Discovery of small-molecule enzyme activators by activity-based protein profiling

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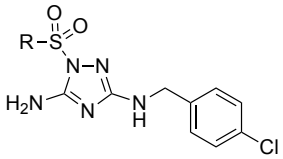
SUPPLEMENTARY INFORMATION

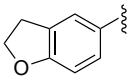
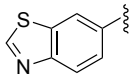
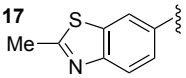
1. Supplementary Tables
2. Supplementary Figures
3. Supplementary Note – Structure-Activity Study & Synthetic Procedures

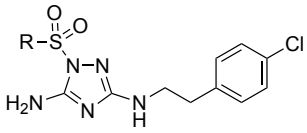
Supplementary Table 1. Small molecule screening data for mouse LYPLAL1

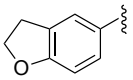
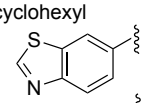
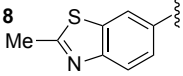
Category	Parameter	Description
Assay	Type of assay	Fluorescence polarization
	Target	LYPLAL1
	Primary measurement	Purified LYPLAL1 is a serine hydrolase that reacts with fluorophosphonate-rhodamine to change spin rate of free probe compared to LYPLAL1-associated probe.
	Key reagents	Fluorophosphonate-rhodamine (FP-Rh), purified recombinant mLYPLAL1, Tris-HCl, NaCl, Pluronic F-127.
	Assay protocol	9.5 μl 4 μM mLYPLAL1 in 50 mM Tris-HCl, 150 mM NaCl, 0.01% Pluronic F-127, pH 7.5 incubated with 50 nl 2 mM compounds for 1.5 h at 37°C. 0.5 μl 1.5 μM FP-Rh probe was added and incubated for 60 min at 37°C followed by end-point fluorescence measurement (Ex. 535 nm, 2 orthogonal measurements at Em. 595 nm).
	Additional comments	Kinetic analysis of purified mLYPLAL1 with FP-Rh for this assay is provided in **Extended Data Fig. 1a**.
Library	Library size	16,000 on 384-well plates as single compounds at 2 mM in DMSO
	Library composition	Small molecules < 500 Da and obeying Lipinski's "Rule of 5".
	Source	Maybridge Hitfinder Collection
	Additional comments	
Screen	Format	384-well plate
	Concentration(s) tested	Final concentration of 10 μM
	Plate controls	DMSO, fluorophosphonate-biotin (FP-biotin, full inhibition).
	Reagent/ compound dispensing system	Bioraptor FRD™ (Beckman Coulter) for mLYPLAL1 and FP-Rh dispensation. Biomek FX (Beckman Coulter) for library compound dispensation.
	Detection instrument and software	EnVision™ 2014 Multilabel plate reader (PerkinElmer); software: EnVision Workstation v. 1.12.
	Assay validation/QC	Intra- and inter-plate CV were 4.61 and 2.68%, respectively. The average S/B ratio was 1.92.
	Correction factors	Milli-polarization ratio (mP) = 1000 x (S - G x P) / (S + G x P) where S = detector channel 2, P = detector channel 1, G = G-factor for channel 1 correction, which is 0.94.
	Normalization	Data is normalized as percent activity, where DMSO = 100%. % activity = 100 x (Compound – FP-biotin) / (DMSO – FP-biotin)
	Additional comments	
Post-HTS analysis	Hit criteria	Inhibitors = compounds that decrease activity <60%; activators = compounds that increase activity >140%.
	Hit rate	Inhibitors = 0.53%, Activators = 0.36% for the fluorescence polarization-based ABPP screen.
	Additional assay(s)	An orthogonal assay (gel-based competitive ABPP) was used for hit validation (**Extended Data Fig. 2**). Two additional assays (4-nitrophenyl acetate and 4-umbelliferyl acetate hydrolysis) were used to confirm the top activator hit.
	Confirmation of hit purity and structure	Purity was assessed by NMR as well as comparison with re-synthesized and purchased hit compound.
	Additional comments	Structure of the initial top validated activator is incorrectly assigned in commercial libraries. Structure of compound **4** corrected based on X-ray crystal structure (**Fig. 1**).

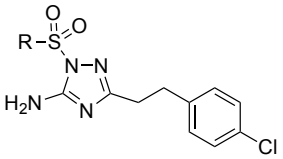
Supplementary Table 2. Compounds examined in exploration of the 1,2,4-triazole C3 linker

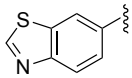
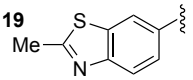
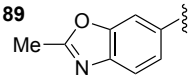


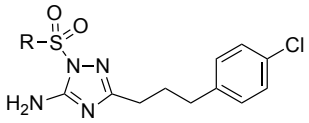
	R	mLYPLAL1 EC_{50} (fold increase)	hLYPLAL1 EC_{50} (fold increase)
15		>20 (1.0)	nd
78	1-naphthyl	2.4 (1.2)	nd
79	8-quinolyl	>20 (1.1)	nd
80	4-MeOPh	>20 (1.1)	nd
81	cyclohexyl	>20 (1.0)	nd
82		>20 (1.0)	nd
17		>20 (1.0)	nd

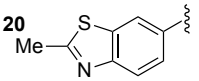


	R	mLYPLAL1 EC_{50} (fold increase)	hLYPLAL1 EC_{50} (fold increase)
16		>20 (1.0)	nd
83	1-naphthyl	2.1 (1.6)	nd
84	3-ClPh	>20 (1.2)	nd
85	3-NO_2Ph	6.2 (2.0)	nd
86	cyclohexyl	>20 (1.0)	nd
87		2.1 (1.6)	nd
18		>20 (1.1)	nd



	R	mLYPLAL1 EC_{50} (fold increase)	hLYPLAL1 EC_{50} (fold increase)
88		9.8 (2.2)	nd
19		2.6 (2.0)	nd
89		6.5 (1.6)	nd



	R	mLYPLAL1 EC_{50} (fold increase)	hLYPLAL1 EC_{50} (fold increase)
20		>20 (1.0)	nd
90	2-naphthyl	>20 (1.0)	nd
91	3-MeCOPh	>20 (1.0)	nd
92	3-NH_2COPh	>20 (1.1)	nd

Supplementary Table 3. *In vitro* ADME properties of **4** and **12**

Metabolic stability:

Half-life in 1 mg/ml hepatic microsomes

	Species (T1/2 in min)	
Compound	Human	Mouse
sunitinib	37.4	12.8
4	3.2	3.0
12	2.5	2.3

Intrinsic clearance

	Species (Cl_{int} - µl/min/mg)	
Compound	Human	Mouse
sunitinib	19	54
4	219	233
12	273	303

Solubility:

Compound	Kinetic Solubility (µM)	Equilibrium Solubility (µM)
4	**6.5**	nd
12	**1.3**	nd

P450 inhibition:

	% inhibition at 10 µM			
Compound	1A2	2C9	2D6	3A4
Furafylline (40 µM)	71	7	-35	-22
Sulfaphenazole	-9	93	-23	-20
Quinidine	-5	15	87	-1
Ketoconazole (1 µM)	-6	24	-22	96
4	34	82	38	91
12	32	88	32	89

Plasma protein binding:

	(% free)				(% bound)	
Compound	Human	Mouse		Compound	Human	Mouse
4	<0.1	0.2		**4**	>99.9	99.8
12	<0.1	0.2	*	**12**	>99.9	99.8
Carbamazepine	31	33.5		Carbamazepine	69.0	66.5
Ritonavir	0.4	0.5		Ritonavir	99.6	99.5

*__12__ degraded slowly in plasma. At 24 hr, 10% of the compound was accounted for in human plasma incubations and 40% in mouse plasma incubations

Supplementary Table 4. Crystal data and structure refinement for **4**.

	Compound **4**	
Identification code	boger30_a	
Empirical formula	C17 H15 Cl N4 O3 S2	
Formula weight	422.90	
Temperature	100.0 K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 5.8358(3) Å	α= 104.054(2)°
	b = 11.3499(5) Å	β= 95.985(2)°
	c = 14.0129(6) Å	γ = 95.776(2)°
Volume	887.80(7) $Å^3$	
Z	2	
Density (calculated)	1.582 Mg/m^3	
Absorption coefficient	0.478 mm^{-1}	
F(000)	436	
Crystal size	0.34 x 0.28 x 0.2 mm^3	
Theta range for data collection	1.512 to 26.427°	
Index ranges	-7<=h<=7, -14<=k<=12, -14<=l<=17	
Reflections collected	17096	
Independent reflections	3647 [R(int) = 0.0388]	
Completeness to theta = 26.000°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.0932 and 0.0604	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3647 / 0 / 244	
Goodness-of-fit on F^2	1.050	
Final R indices [I>2sigma(I)]	R1 = 0.0300, wR2 = 0.0735	
R indices (all data)	R1 = 0.0372, wR2 = 0.0777	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.413 and -0.314 $e.Å^{-3}$	

Supplementary Table 5. Crystal data and structure refinement for **12**.

	Compound **12**	
Identification code	SG1-91	
Empirical formula	C17 H14 Cl N5 O2 S3	
Molecular formula	C17 H14 Cl N5 O2 S3	
Formula weight	451.96	
Temperature	100.0 K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 1 21/n 1	
Unit cell dimensions	a = 11.0577(6) Å	α= 90°.
	b = 5.8200(4) Å	β= 96.005(3)°
	c = 29.2403(19) Å	γ = 90°
Volume	1871.5(2) $Å^3$	
Z	4	
Density (calculated)	1.604 Mg/m^3	
Absorption coefficient	0.565 mm^{-1}	
F(000)	928	
Crystal size	0.317 x 0.291 x 0.255 mm^3	
Crystal color, habit	Colorless Block	
Theta range for data collection	1.400 to 25.740°	
Index ranges	-13<=h<=13, -7<=k<=7, -31<=l<=35	
Reflections collected	23239	
Independent reflections	3566 [R(int) = 0.0581]	
Completeness to theta = 25.000°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.0921 and 0.0601	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3566 / 2 / 262	
Goodness-of-fit on F^2	1.038	
Final R indices [I>2sigma(I)]	R1 = 0.0325, wR2 = 0.0734	
R indices (all data)	R1 = 0.0451, wR2 = 0.0796	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.347 and -0.392 $e.Å^{-3}$	

Supplementary Table 6. Crystal data and structure refinement for **34**.

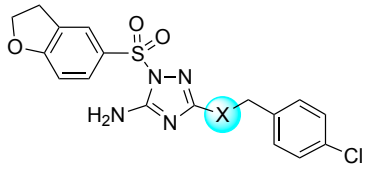
	Compound **34**	
Identification code	SG1-15	
Empirical formula	C15 H12 Br Cl N4 O2 S2	
Molecular formula	C15 H12 Br Cl N4 O2 S2	
Formula weight	459.77	
Temperature	100.0 K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 5.268(2) Å	α= 80.697(9)°
	b = 8.623(3) Å	β= 87.437(11)°
	c = 19.447(7) Å	γ = 87.839(10)°
Volume	870.5(5) $Å^3$	
Z	2	
Density (calculated)	1.754 Mg/m^3	
Absorption coefficient	2.772 mm^{-1}	
F(000)	460	
Crystal size	0.135 x 0.031 x 0.021	
Crystal color, habit	Colorless Rod	
Theta range for data collection	2.124 to 25.391°	
Index ranges	-6<=h<=6, -10<=k<=9, -23<=l<=23	
Reflections collected	10538	
Independent reflections	3181 [R(int) = 0.0619]	
Completeness to theta = 25.000°	99.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.0916 and 0.0651	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3181 / 0 / 226	
Goodness-of-fit on F^2	1.011	
Final R indices [I>2sigma(I)]	R1 = 0.0432, wR2 = 0.0738	
R indices (all data)	R1 = 0.0804, wR2 = 0.0826	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.390 and -0.411 $e.Å^{-3}$	

Supplementary Table 7. Crystal data and structure refinement for **37**.

