro (SARS-CoV-2)	50 μM 3CL Protease Substrate	GC376	5	8	48	92

[a] IC_{50} of the control nsP2 protease inhibitor.

Figure S1. Chemical stability of **3** and **4** at room temperature

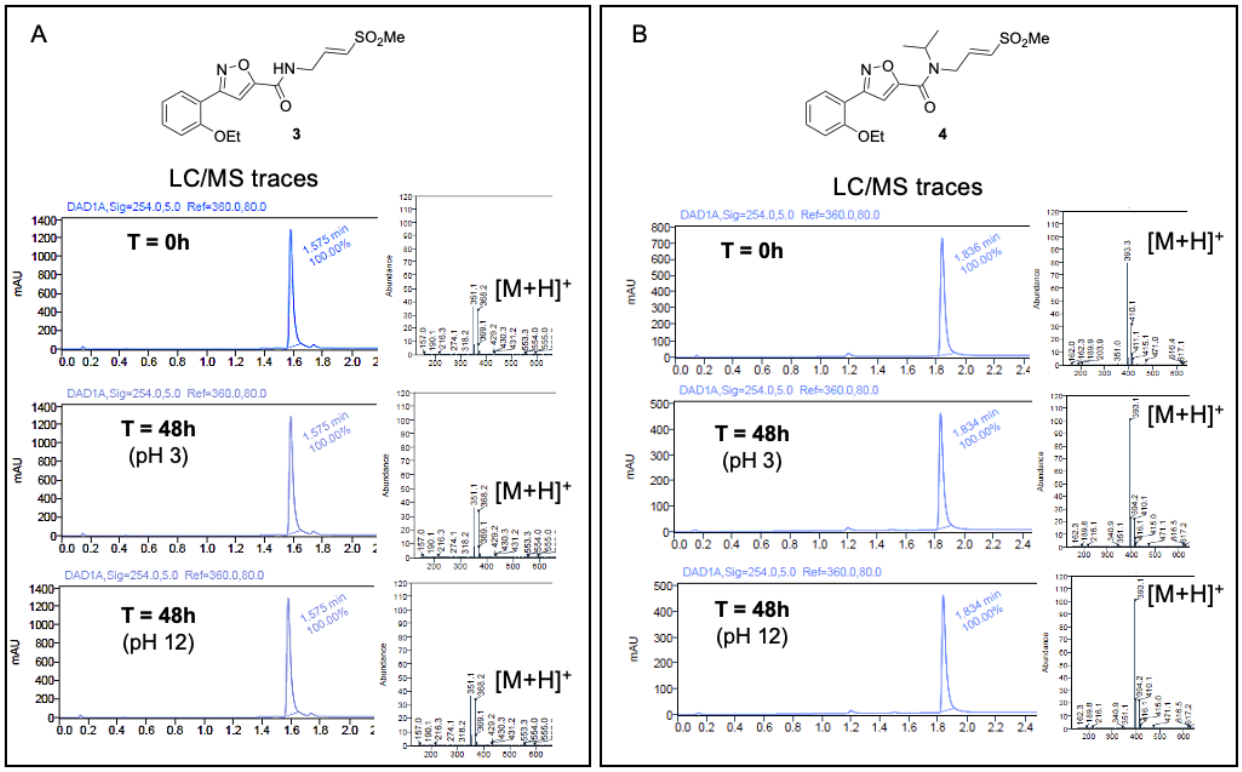
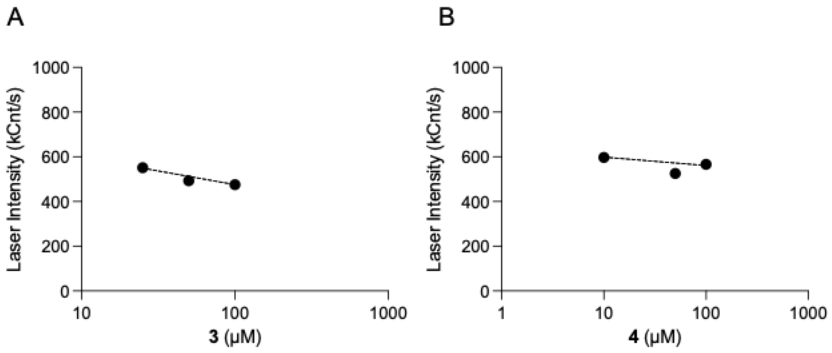


Figure S2. DLS data for **3** and **4**



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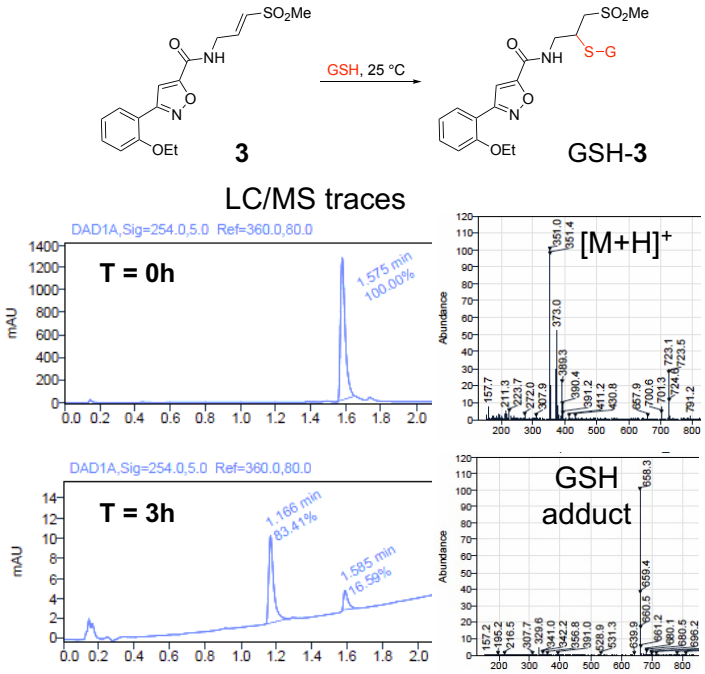
Dynamic Light Scattering (DLS) Assay. Aggregation behavior of chemical probe **3** and negative control **4** were determined by DLS in duplicate at 25 ºC in 25 mM HEPES buffer (pH 7.4) containing 0.003% Tween-20 and 1 mM DTT, using a 10 mM stock solution containing 2% DMSO. Light scattering was measured using a DynaPro Plate Reader III. Buffer with 2% DMSO control produced an average laser intensity of 858 kCnts/s.

Figure S3. GSH stability of **3**



Method for GSH stability. A 10 mM solution of GSH (Sigma Aldrich Cat# G4251 (St. Louis, MO, USA)) was prepared in a pH 7.4 phosphate buffer. A 10 mM DMSO solution of Compound **3** was diluted in phosphate buffer to give a solution at 100 µM with 1% DMSO. At time zero (t = 0), 50 µL of the 100 µM test compound solution was added to an Eppendorf tube containing 50 µL phosphate buffer and 50 µL of 10 mM GSH solution. The final concentrations of the compound and GSH were maintained at 50 µM and 5 mM, respectively. The Eppendorf tube was vortexed, and then the sample was transferred to a high-recovery autosampler vial for LCMS analysis. Analysis was performed at 0, 1, and 3 h time points, and the percentage of GSH adduct formation was calculated using Agilent LCMS software (OpenLab CDS Version 2.7).

Figure S4. Dose response of **3** in the EEEV-nLuc replicon assay

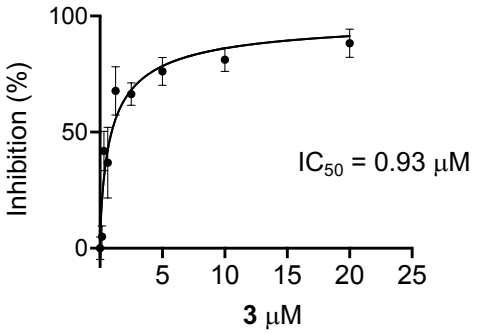
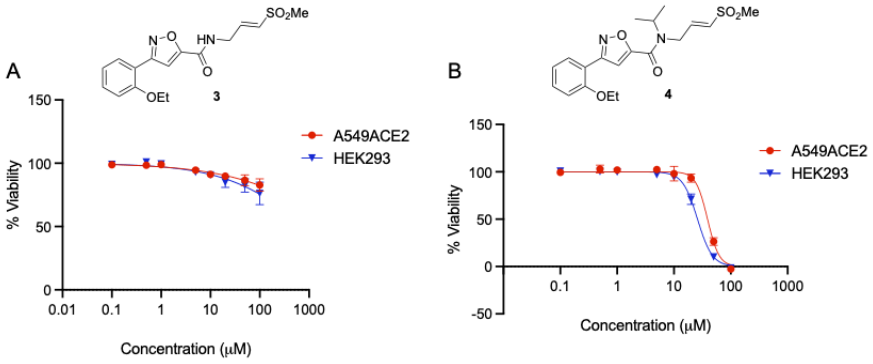


Figure S5. Cell toxicity data upon 48h exposure of **3** and **4**



Cell viability assay. Cells were plated on a 384 well plate at a seeding density of 2000 cells/well for A549ACE2 (in DMEM high-glucose, 10% fetal bovine serum, 1X non-essential amino acids, and 2 mM L-glutamine) and HEK293 (in DMEM high-glucose, 10% fetal bovine serum) and allowed to adhere overnight. Cells were treated with dose response of compound, 1% DMSO (negative control), or 10% DMSO (positive control) for 48 h. CellTiter-Glo reagent (Promega) was added to each well and luminescence was detected on GloMax plate reader. There were two biological replicates containing treatments in quadruplicate. Cell viability was calculated using the following formula: ((raw RLU value – average of positive control wells) / (average of negative control wells – average of positive control wells))*100. Plots were generated using GraphPad Prism.

Figure S6. Site of covalent modification of CHIKV nsP2pro. MS/MS spectrum of the doubly charged ion (*m/z* 571.2441) corresponding to CHIKV nsP2 tryptic peptide ANVCWAK (aa 475–481). C478 was modified by a mass shift of +350.094 Da resulting from addition of SGC-NSP2PRO-1 (**3**). MS/MS spectra corresponding to a modified ANVCWAK (aa 475–481) peptide were observed in two independent samples (rep1 and rep2) treated with SGC-NSP2PRO-1 (**3**). The figure shows the MS/MS spectrum from rep2.

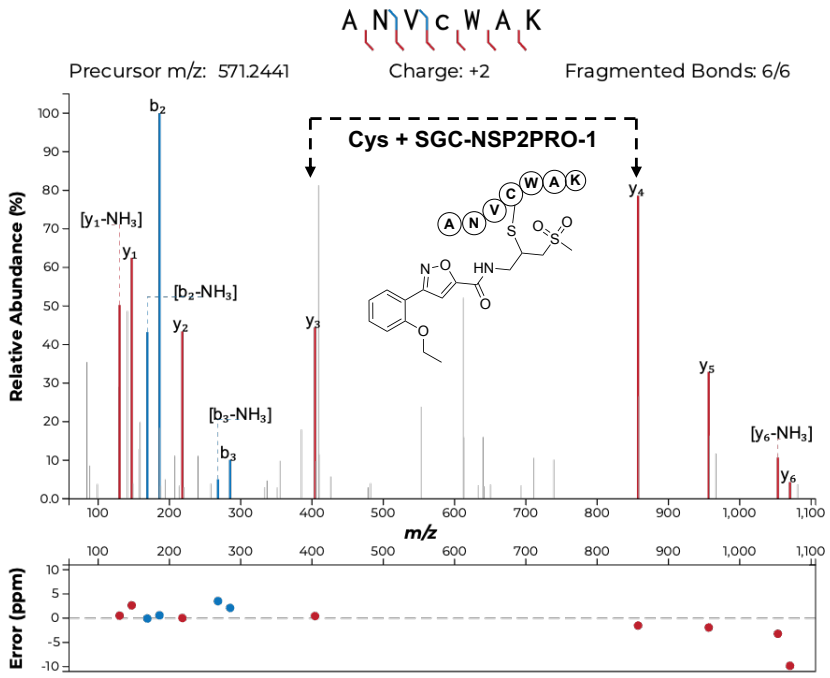


Figure S7. Dose-response curves of **VS**. A: Inhibition of CHIKV nsP2pro following a 30 min incubation. Values are the mean of triplicate determinations. B: Inhibition of CHIKV-nLuc replication in human fibroblast MRC5 cells at 6 h post-inoculation with the virus. Values are the average of duplicate determinations.