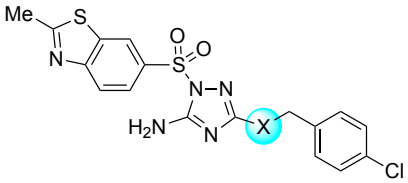
	Compound **37**	
Identification code	SG1-11	
Empirical formula	C16 H15 Cl N4 O3 S2	
Molecular formula	C16 H15 Cl N4 O3 S2	
Formula weight	410.89	
Temperature	100.0 K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	C 1 2/c 1	
Unit cell dimensions	a = 20.482(2) Å	α= 90°
	b = 17.972(2) Å	β= 110.176(2)°
	c = 10.6391(12) Å	γ = 90°
Volume	3675.9(7) $Å^3$	
Z	8	
Density (calculated)	1.485 Mg/m^3	
Absorption coefficient	0.459 mm^{-1}	
F(000)	1696	
Crystal size	0.213 x 0.053 x 0.021 mm^3	
Crystal color, habit	Colorless Rod	
Theta range for data collection	2.119 to 25.395°	
Index ranges	-24<=h<=24, -21<=k<=21, -12<=l<=12	
Reflections collected	21918	
Independent reflections	3384 [R(int) = 0.0499]	
Completeness to theta = 25.000°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.0916 and 0.0661	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3384 / 0 / 236	
Goodness-of-fit on F^2	1.023	
Final R indices [I>2sigma(I)]	R1 = 0.0337, wR2 = 0.0689	
R indices (all data)	R1 = 0.0518, wR2 = 0.0763	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.237 and -0.283 $e.Å^{-3}$	

Supplementary Table 8. Crystal data and structure refinement for **78**.

	Compound **78**	
Identification code	SG1-111	
Empirical formula	C19 H16 Cl N5 O2 S	
Molecular formula	C19 H16 Cl N5 O2 S	
Formula weight	413.88	
Temperature	100.0 K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	Pbca	
Unit cell dimensions	a = 24.7536(10) Å	α= 90°
	b = 8.2870(4) Å	β= 90°
	c = 36.1110(13) Å	γ = 90°
Volume	7407.6(5) $Å^3$	
Z	16	
Density (calculated)	1.484 Mg/m^3	
Absorption coefficient	0.346 mm^{-1}	
F(000)	3424	
Crystal size	0.153 x 0.147 x 0.131 mm^3	
Crystal color, habit	Colorless Block	
Theta range for data collection	1.645 to 25.350°	
Index ranges	-29<=h<=28, -6<=k<=9, -43<=l<=43	
Reflections collected	46733	
Independent reflections	6755 [R(int) = 0.0578]	
Completeness to theta = 25.000°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7453 and 0.5393	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	6755 / 6 / 529	
Goodness-of-fit on F^2	1.067	
Final R indices [I>2sigma(I)]	R1 = 0.0435, wR2 = 0.0865	
R indices (all data)	R1 = 0.0680, wR2 = 0.0964	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.307 and -0.396 $e.Å^{-3}$	



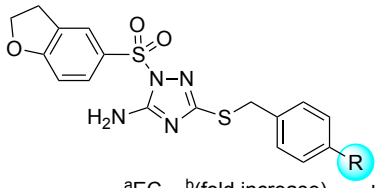
Compd	X	[a]EC_{50} [b](fold increase) mLYPLAL1	hLYPLAL1	rel activation HEK293[c]
4[d]	S	0.49 (2.3)	0.44 (2.5)	100%
15	NH	>20 (1.0)	nd[e]	0%
16	$NHCH_2$	>20 (1.0)	nd	0%



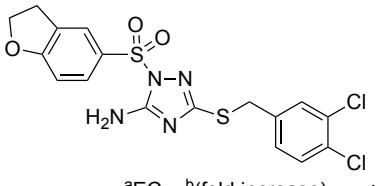
Compd	X	[a]EC_{50} [b](fold increase) mLYPLAL1	hLYPLAL1	rel activation HEK293[c]
12[d]	S	0.10 (2.8)	nd (2.5)	206%
17	NH	>20 (1.0)	nd[e]	1%
18	$NHCH_2$	>20 (1.1)	nd	0%
19	CH_2	2.6 (2.0)	nd	11%
20	CH_2CH_2	>20 (1.0)	nd	1%

[a]EC_{50} (μM). [b]Fold increase in FP-Rh labeling of LYPLAL1 by ABPP. [c]Relative activation of mLYPLAL1 in HEK293 proteome by the compound (10 μM) expressed as a % of **4** (2.5–5 fold activation, 10 μM). [d]Structure determined by X-ray. [e]Not determined.

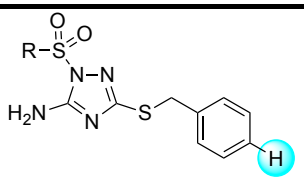
Supplementary Figure 1. Sulfur atom replacements in the C3 linker.



Compd	R	[a]EC_{50} [b](fold increase) mLYPLAL1	[a]EC_{50} [b](fold increase) hLYPLAL1	rel activation HEK293[c]
4[d]	Cl	0.49 (2.3)	0.44 (2.5)	100%
21	H	>20 (1.3)	6.3 (2.3)	26%
22	Br	0.91 (2.4)	0.33 (2.4)	96%
23	I	1.2 (1.5)	4.3 (2.4)	48%
24	Me	1.3 (1.8)	2.2 (2.4)	57%
25	NO_2	6.3 (1.3)	1.9 (2.5)	11%



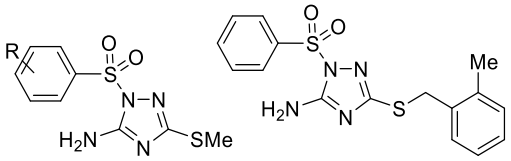
Compd	[a]EC_{50} [b](fold increase) mLYPLAL1	[a]EC_{50} [b](fold increase) hLYPLAL1	rel activation HEK293[c]
26	0.77 (2.2)	0.45 (2.4)	74%



	R	mLYPLAL1 [a]EC_{50} [b](fold increase)	hLYPLAL1 [a]EC_{50} [b](fold increase)
27	4-FPh	>20 (1.0)	>20 (1.2)
28	4-F,2-ClPh	>20 (1.3)	>20 (1.2)
29	5-F,2-MePh	>20 (1.0)	>20 (1.2)

[a]EC_{50} (μM). [b]Fold increase in FP-Rh labeling of LYPLAL1 by ABPP. [c]Relative activation of mLYPLAL1 in HEK293 proteome by the compound (10 μM) expressed as a % of **4** (2.5–5 fold activation, 10 μM). [d]Structure determined by X-ray.

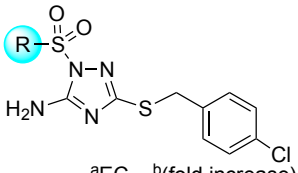
Supplementary Figure 2. Importance of the benzyl group and its substituent.



R = 4-Cl (AMB9827430)
R = 4-Me (AMB9827429)

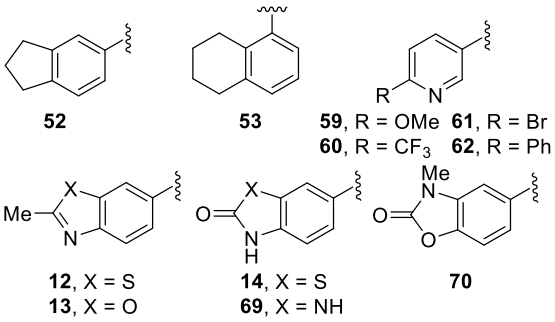
AMB2446039

Supplementary Figure 3. Representative inactive analogues of **4** probing the importance of the C3 thiobenzyl group. The corresponding compounds containing the 4-chlorobenzylthio substituent are active.

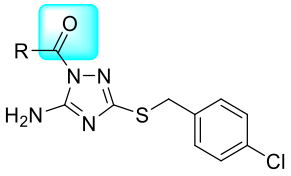


R	[a]EC$_{50}$ [b](fold increase) mLYPLAL1	hLYPLAL1	rel activation HEK293[c]
4[d]	0.49 (2.3)	0.44 (2.5)	100% (2.5-5)
30, Me	>20 (1.3)	>20 (2.5)	18%
31, *i*-Pr	4.9 (2.1)	15 (2.2)	34%
32, cyclohexyl	0.41 (2.0)	0.26 (2.2)	29%
5, Ph	1.40 (2.3)	0.70 (2.7)	70%
33, 4-FPh	1.1 (2.1)	0.57 (3.4)	nd[e]
6, 4-ClPh	0.74 (2.0)	1.1 (2.3)	76%
34, 4-BrPh[d]	1.1 (2.3)	0.57 (2.3)	35%
35, 4-MePh	0.76 (2.2)	0.37 (2.2)	21%
36, 4-EtPh	0.80 (1.9)	nd[e] (2.2)	45%
37, 4-MeOPh[d]	0.97 (2.4)	0.30 (2.2)	63%
38, 4-CNPh	0.77 (2.2)	nd (2.1)	83%
39, 4-O_2NPh	0.32 (2.4)	nd (2.1)	53%
40, 4-PhPh	>20 (1.3)	nd (1.7)	8%
7, 3-ClPh	0.19 (2.7)	nd (2.5)	140%
41, 3-MeOPh	0.62 (2.4)	nd (nd)	73%
42, 3-O_2NPh	0.17 (2.7)	0.11 (2.5)	128%
8, 3-MeO_2CPh	0.16 (3.1)	nd (nd)	168%
9, 3-EtO_2CPh	0.35 (2.7)	nd (nd)	145%
43, 3-MeO_2C,2-MePh	0.38 (1.8)	nd (nd)	40%
44, 3-AcPh	0.48 (2.4)	nd (1.4)	52%
45, 3-NH_2COPh	1.4 (2.4)	nd (1.6)	121%
46, 3-AcNHPh	0.65 (2.8)	nd (nd)	98%
10, 3-BzNHPh	0.37 (3.1)	nd (nd)	152%
47, 3-PhPh	0.48 (2.2)	nd (2.1)	45%
48, 2-FPh	0.81 (2.6)	nd (2.3)	66%
49, 2-O_2NPh	1.3 (2.4)	nd (2.0)	38%
50, 1-naphthyl	0.25 (2.5)	nd (nd)	68%
51, 2-naphthyl	0.10 (1.6)	nd (1.6)	11%
52	0.79 (2.5)	nd (2.2)	49%
53	1.3 (2.5)	nd (2.4)	44%
54, 3-furanyl	>20 (2.4)	nd (nd)	49%
55, 3-thienyl	0.55 (2.7)	nd (nd)	47%
56, 2-thienyl	0.56 (1.8)	0.65 (3.2)	nd
57, 5-Ph-2-thienyl	0.21 (1.6)	nd (1.8)	16%
58, 5-(2-py)-2-thienyl	0.06 (1.4)	0.02 (1.5)	nd
59	2.2 (2.0)	0.71 (1.9)	32%
60	2.4 (1.3)	nd (1.8)	7%
61	nd (1.1)	nd (nd)	23%
62	0.19 (1.3)	nd (nd)	5%
63, 8-quinolyl	0.94 (2.4)	nd (nd)	53%
64, NMe-indol-4-yl	0.25 (1.4)	nd (nd)	19%
65, 5-benzimidazolyl	0.38 (2.7)	nd (nd)	124%
66, 2-benzthiazolyl	>20 (1.1)	nd (nd)	5%
67, 4-benzthiazolyl	0.43 (2.1)	nd (nd)	47%
11, 6-benzthiazolyl	0.25 (2.8)	0.35 (2.8)	170%
68, 4-benzoxadiazoyl	0.44 (2.0)	0.23 (3.0)	nd
12[d]	0.10 (2.8)	nd (2.5)	206%
13	0.19 (2.9)	nd (nd)	183%
14	0.06 (2.7)	nd (nd)	243%
69	0.74 (2.7)	nd (nd)	89%
70	0.38 (2.4)	nd (nd)	78%

[a]EC$_{50}$ (μM). [b]Fold increase in FP-Rh labeling of LYPLAL1 by ABPP. [c]Relative activation of mLYPLAL1 in HEK293 proteome by the compound (10 μM) expressed as a % of **4** (2.5–5 fold activation, 10 μM). [d]Structure confirmed by X-ray. [e]Not determined.



Supplementary Figure 4. Modifications to the N1 sulfonamide substituent.