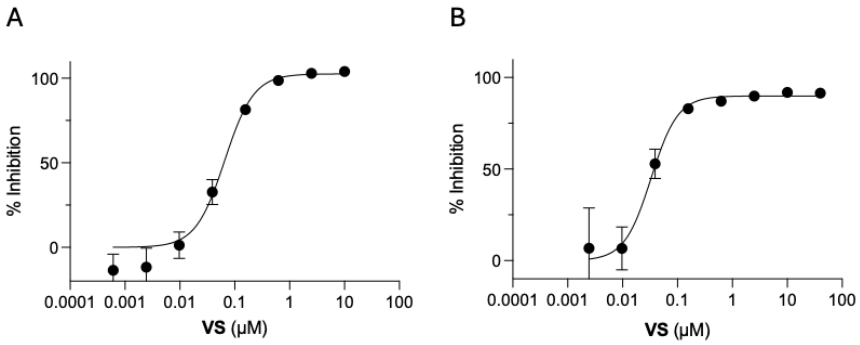


Figure S8. TAMRA fluorescence chemoproteomics of the vinyl sulfone warhead. **a.** Chemical structures of the simplified vinyl sulfone (**VSS**) and pyrazole chloroacetamide (**CA**). **b.** Uncropped fluorescent imaging of SDS-PAGE gel following covalent labeling of purified nsP2 (4 µg, lane P) by **TVS** or HEK293 cell lysates (140 µg total protein each, lanes 1 and 2) by **VSS** or **CA**. In lanes 1, **VSS** or **CA** were incubated at the indicated concentrations with cell lysates prior to click reaction to append the TAMRA fluorophore. In lanes 2 HEK293 cell lysates was supplemented with purified CHIKV nsP2. The expected M.Wt. of the nsP2 band is marked. No labelling of HEK293 cell lysate or nsP2 was observed with **VSS** (10 or 100 µM), while multiple proteins in the HEK293 cell lysate were weakly labelled by **CA** (10 µM). M lane contains molecular weight markers.

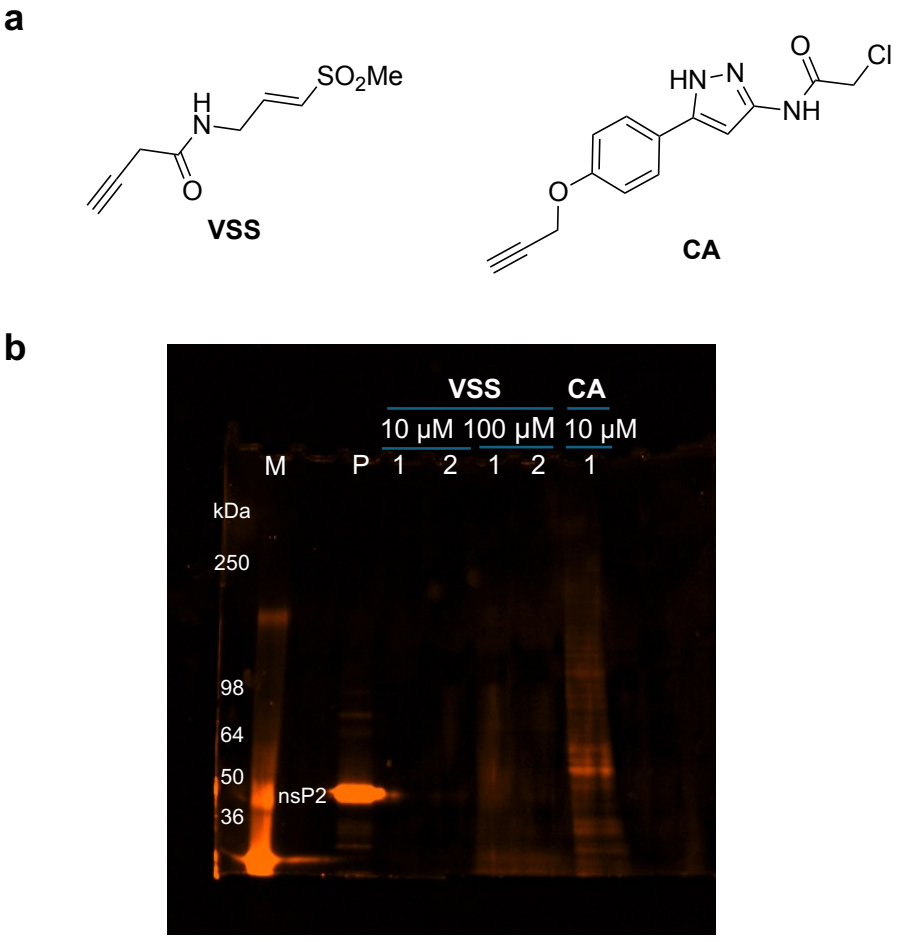


Figure S9. Structure of MOM-**1**

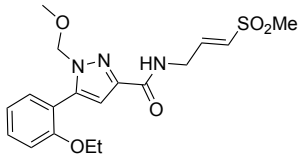


Figure S10. ^{1}H NMR (400 MHz, DMSO-d_6) for SGC-NSP2PRO-1N (**4**)

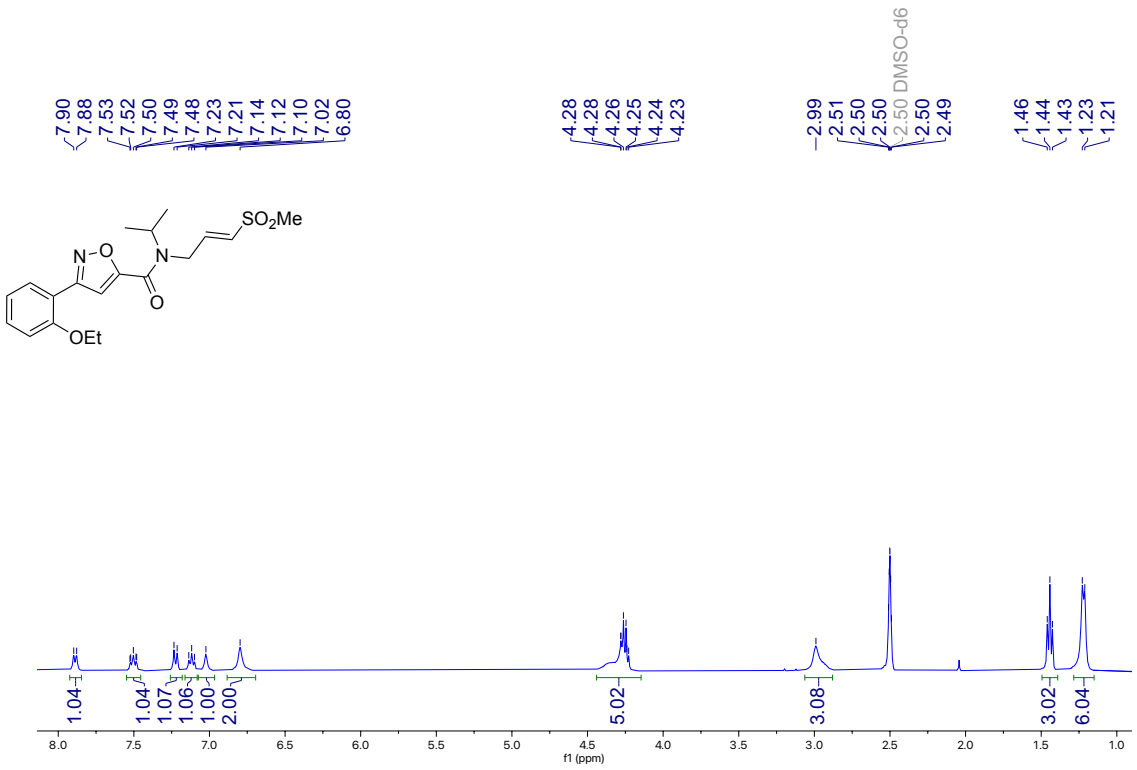


Figure S11. ^{13}C NMR (126 MHz, DMSO-d_6) for SGC-NSP2PRO-1N (**4**)