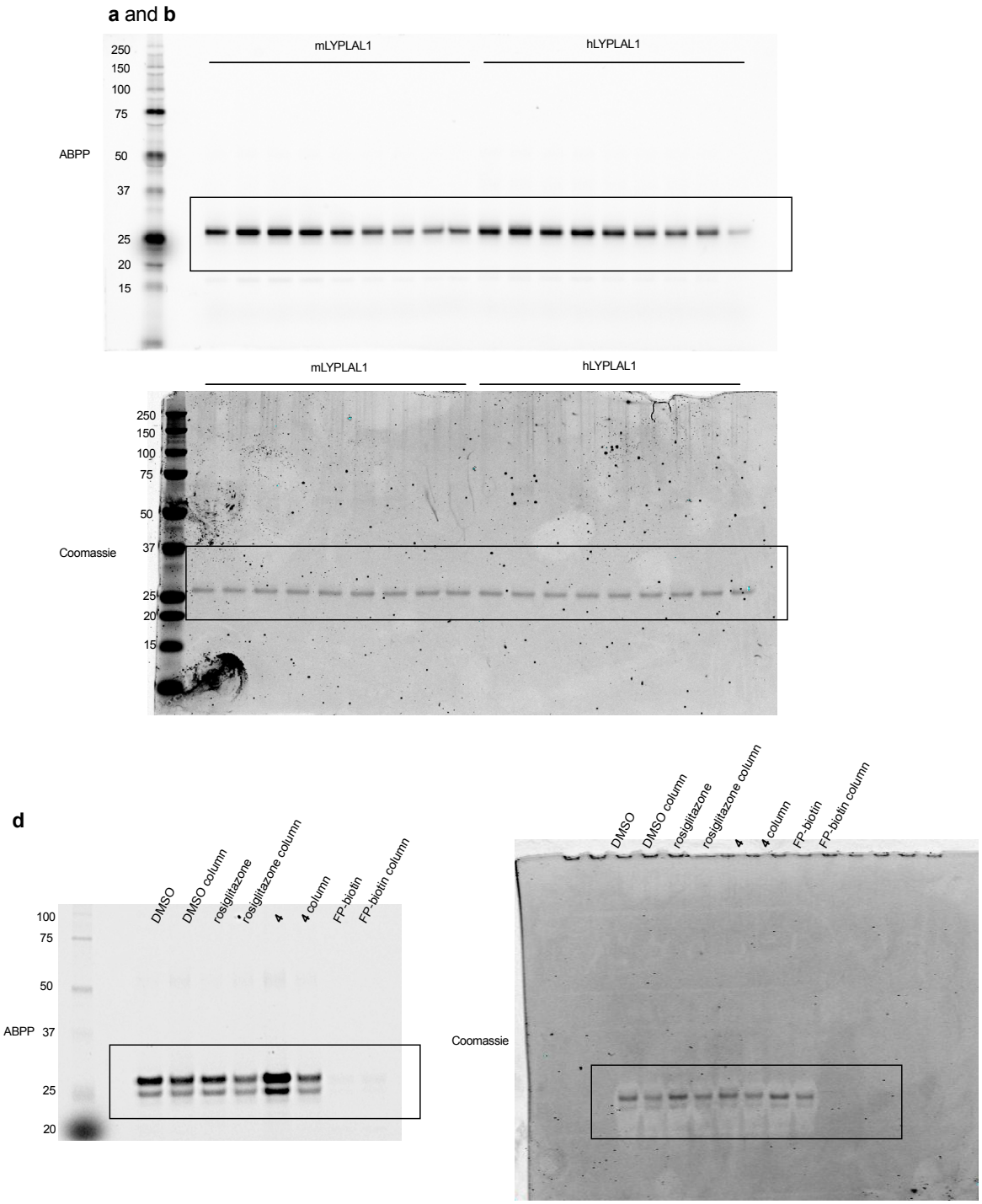


	R	mLYPLAL1 $^{a}EC_{50}$ ^{b}(fold increase)	hLYPLAL1 $^{a}EC_{50}$ ^{b}(fold increase)
71	Ph	>20 (1.0)	>20 (1.0)
72	4-NO_2Ph	>20 (1.0)	nd^{c}
73	4-FPh	>20 (1.0)	nd
74	3-FPh	>20 (1.0)	nd
75	3-NO_2Ph	>20 (1.0)	nd
76	cyclohexyl	>20 (1.0)	nd
77	1-naphthyl	>20 (1.0)	nd

$^{a}EC_{50}$ (μM). ^{b}Fold increase in FP-Rh labeling of LYPLAL1 by ABBP. ^{c}Not determined.

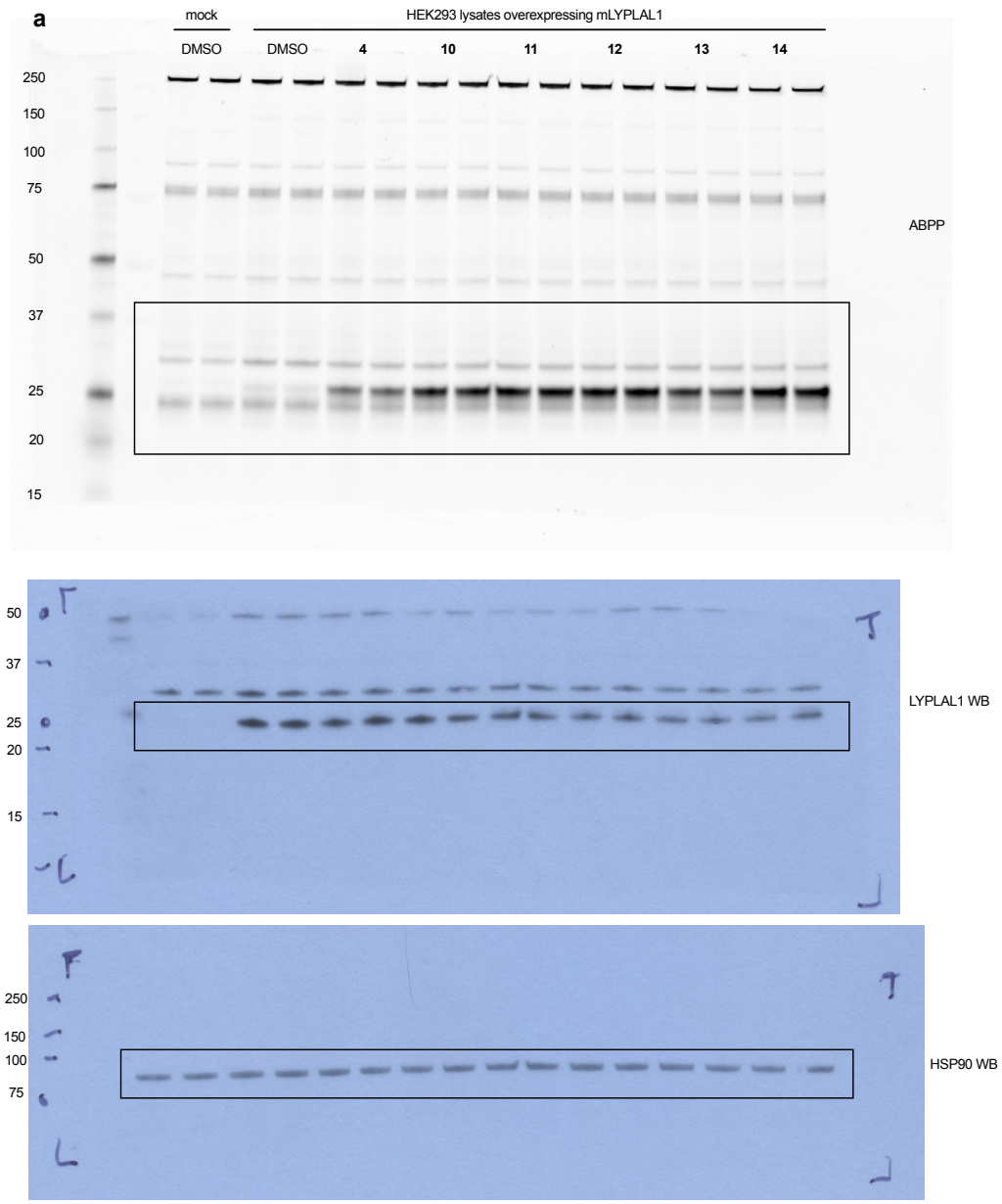
Supplementary Figure 5. N1 amide versus sulfonamide substitution

Uncropped gels from Fig. 2



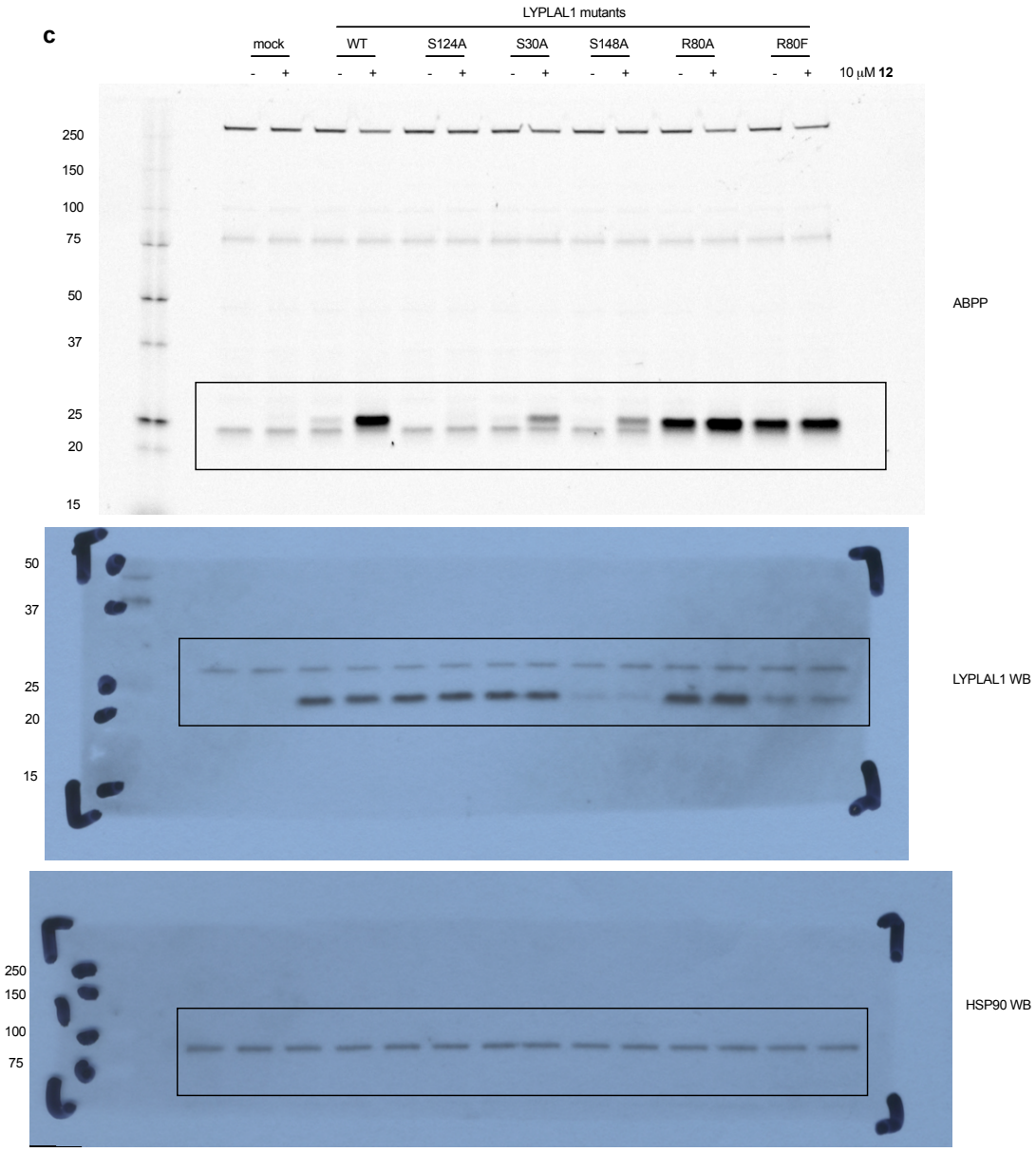
Supplementary Figure 6. Uncropped gels. Boxes indicate data shown in the respective main figure panels.