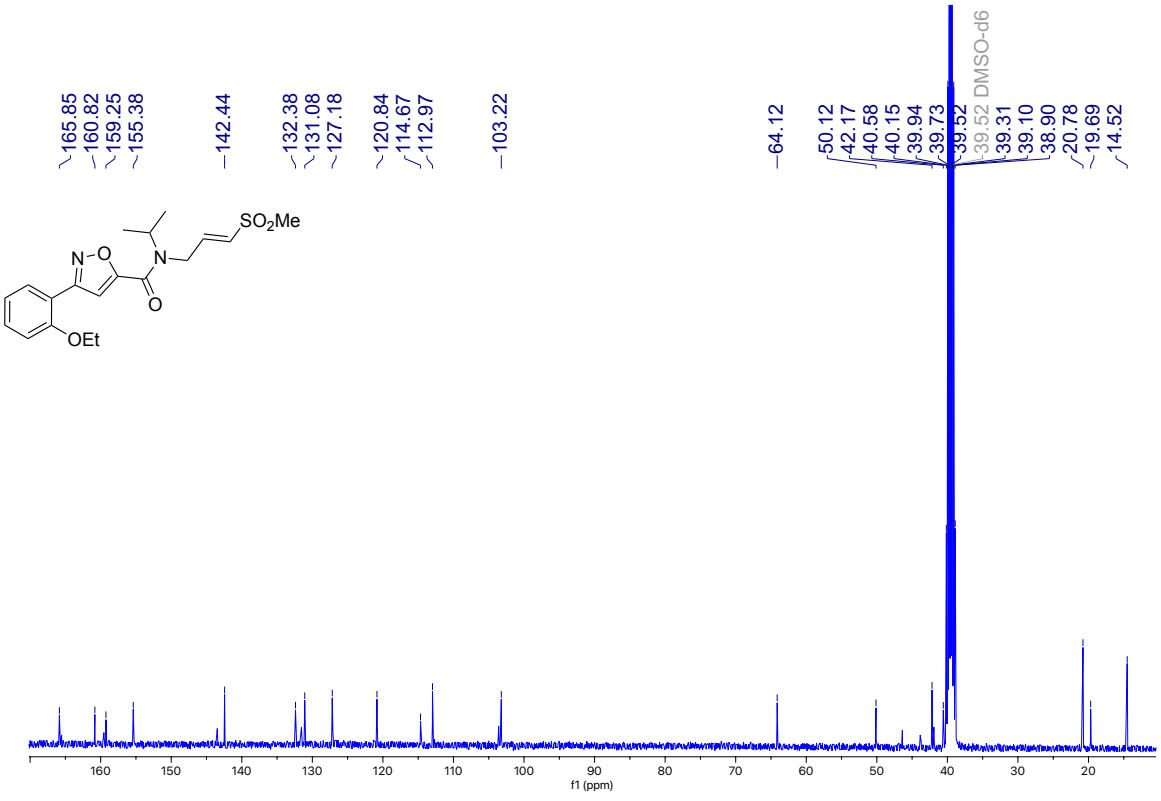


Figure S12. ^{1}H NMR (400 MHz, DMSO-d_6) for **VS**

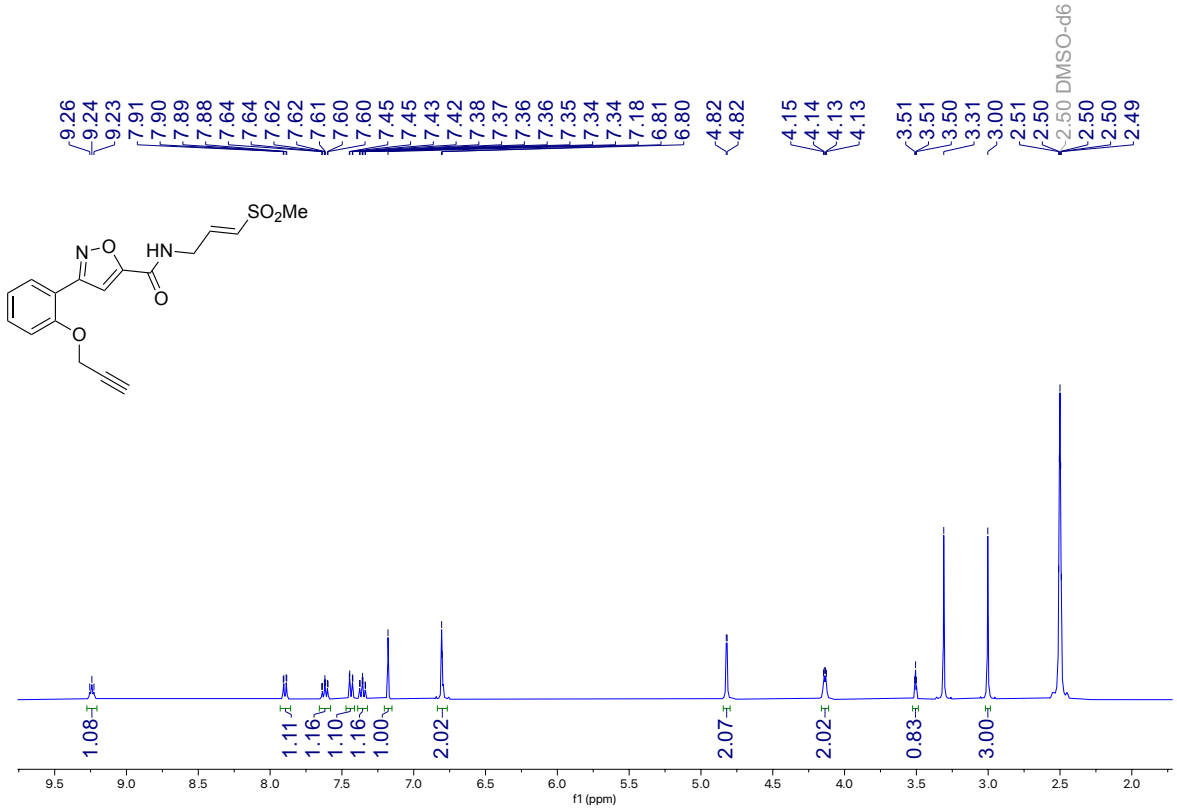


Figure S13. ^{13}C NMR (101 MHz, DMSO-d_6) for **VS**

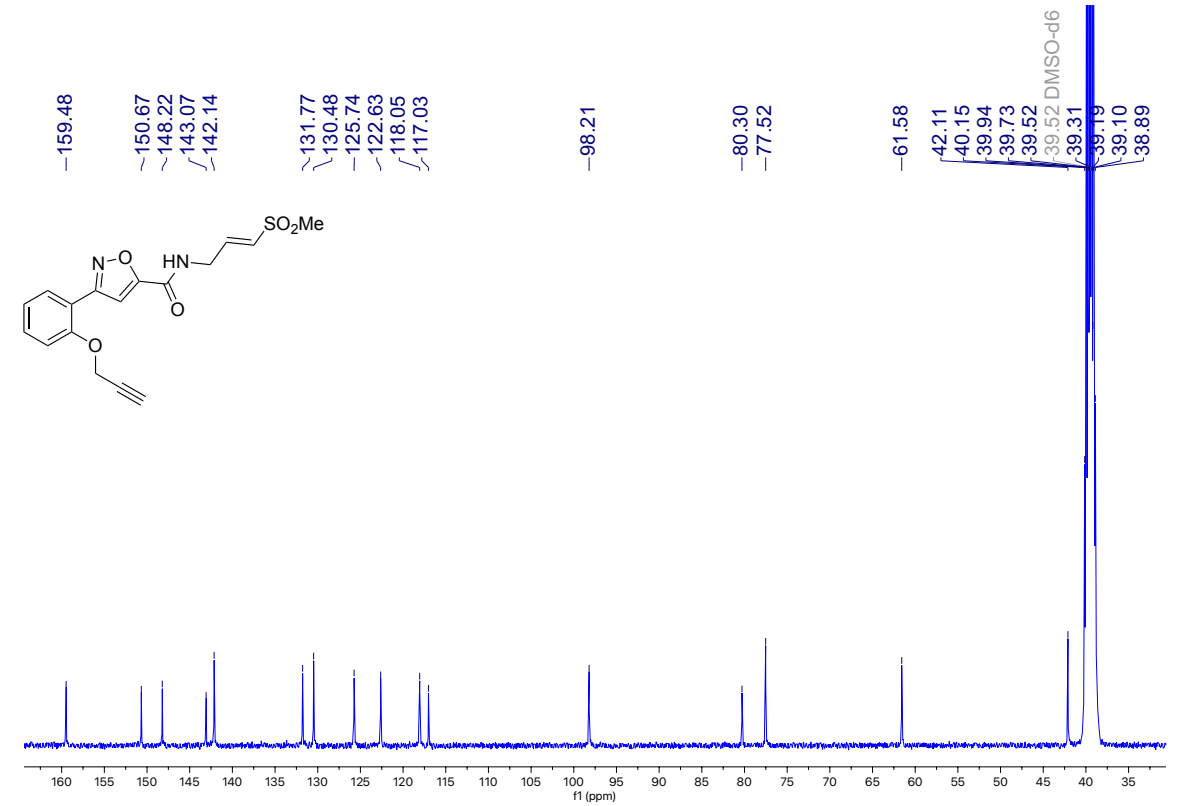


Figure S14. ^{1}H NMR (700 MHz, CD_3CN) for **TVS**

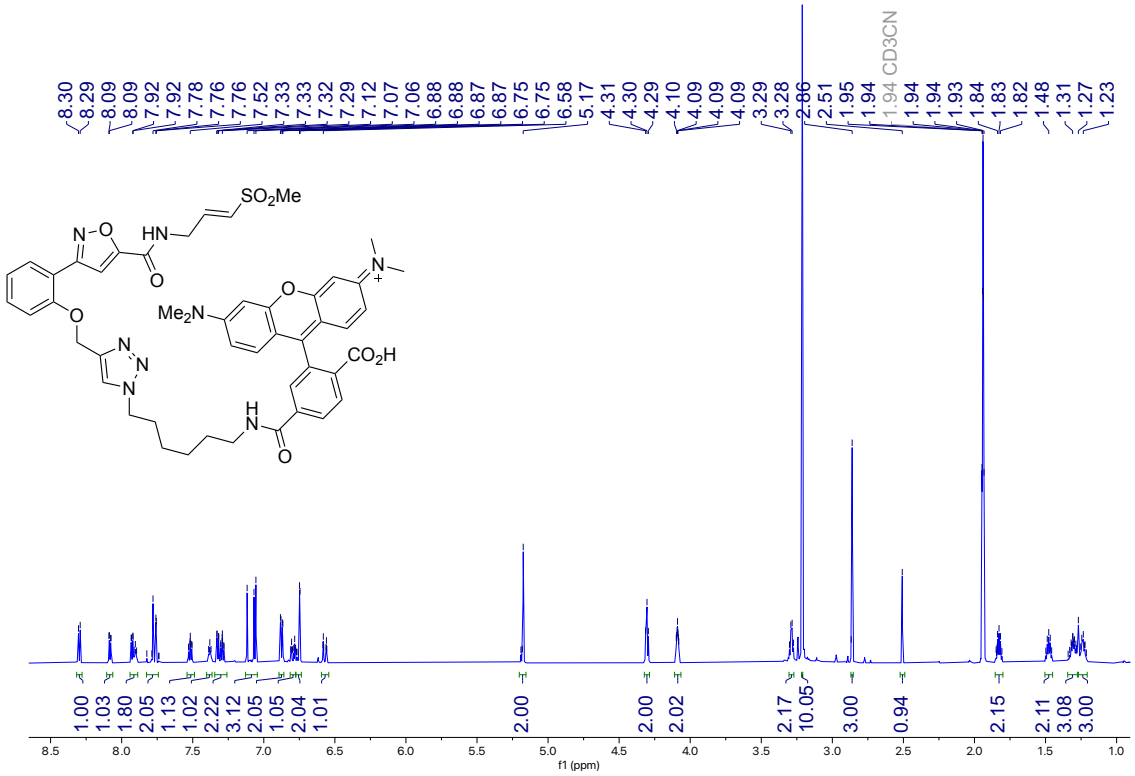


Figure S15. ^{13}C NMR (176 MHz, CD_3CN) for **TVS**