Uncropped gels from Fig. 4



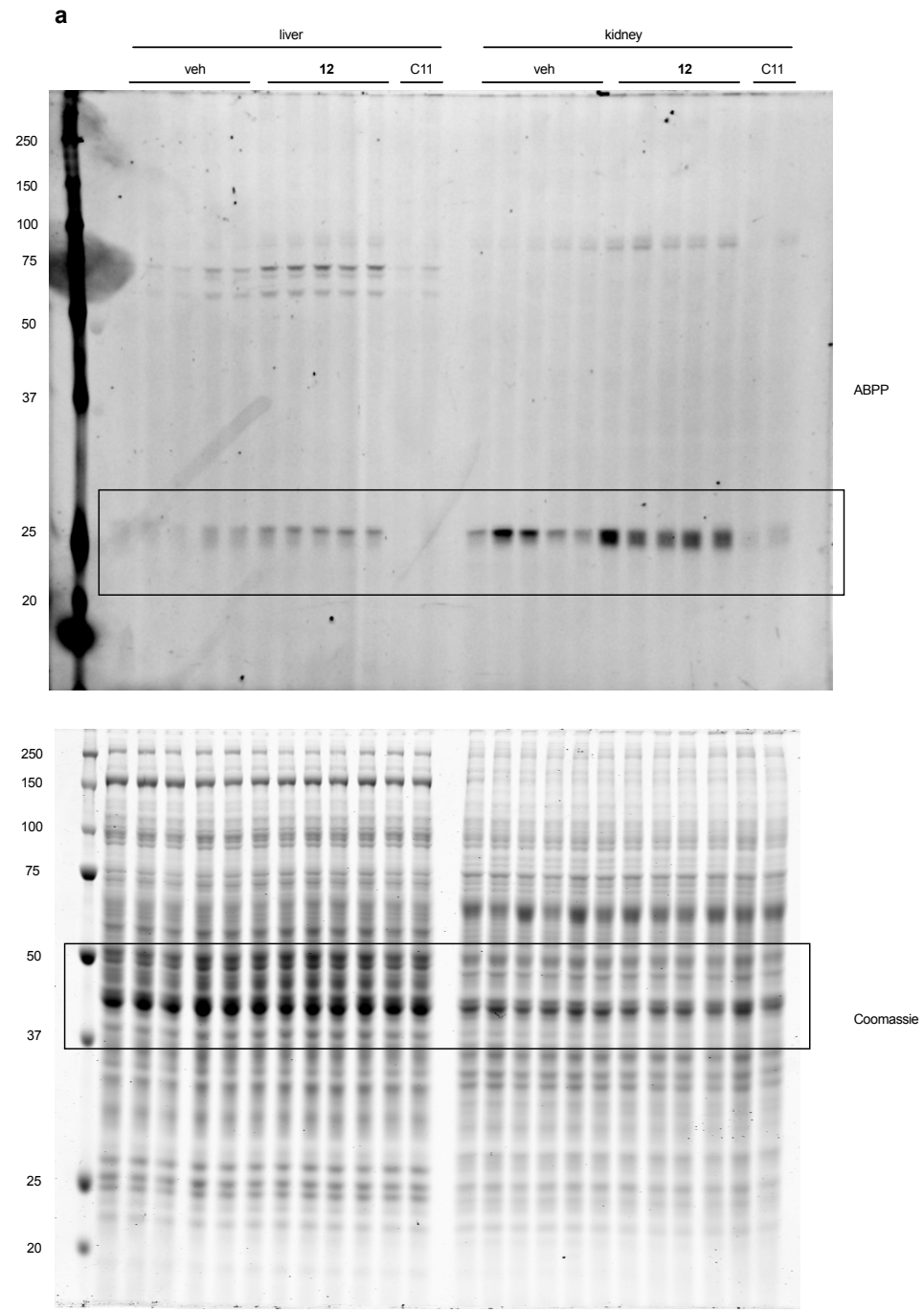
Supplementary Figure 6 (cont.)

Uncropped gels from Fig. 5



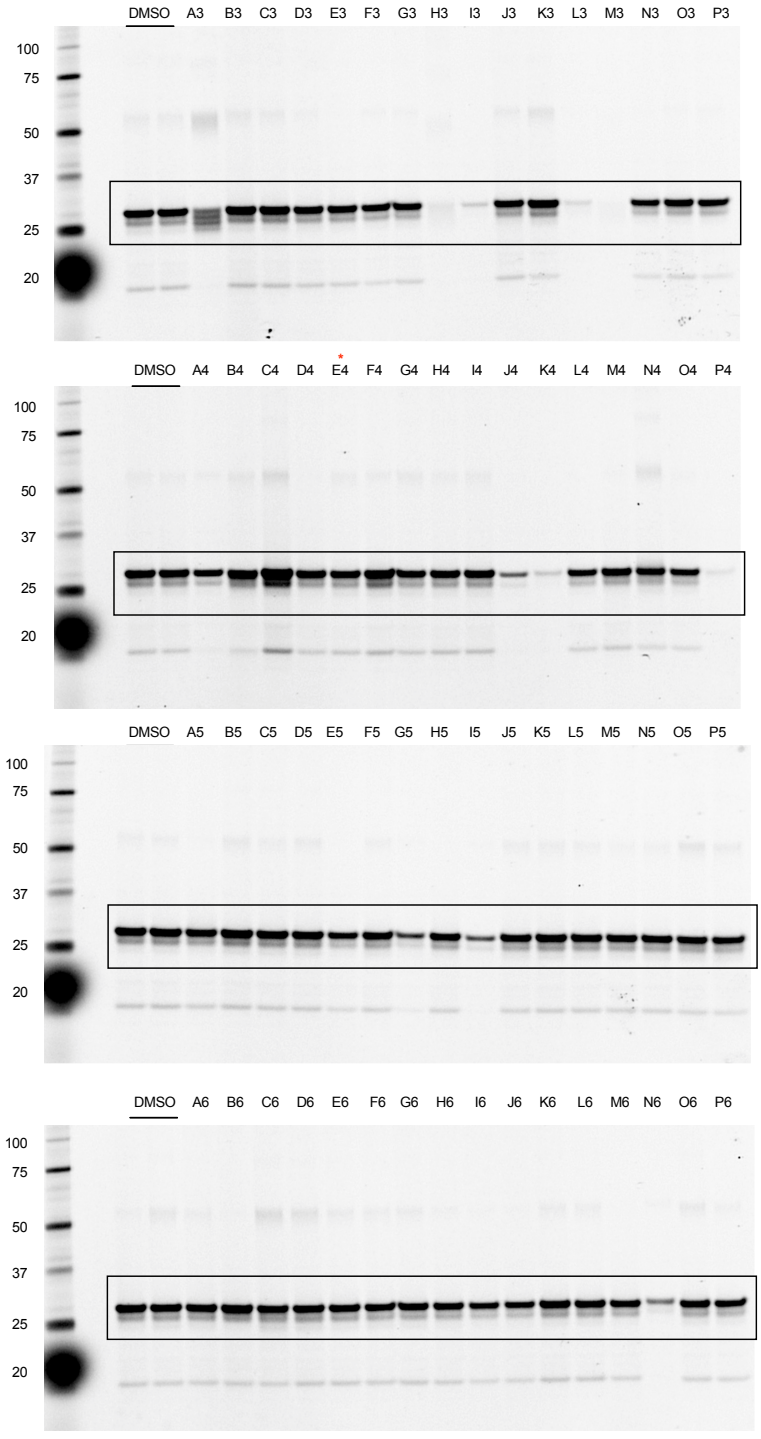
Supplementary Figure 6 (cont.)

Uncropped gels from Fig. 6



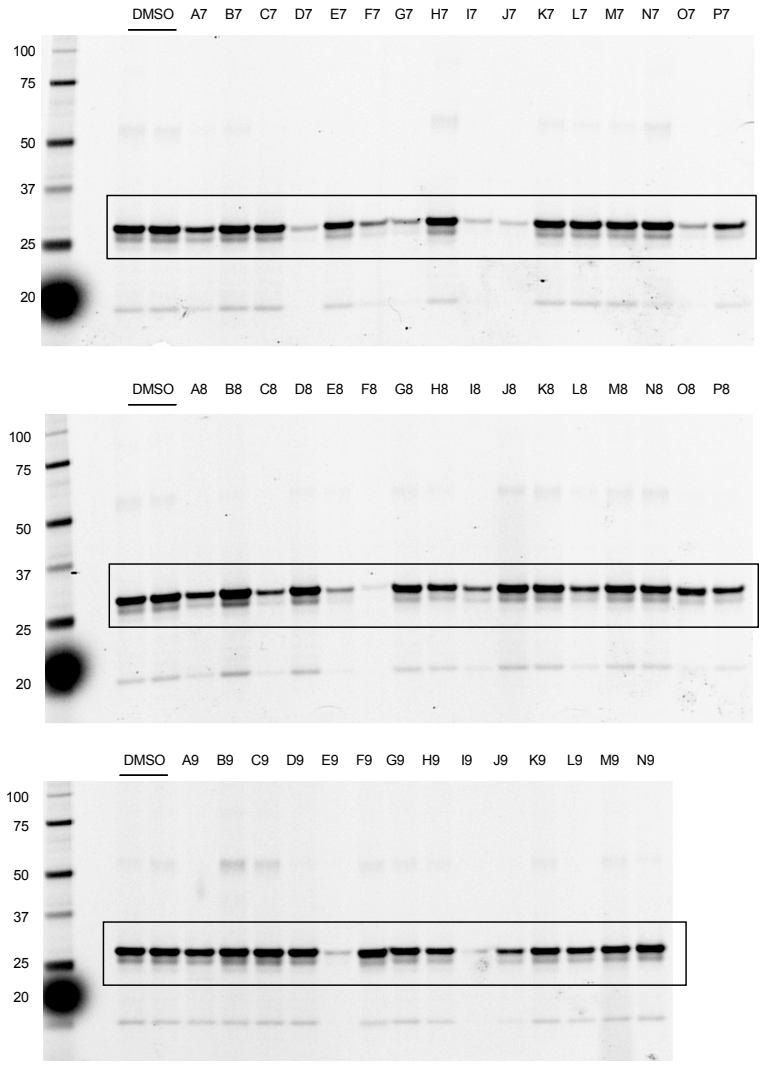
Supplementary Figure 6 (cont.)

Uncropped gels from Extended Data Fig. 2



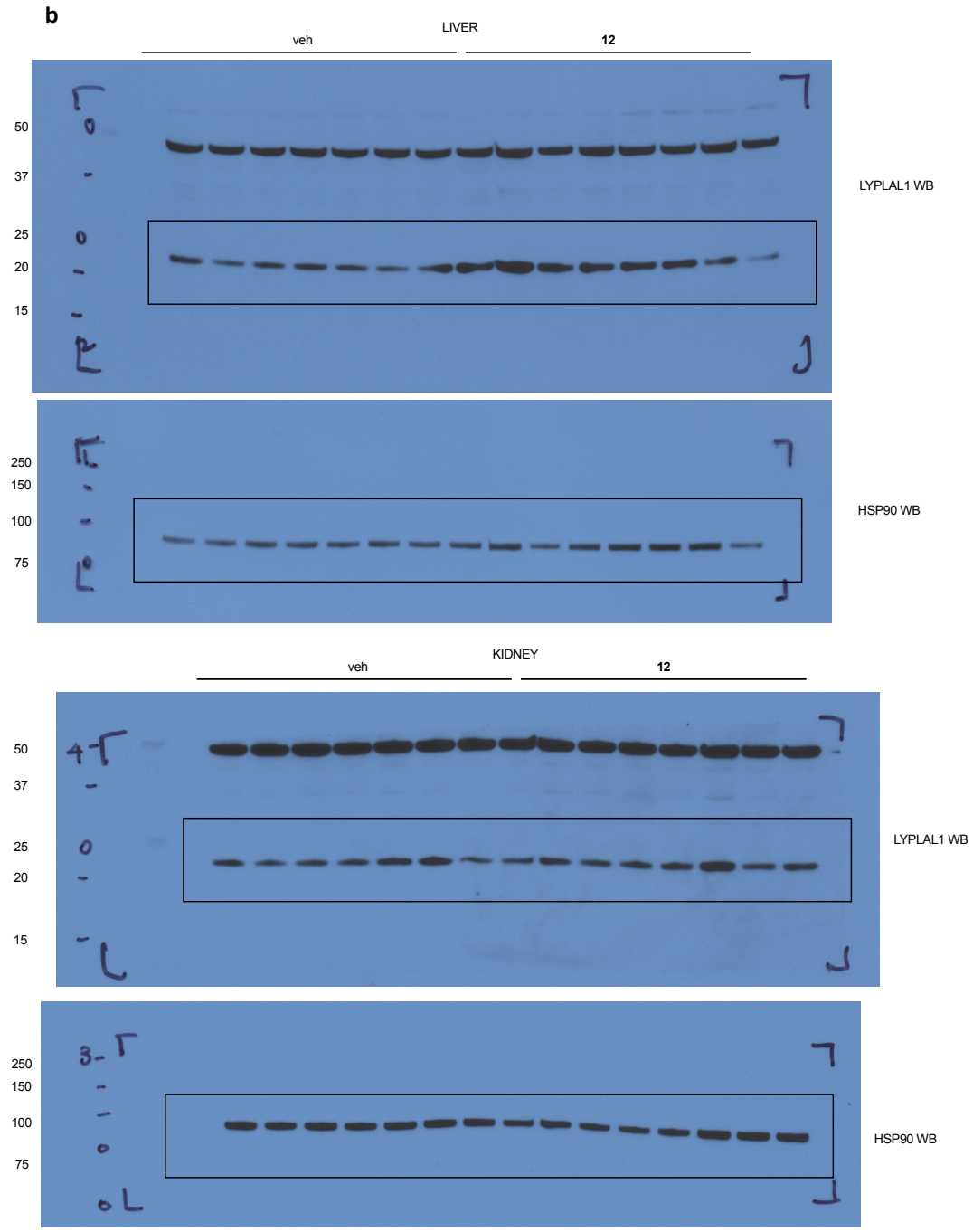
Supplementary Figure 6 (cont.)

Uncropped gels from Extended Data Fig. 2 (continued)



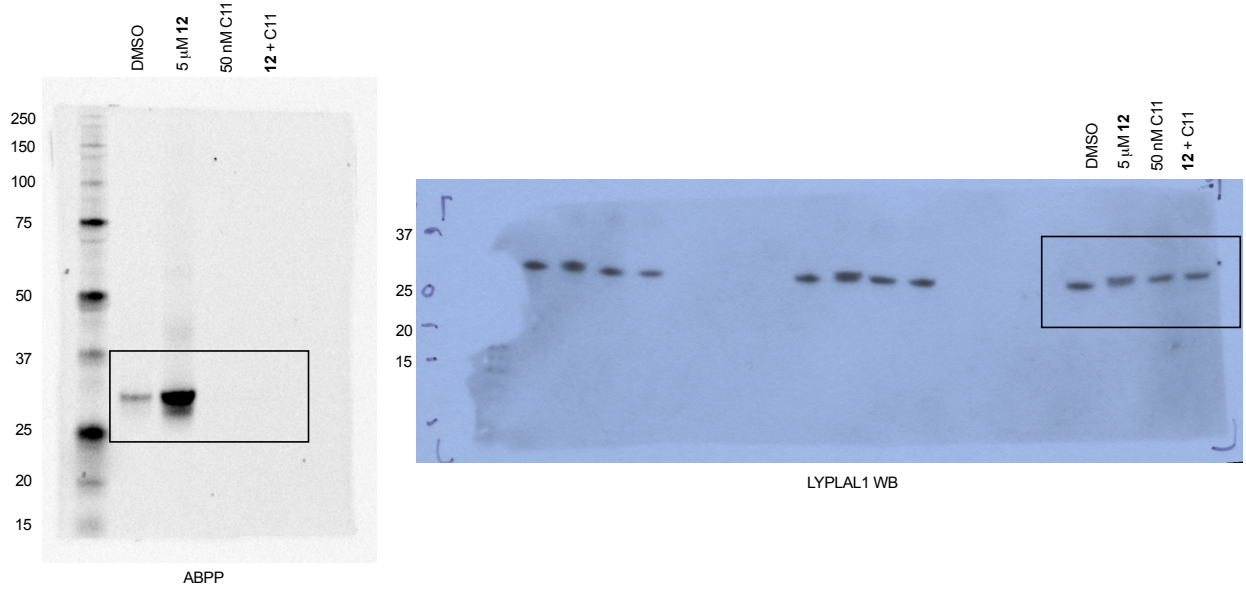
Supplementary Figure 6 (cont.)

Uncropped gels from Extended Data Fig. 5



Supplementary Figure 6 (cont.)

Uncropped gel from Extended Data Fig. 6



Supplementary Figure 6 (cont.)

Structure-Activity Study

To our knowledge, this represents the first report of the discovery and optimization of enzyme activation by a small molecule using ABPP. Further, it was conducted on an enzyme whose endogenous substrates are unknown. Because inhibitors of this enzyme were found to increase glucose production in primary hepatocytes[35], a property that *in vivo* would exacerbate metabolic disease, stimulation of LYPLAL1 activity might be predicted to ameliorate metabolic dysfunction. With this goal in mind, we set out to define the structural features of **4** (which we have named PAL-4) responsible for LYPLAL1 activation and conducted an initial structure-activity relationship study to explore and improve this activity. This analysis was performed with compound assessments against purified mLYPLAL1, often with additional assessments against purified hLYPLAL1. We have not seen substantial differences in activity between mLYPLAL1 and hLYPLAL1 with this compound series. Compounds were prepared by approaches identical to that detailed for **4** in **Fig. 1** and involved sulfonylation or acylation of **3** or related 5-amino-1,2,4-triazoles as the last step. The C3 4-chlorobenzylthio substituent proved key to the activity of compounds. This was initially examined with compounds **15** and **16** that not only contain the lead compound dihydrobenzfuran-5-sulfonyl substituent, but also other N1 sulfonyl substituents that proved more active, including one shown in **Supplementary Fig. 1**. Replacement of the sulfur atom (S) in the linker to the 4-chlorophenyl group with a nitrogen (NH) or a methylene (CH_2) led to a complete loss in activity (NH, **15** and **17**) or >25-fold loss in activity (CH_2, **19** vs. **12**). To ensure that this was not due to the larger size of the sulfur atom, approaching the size of CH_2CH_2, analogues with $NHCH_2$ and CH_2CH_2 replacements were prepared and examined. These proved to even less active or inactive (**Supplementary Fig. 1** and **Supplementary Table 2**).

The importance of the 4-chlorophenyl group was established with the representative series **21**-**25**, where **4** was found to be the most active compound in the set examined (**Supplementary Fig. 2**). Removal of the 4-chloro substituent (**21**) led to a substantial (>10-40 fold) reduction in the potency of the LYPLAL1 activator, while its replacement with conservative alternative substituents reduced (**23**-**25**) or perhaps maintained (**22**) this activity. Interestingly, the 3,4-dichlorophenyl analogue **26** essentially maintained both the efficacy and potency of **4**, suggesting there may be opportunities to further modulate the activity and properties of the lead structure with additional modifications.

What was clear from this series and additional compounds also examined at an early stage was that: (1) the phenyl group was required for activity since the corresponding C3 thiomethyl analogues were inactive (**Supplementary Fig. 3**), (2) not all closely related thiobenzyl substituents conveyed activity (e.g., 2-Me$PhCH_2S$-, **Supplementary Fig. 3**), (3) the 4-chloro substituent on the benzyl group substantially enhanced potency and was the most effective of the small set of substituents examined (**Supplementary Fig. 2**), and (4) the sulfur atom in the linker essentially was required (**Supplementary Fig. 1**).

By contrast, the N1 sulfonamide proved to be a group that could be extensively modified with maintenance or improvements in the potency (EC_{50}) and occasionally the efficacy (fold increase) of the compound (**Supplementary Fig. 4**). Although a simple methylsulfonamide (**30**) was largely inactive, larger aliphatic (**31**) and the cyclohexyl (**32**) sulfonamides were active, nearly matching the activity of **4**. Phenylsulfonamide **5** nearly matched the potency of **4** and approached its efficacy. As a result, a range of substituted phenylsufonamides were explored (**5**-**10** and **33**-**49**) and found to display good activity that was widely tolerant of the nature (electron-withdrawing or electron-donating), size, polarity, and position of the added substituent. Typically, substituents at the meta position were better tolerated and often led to greater enhancements in activity. Several such compounds (**7**-**10**) exhibited improved potencies (EC_{50} = 0.19, 0.16, 0.35 and 0.37 μM) and improved activations (2.7, 3.1, 2,7 and 3.1-fold) over **4**. Bicyclic aromatic sulfonamides (**50**-**53**), and mono and bicyclic heterocyclic sulfonamides (**11**-**14** and **51**-**70**) were also generally, but not always, well tolerated. The latter bicyclic heterocyclic series examined typically matched or exceeded the potency of **4**, and several substantially surpassed the potency of **4** with three (**12**-

14) exhibiting both improved potencies (EC_{50} = 0.060-0.19 μM or 60-190 nM) and superb activations of 2.7 to 2.9-fold.

Finally, a series of N1 amides **71**-**77** versus sulfonamides were prepared for examination and all were found to be inactive (**Supplementary Fig. 5**), indicating that the sulfonamide moiety plays an integral role in the modulation of the enzyme activation.

These studies provided a range of compounds with which the impact of activating LYPLAL1 enzymatic activity could be further studied. To complement our work using pure LYPLAL1 protein, mLYPLAL1 was overexpressed in HEK293 cells and activation of the enzyme examined by gel-based ABPP in total proteome prepared from transfected cells. In this way, we could determine the extent to which mLYPLAL1 activation would be observed even in a complex proteome where competitive binding to other proteins might adversely impact enzyme binding and activation. For an initial screen, this was conducted at a single arbitrary concentration of compound (10 μM) under defined conditions where the lead compound **4** was found to display substantial activation and a prominent increase (typically 2.5 to 5-fold) in FP-rhodamine labeling of the gel band corresponding to mLYPLAL1. Interestingly, the compounds, including **4**, generally were found to enhance LYPLAL1 labeling and activation better in the complex proteome than in assays with the purified enzyme. For the results reported in **Supplementary Fig. 4**, the activation displayed by the analogues in the HEK293 proteome has been normalized to the FP-rhodamine labeling observed with **4** (normalized to 100%, 2.5 to 5-fold activation). In general, the results in the complex HEK293 proteome followed those observed with the purified enzyme where the more potent and more efficacious analogues (**7**-**14**) exceeded the activity of **4** observed under these conditions and several additional analogues (**45** and **65**) further differentiated themselves from **4** itself. Impressive improvements were observed in this regard with **11**-**14**, which displayed substantial 2 to 3-fold enhancements in mLYPLAL1 activation over **4** itself (2.5 to 5-fold enhancement) in the presence of the HEK293 proteome.

We also determined the basic *in vitro* ADME properties of **4** and **12** (**Supplementary Table 3**). Given that these properties are not auspicious for use of **4** or **12** *in vivo*, we used ABPP to directly assess target engagement in tissues. In spite of its poor *in vitro* ADME profile, a single dose of **12** (100 mg/kg) increased LYPLAL1 activity ~2 fold in mouse liver and kidney (**Fig. 6a**).