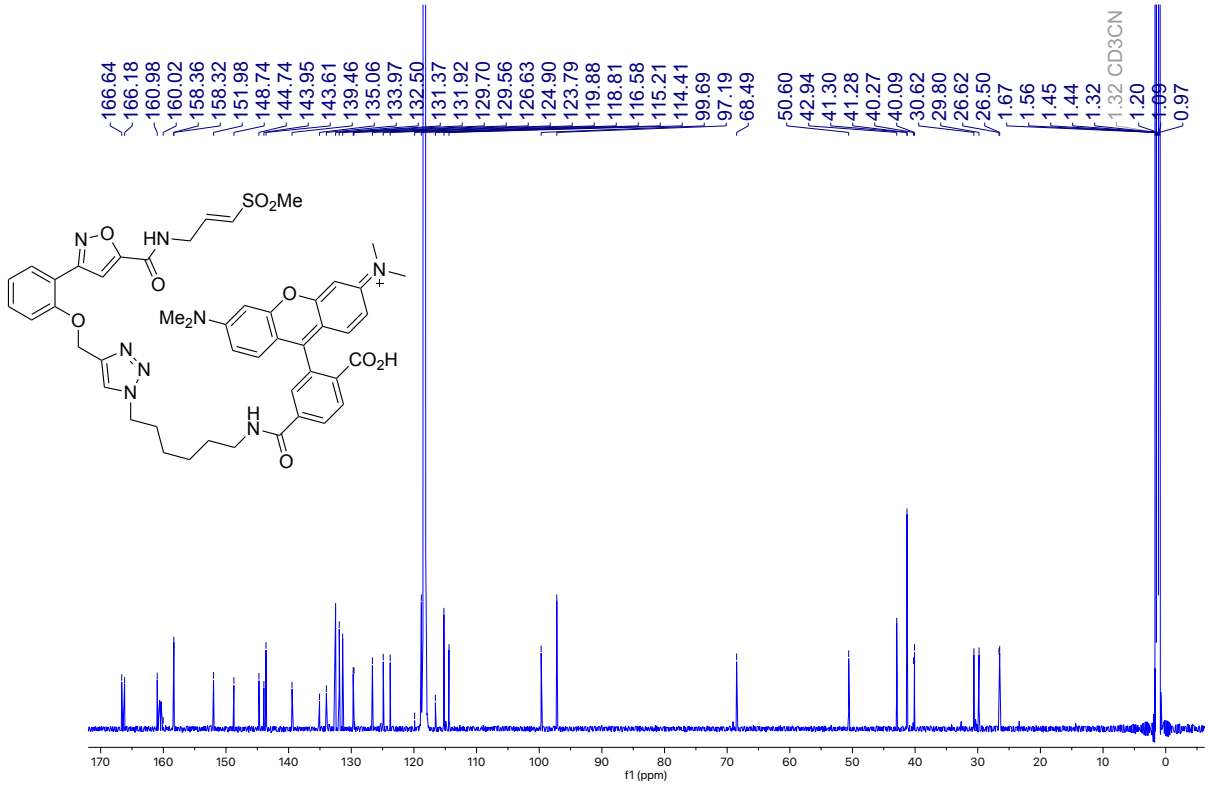


Figure S16. ^{1}H NMR (400 MHz, DMSO-d_6) for **VSC**

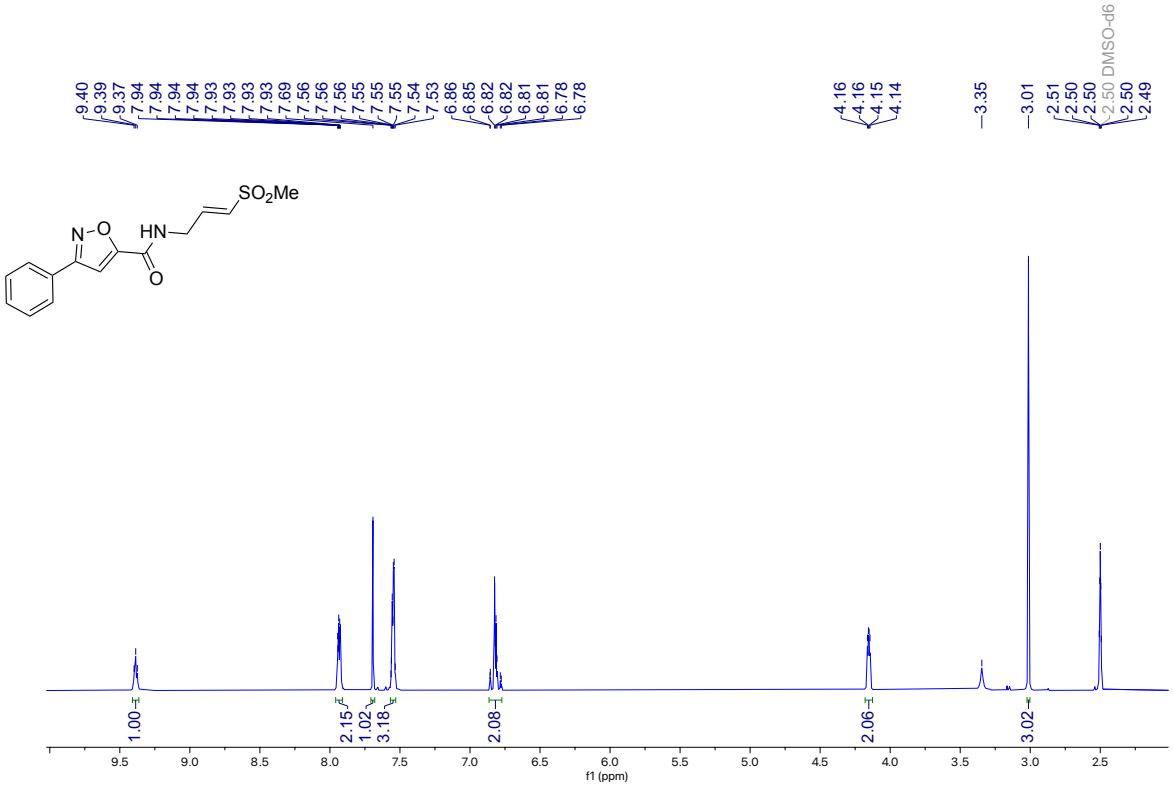
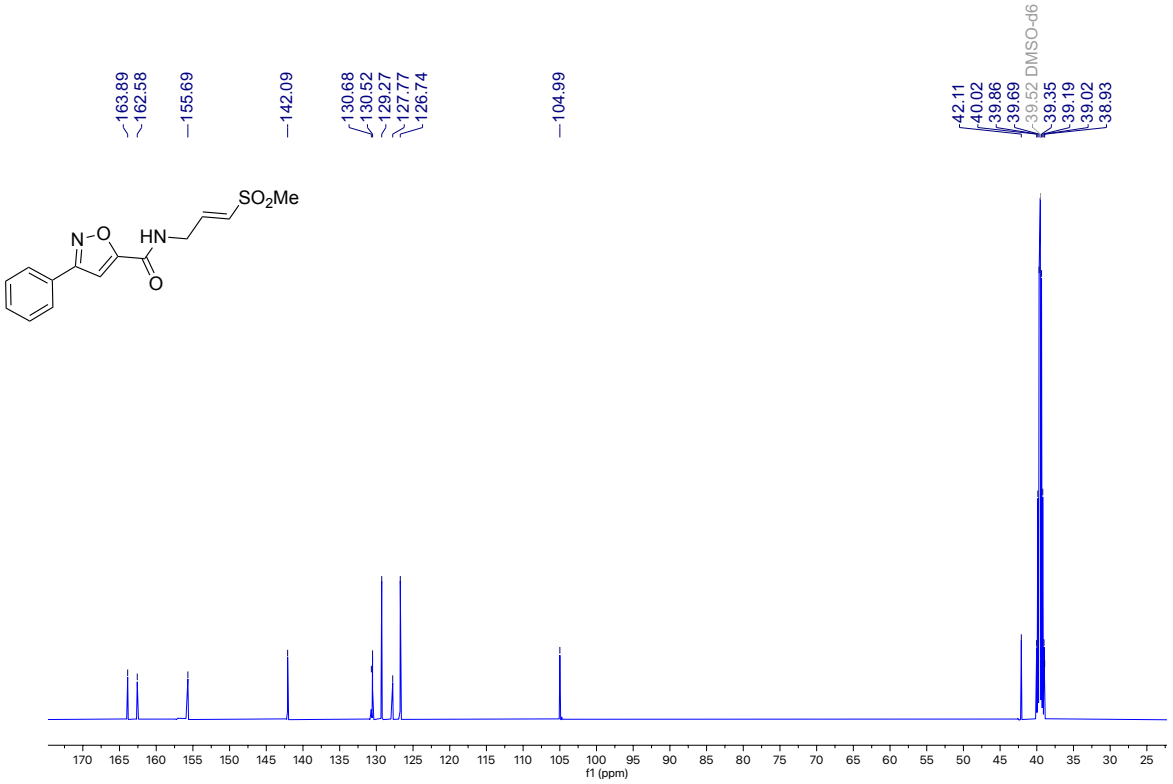
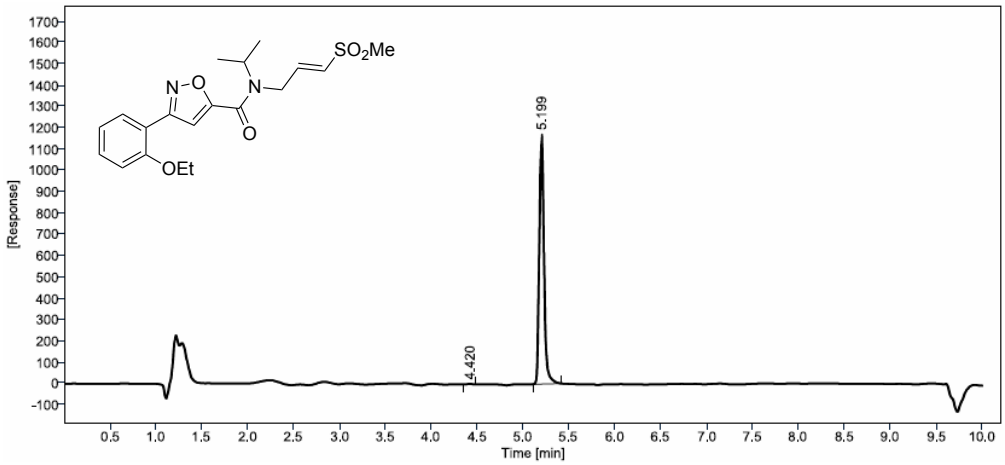