Synthetic Procedures

1. General Procedures
2. Synthesis of candidate LYPLAL1 activator cores
   a) 3-Amino-5-(4-chlorobenzyl)thio-1,2,4-triazole synthesis
   b) 1,2,4-Triazole C2 and C3 linker intermediates synthesis
   c) 1,2,4-Triazole C1 and C2 amino substituted intermediates synthesis
3. General procedure for sulfonylation or acylation

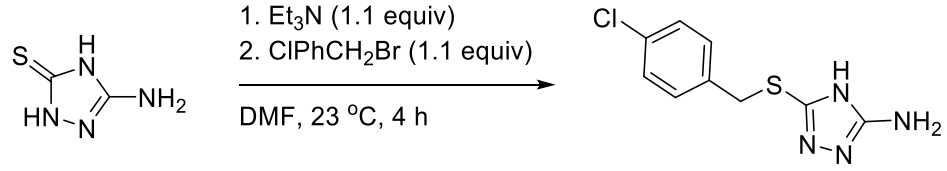
1. General procedures

All commercial reagents were used without further purification unless otherwise noted. THF was distilled prior to use. All reactions were performed in oven-dried (200°C) glassware and under an inert atmosphere of anhydrous N_2 unless otherwise noted. Anhydrous acetonitrile and N,N-dimethylformamide were purchased from Acros Organics. Column chromatography was performed with silica gel 60. TLC was performed on Whatman silica gel (250 μm) F254 glass plates and spots were visualized by UV. PTLC was performed on Whatman silica gel (250 and 500 μm) F254 glass plates. ^{1}H NMR was recorded on a Bruker 500 MHz spectrometer. Chemical shifts are reported in ppm (δ) from an internal standard of residual $CHCl_3$ (δ 7.26 for ^{1}H) or DMSO

(δ 2.50 for ^{1}H). Coupling constants (*J*) (H,H) are given in Hz. ^{1}H NMR data are reported as follows: chemical shift (δ), multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant, and integration. Mass spectra were obtained on an Agilent G1969A ESI-TOF mass spectrometer (high resolution) or Agilent Technologies LC/MSD-SL (low resolution), and the detected masses given as *m/z* with m representing the molecular ion. The purity of assayed compounds was confirmed to >95% by ^{1}H NMR.

2. Synthesis of candidate LYPLAL1 activator cores

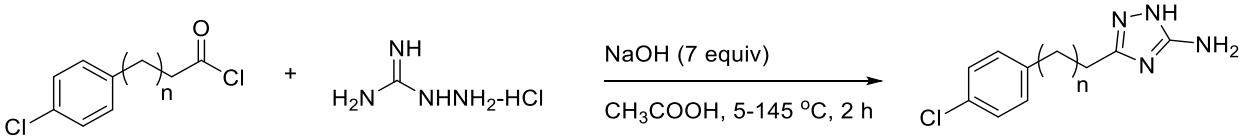
2a. 5-Amino-3-(4-chlorobenzyl)thio-1,2,4-triazole (3)



2 **3**

A solution of 5-amino-3-mercapto-1,2,4-triazole (**2**, 1.0 equiv, 8.61 mmol, 1.0 g) in DMF (12 mL) was treated with triethylamine (1.10 equiv, 9.47 mmol, 1.33 mL) dropwise at room temperature and stirred for 15 min. 4-Chlorobenzyl bromide (1.10 equiv, 9.47 mmol, 1.9 g) was added and the mixture was stirred for 4 h at room temperature. The solvent was evaporated to dryness and the residue was dissolved in ethyl acetate, washed with water and saturated aqueous NaCl, dried over anhydrous Na_2SO_4 and concentrated. Purification by flash column chromatography (SiO_2, 75% EtOAc/hexanes) afforded **3** (1.97 g, 95%) as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.37–7.32 (m, 4H), 6.04 (s, 2H), 4.18 (s, 2H). HRMS-ESI-TOF *m/z* calculated for $C_9H_{10}ClN_4S^+$ [M + H^+]: 241.0309, found: 241.0314.

2b. 1,2,4-Triazole C2 and C3 linker intermediates synthesis



S2: n=1
S3: n=2

S1

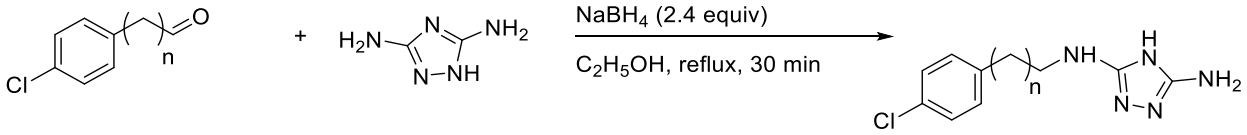
S4: n=1
S5: n=2

S4: Aminoguanidine hydrochloride (**S1**, 2.0 equiv, 0.98 mmol, 108 mg) was powdered using a mortar pestle and mixed with 3-(4-chlorophenyl)propanoyl chloride (**S2**, 1.0 equiv, 0.49 mmol, 100 mg) in a microwave tube. The tube was heated (145–150°C) for 15 min in an oil bath and allowed to cool to room temperature. The reaction mixture was dissolved by the addition of saturated aqueous sodium hydroxide and warmed at reflux for 30 min. The reaction mixture was cooled to room temperature, neutralized by addition of glacial acetic acid, and cooled to 5 °C to complete precipitation. The precipitate was collected by filtration, washed with cold water and then recrystallized from acetonitrile. The acetonitrile was removed, and the residue was dissolved in ethyl acetate, dried with anhydrous Na_2SO_4, and concentrated to obtain the product as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.57 (s, 1H), 7.30 (d, *J* = 8.3 Hz, 2H), 7.22 (d, *J* = 8.3 Hz, 2H), 5.78 (s, 2H), 2.89 (t, *J* = 7.0 Hz, 2H), 2.67 (br s, 2H). LRMS-ESI *m/z* calculated for $C_{10}H_{12}ClN_4^+$ [M + H^+]: 223.1, found: 223.0

S5 was prepared in the same manner as **S4** from aminoguanidine hydrochloride (**S1**) and 4-(4-chlorophenyl)butanoyl chloride (**S3**) and was obtained as a white solid (400 mg, 81%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.25 (d, *J* = 8.4 Hz, 2H), 7.11 (d, *J* = 8.3 Hz, 2H), 4.18 (s, 2H), 2.64 (p, *J* =

7.7 Hz, 4H), 2.04 (p, *J* = 7.5 Hz, 2H). LRMS-ESI *m/z* calculated for $C_{11}H_{14}ClN_4^+$ [M + H^+]: 237.1, found: 237.0.

2c. 1,2,4-Triazole C1 and C2 amino substituted intermediates synthesis



S7: n=0
S8: n=1

S6

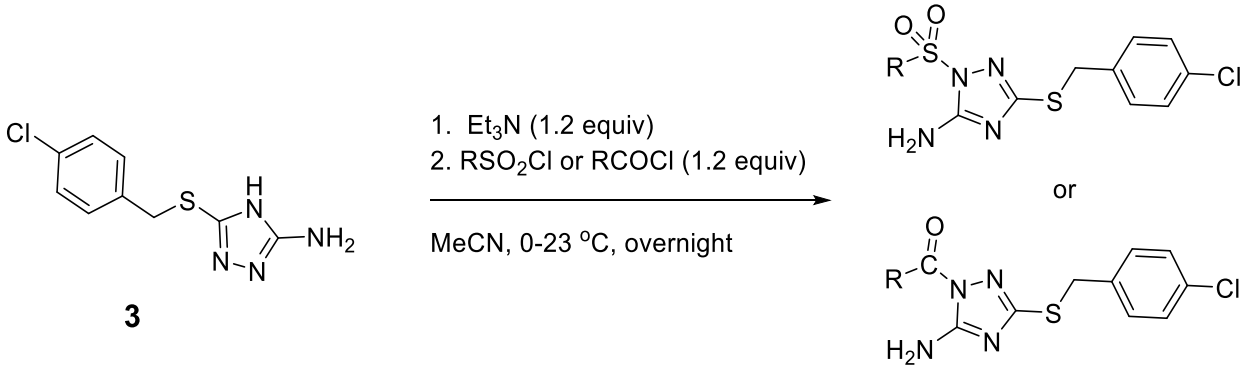
S9 : n=0
S10: n=1

S9: A solution of 3,5-diamino-1,2,4-triazole (**S6**, 1.1 equiv, 2.02 mmol, 200 mg) in ethanol (1.0 mL) was treated with 4-chlorobenzaldehyde (**S7**, 1.0 equiv, 1.8 mmol, 257 mg) in ethanol (1 mL) at room temperature and the mixture was warmed at reflux for 1 h. The reaction mixture was cooled to room temperature, $NaBH_4$ (1.36 equiv, 2.75 mmol, 104 mg) was added and the mixture was warmed at reflux for 30 min. The ethanol volume was reduced, and water was added. A white solid formed that was collected by filtration. The obtained solid was recrystallized from a small volume of ethanol to provide the product as a white solid (137 mg, 34%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.70 (s, 1H), 7.34–7.30 (m, 4H), 6.07 (br s, 1H), 5.37 (br s, 2H), 4.38 (d, *J* = 6.0 Hz, 2H). LRMS-ESI *m/z* calculated for $C_9H_{11}ClN_5^+$ [M + H^+]: 224.1, found: 224.0

S10 was prepared in the same manner as **S9** from 3,5-diamino-1,2,4-triazole (**S6**, 1.1 equiv, 2.02 mmol, 200 mg) and 2-(4-chlorophenyl)acetaldehyde (**S8**, 1.0 equiv, 1.82 mmol, 280 mg). The crude product was purified by flash chromatography (SiO_2, 5% MeOH/CH_2Cl_2) and **S10** was obtained as a white solid (45 mg, 10%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.71 (s, 1H), 7.33 (d, *J* = 8.2 Hz, 2H), 7.23 (d, *J* = 8.3 Hz, 2H), 5.32 (br s, 2H), 3.19 (m, 2H), 2.77 (t, *J* = 7.3 Hz, 2H). LRMS-ESI *m/z* calculated for $C_{10}H_{13}ClN_5^+$ [M + H^+]: 238.1, found: 238.0.

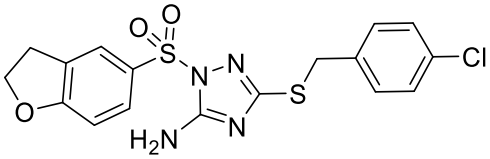
3. General procedure for sulfonylation or acylation

A stirred solution of **3** (1.0 equiv, 0.21 mmol, 50 mg) in acetonitrile (2 mL) was treated with triethylamine (1.2 equiv, 0.25 mmol, 35 μL) dropwise at 0°C. The desired sulfonyl chloride or acyl chloride (1.2 equiv) was then added, and the mixture was stirred at room temperature overnight. The solvent was removed and the crude product purified by column chromatography (SiO_2, EtOAc/hexanes).



3

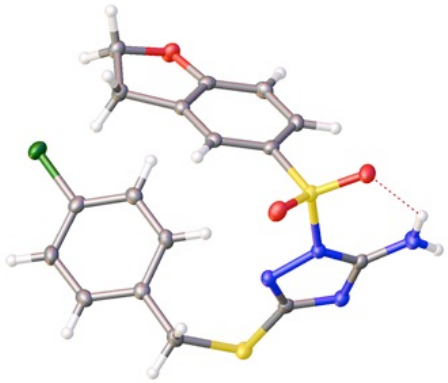
Compound 4: 3-((4-chlorobenzyl)thio)-1-((2,3-dihydrobenzofuran-5-yl)sulfonyl)-5-amino-1*H*-1,2,4-triazole. Trivial name: PAL-4 (Pharmacological Activator of LYPLAL1-4).



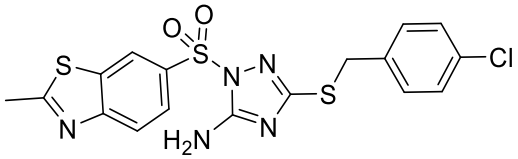
Compound **4** was prepared from 2,3-dihydrobenzofuran-5-sulfonyl chloride (1.2 equiv, 0.25 mmol, 54 mg) using the general procedure. Flash chromatography (SiO_2, 40% EtOAc/hexanes) yielded the desired product as a white solid (30 mg, 34%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.75 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.71 (m, 1H), 7.25 (d, *J* = 8.6 Hz, 2H), 7.19 (d, *J* = 8.6 Hz, 2H), 6.86 (d, *J* = 8.5 Hz, 1H), 5.77 (s, 2H), 4.73 (t, *J* = 8.7 Hz, 2H), 4.18 (s, 2H), 3.26 (t, *J* = 8.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.10, 161.63, 156.23, 136.00, 133.20, 130.53, 130.51, 129.25, 128.60, 127.64, 125.50, 110.11, 72.92, 34.66, 28.98. HRMS-ESI-TOF *m/z* calculated for $C_{17}H_{16}ClN_4O_3S_2^+$ [M + H^+]: 423.0347, found: 423.0351.

This compound is also commercially available from Ambinter (AMB2745087) and Aurora Chemicals (K13.701.587).

The structure of **4** was confirmed by X-ray structure determination (CCDC 1825320; **Supplementary Table 4**).

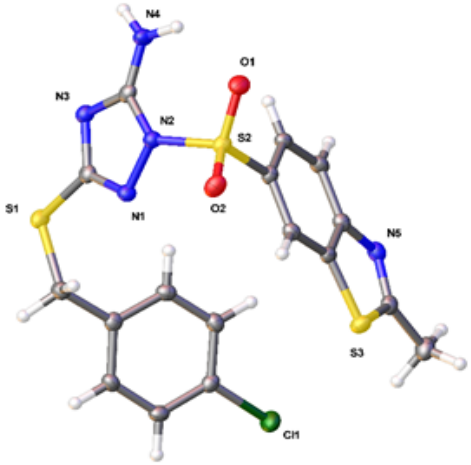


Compound 12: 3-((4-chlorobenzyl)thio)-1-((2-methylbenzo[*d*]thiazol-6-yl)sulfonyl)-5-amino-1*H*-1,2,4-triazole. Trivial name: PAL-12 (Pharmacological Activator of LYPLAL1-12).



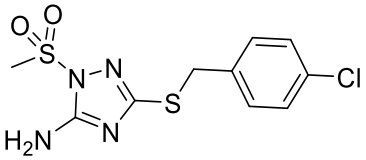
Compound **12** was prepared from 2-methyl-1,3-benzothiazole-6-sulfonyl chloride (1.2 equiv, 0.25 mmol, 62 mg) using the general procedure. Flash chromatography (SiO_2, 50% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.47 (d, *J* = 1.7 Hz, 1H), 8.06 (d, *J* = 8.6 Hz, 1H), 7.95 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.11 (d, *J* = 8.4 Hz, 2H), 5.93 (s, 2H), 4.16 (s, 2H), 2.93 (s, 3H). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 173.54, 162.34, 157.43, 156.42, 136.53, 135.76, 133.25, 132.45, 130.37, 128.59, 125.33, 123.46, 122.84, 34.61, 20.82. HRMS-ESI-TOF *m/z* calculated for $C_{17}H_{15}ClN_5O_2S_3^+$ [M + H^+]: 452.0071, found: 452.0060.

The structure of **12** was confirmed by X-ray structure determination (CCDC 1825321; **Supplementary Table 5**).



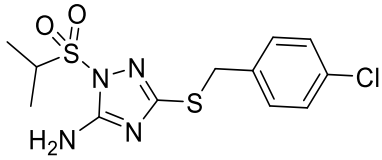
Compounds **27** (Z1410911355), **28** (Z1410911378), and **29** (Z1410911371) were purchased from Enamine.

Compound 30: 3-((4-chlorobenzyl)thio)-1-(methylsulfonyl)-5-amino-1*H*-1,2,4-triazole



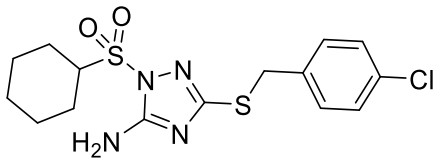
Compound **30** was prepared from methylsulfonyl chloride (1.2 equiv, 0.25 mmol, 20 µL) using the general procedure. Flash chromatography (SiO_2, 40% EtOAc/hexanes) yielded the desired product as a white solid (28 mg, 42%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.32 (m, 4H), 4.43 (s, 2H), 3.36 (s, 3H). HRMS-ESI-TOF *m/z* calculated for $C_{10}H_{12}ClN_4O_2S_2^+$ [M - H^+]: 316.9939, found: 316.9936.

Compound 31: 3-((4-chlorobenzyl)thio)-1-(isopropylsulfonyl)-5-amino-1*H*-1,2,4-triazole



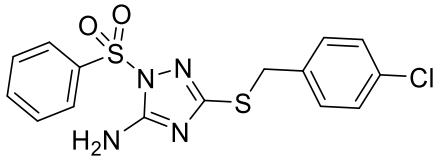
Compound **31** was prepared from propane-2-sulfonyl chloride (1.2 equiv, 0.25 mmol, 30 µL) using the general procedure. Flash chromatography (SiO_2, 50% EtOAc/hexanes) yielded the desired product as a white solid (56 mg, 79%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.34 (d, *J* = 8.3 Hz, 2H), 7.26 (d, *J* = 8.2 Hz, 2H), 5.67 (s, 2H), 4.25 (s, 2H), 3.65 (m, 1H), 1.34 (d, *J* = 6.9 Hz, 6H). HRMS-ESI-TOF *m/z* calculated for $C_{12}H_{16}ClN_4O_2S_2^+$ [M + H^+]: 347.0398, found: 347.0401.

Compound 32: 3-((4-chlorobenzyl)thio)-1-(cyclohexylsulfonyl)-5-amino-1*H*-1,2,4-triazole



Compound **32** was prepared from cyclohexylsulfonyl chloride (1.2 equiv, 0.25 mmol, 35 μL) using the general procedure. Flash chromatography (SiO_2, 30% EtOAc/hexanes) yielded the desired product as a white solid (62 mg, 77%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36 (d, *J* = 8.5 Hz, 2H), 7.26 (d, *J* = 8.4 Hz, 2H), 5.65 (s, 2H), 4.24 (s, 2H), 3.35 (tt, *J* = 12.2, 3.4 Hz, 1H), 1.90 (m, 4H), 1.50 (qd, *J* = 12.5, 3.0 Hz, 2H), 1.21 (m, 4H). LRMS-ESI *m/z* calculated for $C_{15}H_{20}ClN_4O_2S_2^+$ [M + H$^+$]: 387.1, found: 387.0.

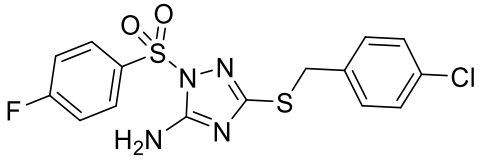
Compound 5: 3-((4-chlorobenzyl)thio)-1-(phenylsulfonyl)-5-amino-1*H*-1,2,4-triazole



Compound **5** was prepared from phenylsulfonyl chloride (1.2 equiv, 0.25 mmol, 32 μL) using the general procedure. Flash chromatography (SiO_2, 40% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.92 (m, 2H), 7.71 (tt, *J* = 7.6, 1.2 Hz, 1H), 7.55 (m, 2H), 7.24 (m, 2H), 7.18 (m, 2H), 5.83 (s, 2H), 4.18 (s, 2H). HRMS-ESI-TOF *m/z* calculated for $C_{15}H_{14}ClN_4O_2S_2^+$ [M + H$^+$]: 381.0241, found: 381.0242.

This compound is also commercially available from Ambinter (AMB2745084).

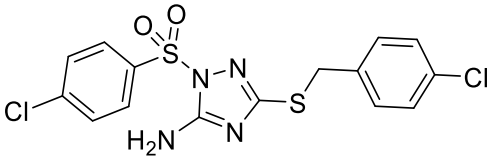
Compound 33: 3-((4-chlorobenzyl)thio)-1-((4-fluorophenyl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



Compound **33** was prepared from 4-fluorophenylsulfonyl chloride (1.2 equiv, 0.25 mmol, 29 μL) using the general procedure. Flash chromatography (SiO_2, 30% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.93 (m, 2H), 7.26 (m, 2H), 7.22 (m, 4H), 5.82 (s, 2H), 4.18 (s, 2H). LRMS-ESI *m/z* calculated for $C_{15}H_{13}ClFN_4O_2S_2^+$ [M + H$^+$]: 398.0, found: 399.0.

This compound is also commercially available from Ambinter (AMB2745085).

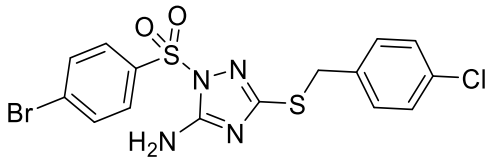
Compound 6: 3-((4-chlorobenzyl)thio)-1-((4-chlorophenyl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



Compound **6** was prepared from 4-chlorophenylsulfonyl chloride (1.2 equiv, 0.25 mmol, 48 mg) using the general procedure. Flash chromatography (SiO_2, 40% EtOAc/hexanes) yielded the desired product as a white solid (72 mg, 83%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.84 (d, *J* = 8.8 Hz, 2H), 7.51 (d, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 8.4 Hz, 2H), 7.22 (d, *J* = 8.5 Hz, 2H), 5.90 (s, 2H), 4.18 (s, 2H). HRMS-ESI-TOF *m/z* calculated for $C_{15}H_{12}Cl_2N_4O_2S_2^+$ [M$^+$]: 414.9857, found: 414.9853.

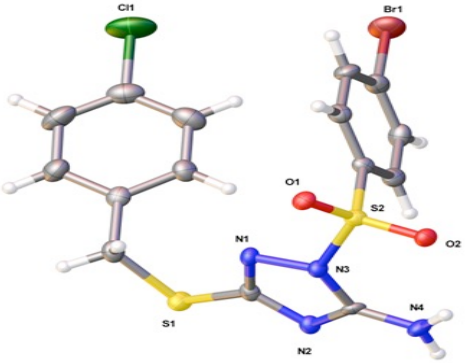
This compound is also commercially available from Ambinter (AMB24475).

Compound 34: 1-((4-bromophenyl)sulfonyl)-3-((4-chlorobenzyl)thio)-5-amino-1*H*-1,2,4-triazole

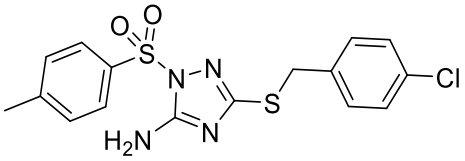


Compound **34** was prepared from 4-bromophenylsulfonyl chloride (1.2 equiv, 0.25 mmol, 63.8 mg) using the general procedure. Flash chromatography (SiO_2, 50% EtOAc/hexanes) yielded the desired product as a white solid (41 mg, 43%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.71 (d, J = 8.6 Hz, 2H), 7.68 (d, J = 8.6 Hz, 2H), 7.26 (d, J = 8.6 Hz, 2H), 7.22 (d, J = 8.6 Hz, 2H), 5.82 (s, 2H), 4.18 (s, 2H). HRMS-ESI-TOF *m/z* calculated for $C_{15}H_{13}BrClN_4O_2S_2^+$ [M + H^+]: 458.9346, found: 458.9351.

The structure of **34** was confirmed by X-ray structure determination (CCDC 1825319; **Supplementary Table 6**).

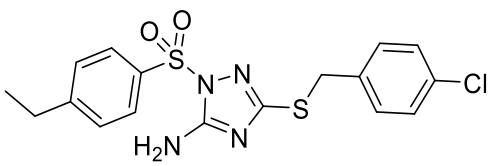


Compound 35: 3-((4-chlorobenzyl)thio)-1-(4-methylphenyl)sulfonyl-5-amino-1*H*-1,2,4-triazole



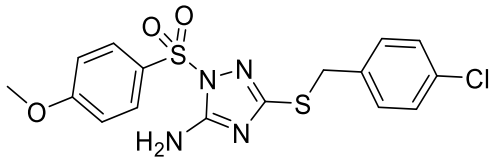
Compound **35** was prepared from 4-methylphenylsulfonyl chloride (1.2 equiv, 0.25 mmol, 48 mg) using the general procedure. Flash chromatography (SiO_2, 30% EtOAc/hexanes) yielded the desired product as a white solid (64 mg, 77%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.79 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8.4 Hz, 2H), 7.24 (d, J = 8.4 Hz, 2H), 7.17 (d, J = 8.4 Hz, 2H), 5.81 (s, 2H), 4.17 (s, 2H), 2.47 (s, 3H). HRMS-ESI-TOF *m/z* calculated for $C_{16}H_{16}ClN_4O_2S_2^+$ [M + H^+]: 395.0398, found: 395.0398.