Figure S17. ^{13}C NMR (101 MHz, DMSO-d_6) for **VSC**



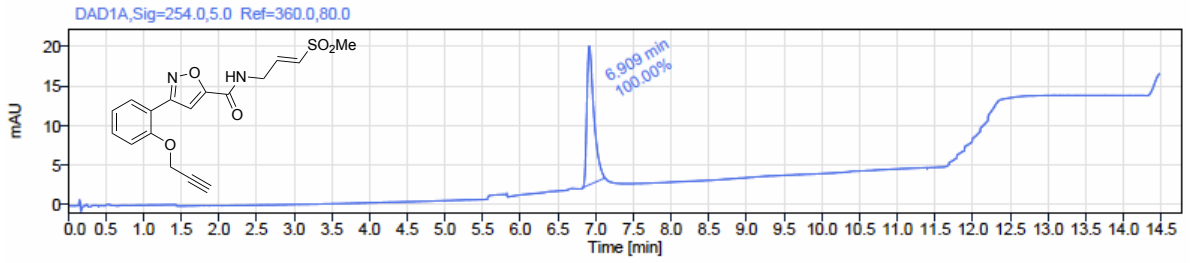
HPLC analyses

Figure S18. HPLC trace of SGC-NSP2PRO-1N (**4**)



Sr.No,	RT	Area	Height	% Area
1	4.42	11	4	0.27
2	5.20	4174	1171	99.73

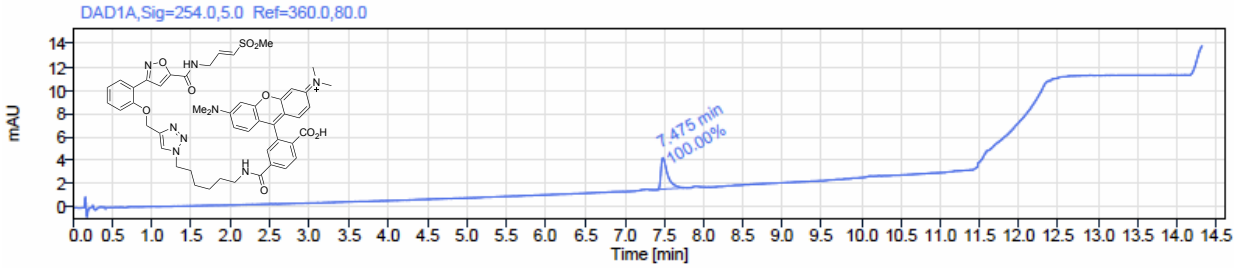
Figure S19. HPLC trace of **VS**



Signal Name DAD1A

RT (min)	Signal description	m/z	Purity UV(‰) / MS(%)	Area	Area%
6.909	DAD1A,Sig=254.0,5.0 Ref=360.0,80.0			114.1	100.00

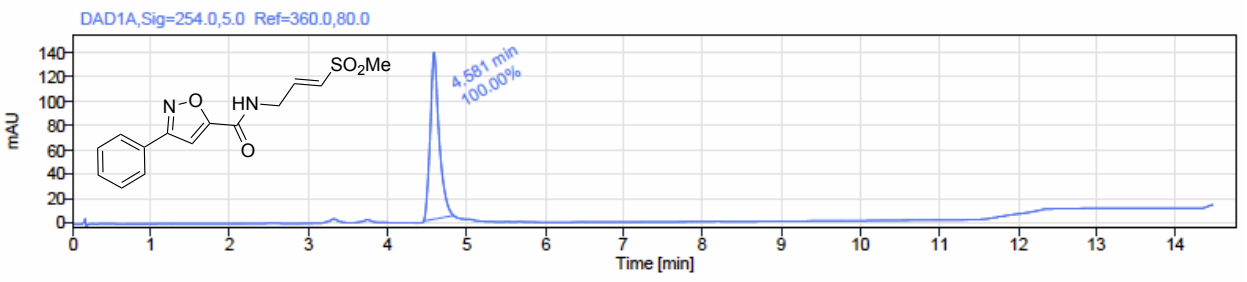
Figure S20. HPLC trace of **TVS**



Signal Name DAD1A

RT (min)	Signal description	m/z	Purity UV(‰) / MS(%)	Area	Area%
7.475	DAD1A,Sig=254.0,5.0 Ref=360.0,80.0			15.8	100.00

Figure S21. HPLC trace of **VSC**

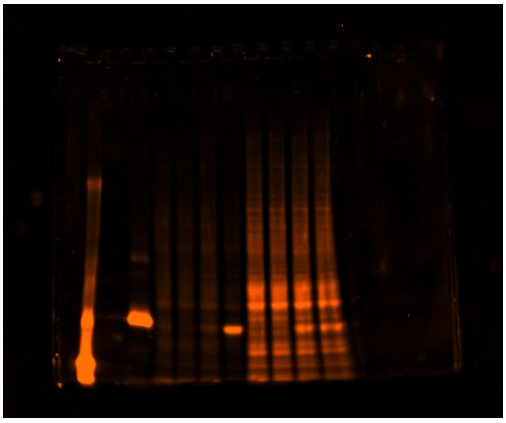


Signal Name DAD1A

RT (min)	Signal description	m/z	Purity UV(‰) / MS(%)	Area	Area%
4.581	DAD1A,Sig=254.0,5.0 Ref=360.0,80.0			1079.2	100.00

Figure S22. Uncropped images of SDS-PAGE gels. **a.** Figure 4b. **b.** Figure 5a.

a Figure 4b



b Figure 5a