Compound 36: 3-((4-chlorobenzyl)thio)-1-((4-ethylphenyl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



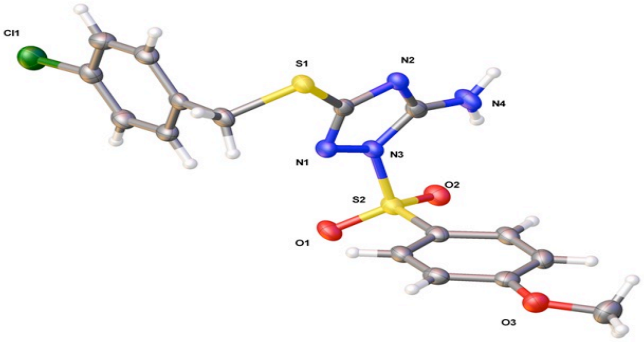
Compound **36** was prepared from 4-ethylphenylsulfonyl chloride (1.2 equiv, 0.25 mmol, 24 μL) using the general procedure. Flash chromatography (SiO_2, 50% EtOAc/hexanes) yielded the desired product as a white solid (51 mg, 84%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.82 (d, *J* = 8.5 Hz, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.26 (d, *J* = 8.5 Hz, 2H), 7.18 (d, *J* = 8.4 Hz, 2H), 5.80 (s, 2H), 4.18 (s, 2H), 2.76 (q, *J* = 7.6 Hz, 2H), 1.29 (t, *J* = 7.6 Hz, 3H). HRMS-ESI-TOF *m/z* calculated for $C_{17}H_{18}ClN_4O_2S_2^+$ [M + H$^+$]: 409.0554, found: 409.0558.

Compound 37: 3-((4-chlorobenzyl)thio)-1-((4-methoxyphenyl)sulfonyl)-5-amino-1*H*-1,2,4-triazole

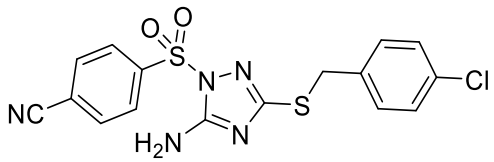


Compound **37** was prepared from 4-methoxyphenylsulfonyl chloride (1.2 equiv, 0.25 mmol, 52 mg) using the general procedure. Flash chromatography (SiO_2, 30% EtOAc/hexanes) yielded the desired product as a white solid (72 mg, 70%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.84 (d, *J* = 9.1 Hz, 2H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.19 (d, *J* = 8.4 Hz, 2H), 6.97 (d, *J* = 9.0 Hz, 2H), 5.82 (s, 2H), 4.17 (s, 2H), 3.90 (s, 3H). HRMS-ESI-TOF *m/z* calculated for $C_{16}H_{16}ClN_4O_3S_2^+$ [M + H$^+$]: 412.0299, found: 412.0299.

The structure of **37** was confirmed by X-ray structure determination (CDCC 1825322; **Supplementary Table 7**).

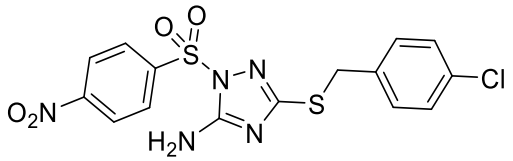


Compound 38: 4-((5-amino-3-((4-chlorobenzyl)thio)-1*H*-1,2,4-triazol-1-yl)sulfonyl)-benzonitrile



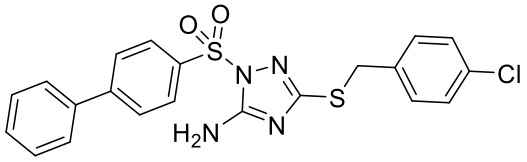
Compound **38** was prepared from 4-cyanophenylsulfonyl chloride (1.2 equiv, 0.25 mmol, 50.2 mg) using the general procedure. Flash chromatography (SiO_2, 50% EtOAc/hexanes) yielded the desired product as a white solid (68 mg, 80%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.00 (d, *J* = 7.8 Hz, 2H), 7.82 (d, *J* = 7.8 Hz, 2H), 7.29 (d, *J* = 8.3 Hz, 2H), 7.24 (d, *J* = 8.3 Hz, 2H), 5.74 (s, 2H), 4.18 (s, 2H). LRMS-ESI *m/z* calculated for $C_{16}H_{13}ClN_5O_2S_2^+$ [M + H$^+$]: 406.0, found: 406.0.

Compound 39: 3-((4-chlorobenzyl)thio)-1-((4-nitrophenyl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



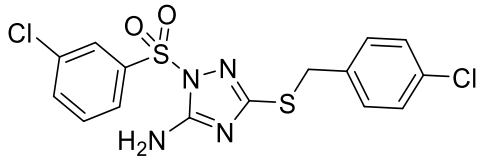
Compound **39** was prepared from 4-nitrophenylsulfonyl chloride (1.2 equiv, 0.25 mmol, 55 mg) using the general procedure. Flash chromatography (SiO_2) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.36 (d, *J* = 8.9 Hz, 2H), 8.09 (d, *J* = 8.9 Hz, 2H), 7.29 (d, *J* = 8.4 Hz, 2H), 7.24 (d, *J* = 8.4 Hz, 2H), 5.73 (s, 2H), 4.18 (s, 2H). HRMS-ESI-TOF *m/z* calculated for $C_{15}H_{13}ClN_5O_4S_2^+$ [M + H$^+$]: 426.0092, found: 426.0092.

Compound 40: 1-([1,1'-biphenyl]-4-ylsulfonyl)-3-((4-chlorobenzyl)thio)-5-amino-1*H*-1,2,4-triazole



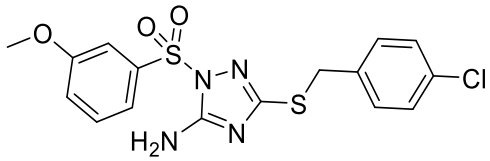
Compound **40** was prepared from (1, 1'-biphenyl)-4-sulfonyl chloride (1.2 equiv, 0.25 mmol, 63.6 mg) using the general procedure. Flash chromatography (SiO_2) yielded the desired product as a white solid (80 mg, 83%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.97 (d, *J* = 8.5 Hz, 2H), 7.73 (d, *J* = 8.5 Hz, 2H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.55–7.42 (m, 4H), 7.29 (d, *J* = 4.5 Hz, 1H), 7.19 (d, *J* = 8.5 Hz, 2H), 5.80 (s, 2H), 4.19 (s, 2H). HRMS-ESI-TOF *m/z* calculated for $C_{21}H_{18}ClN_4O_2S_2^+$ [M + H$^+$]: 457.0554, found: 457.0562.

Compound 7: 3-((4-chlorobenzyl)thio)-1-((3-chlorophenyl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



Compound **7** was prepared from 3-chlorophenylsulfonyl chloride (1.2 equiv, 0.25 mmol, 35 μL) using the general procedure. Flash chromatography (SiO_2, 30% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR (CD_2Cl_2, 400 MHz): δ 7.16 (s, 1H), 7.82 (d, *J* = 7.8 Hz, 1H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.54 (t, *J* = 7.8 Hz, 1H), 7.28 (d, *J* = 8.5 Hz, 2H), 7.23 (d, *J* = 8.5 Hz, 2H), 6.02 (s, 2H), 4.20 (s, 2H). HRMS-ESI-TOF *m/z* calculated for $C_{15}H_{13}Cl_2N_4O_2S_2^+$ [M + H$^+$]: 414.9851, found: 414.9855.

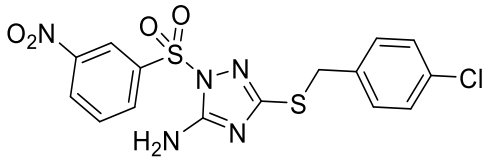
Compound 41: 3-((4-chlorobenzyl)thio)-1-((3-methoxyphenyl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



Compound **41** was prepared from 3-methoxyphenylsulfonyl chloride (1.2 equiv, 0.25 mmol, 35 μL) using the general procedure. Flash chromatography (SiO_2, 30% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.51–7.42 (m, 3H), 7.25–7.15 (m,

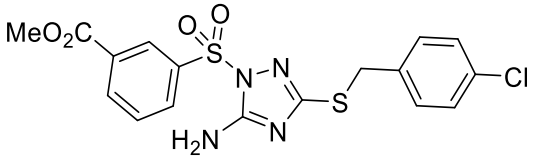
5H), 5.84 (s, 2H), 4.18 (s, 2H), 3.86 (s, 3H). HRMS-ESI-TOF *m/z* calculated for $C_{16}H_{16}ClN_4O_3S_2^+$ [M + H^+]: 411.0347, found: 411.0354.

Compound 42: 3-((4-chlorobenzyl)thio)-1-((3-nitrophenyl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



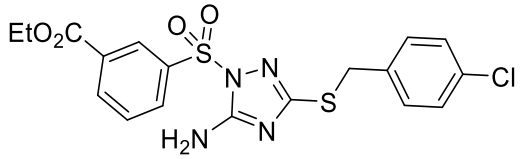
Compound **42** was prepared from 3-nitrophenylsulfonyl chloride (1.2 equiv, 0.25 mmol, 55 mg) using the general procedure. Flash chromatography (SiO_2, 50% EtOAc/hexanes) yielded the desired product as a white solid (76 mg, 86%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.67–8.62 (m, 2H), 8.33 (ddd, *J* = 7.9, 1.7, 1.0 Hz, 1H), 7.98 (t, *J* = 8.1 Hz, 1H), 7.67 (s, 2H), 7.31 (m, 4H), 4.21 (s, 2H). LRMS-ESI *m/z* calculated for $C_{15}H_{13}ClN_5O_4S_2^+$ [M + H^+]: 426.0, found: 426.0.

Compound 8: methyl 3-((5-amino-3-((4-chlorobenzyl)thio)-1*H*-1,2,4-triazol-1-yl)sulfonyl)-benzoate



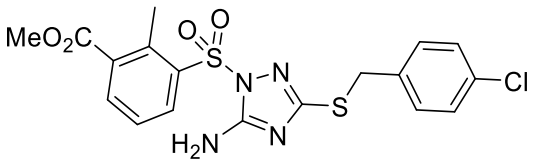
Compound **8** was prepared from methyl 3-chlorosulfonylbenzoate (1.2 equiv, 0.25 mmol, 58 mg) using the general procedure. Flash chromatography (SiO_2, 40% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.61 (t, *J* = 1.8 Hz, 1H), 8.36 (dt, *J* = 7.8, 1.4 Hz, 1H), 8.08 (m, 1H), 7.64 (t, *J* = 7.8 Hz, 1H), 7.24 (d, *J* = 8.5 Hz, 2H), 7.18 (d, *J* = 8.5 Hz, 2H), 5.85 (s, 2H), 4.17 (s, 2H), 3.97 (s, 3H). LRMS-ESI *m/z* calculated for $C_{17}H_{16}ClN_4O_4S_2^+$ [M + H^+]: 439.0, found: 439.0.

Compound 9: ethyl 3-((5-amino-3-((4-chlorobenzyl)thio)-1*H*-1,2,4-triazol-1-yl)sulfonyl)-benzoate



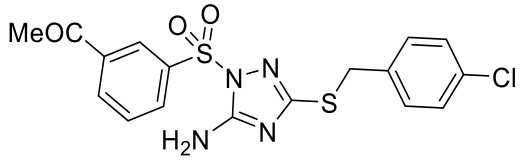
Compound **9** was prepared from ethyl 3-chlorosulfonylbenzoate (1.2 equiv, 0.25 mmol, 62 mg) using the general procedure. Flash chromatography (SiO_2, 35% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.62 (s, 1H), 8.37 (d, *J* = 8.4 Hz, 1H), 8.07 (d, *J* = 8.0 Hz, 1H), 7.63 (t, *J* = 7.9 Hz, 1H), 7.21 (m, 4H), 5.82 (s, 2H), 4.43 (q, *J* = 7.2 Hz, 2H), 4.19 (s, 2H), 1.41 (t, *J* = 7.2 Hz, 3H). LRMS-ESI *m/z* calculated for $C_{18}H_{18}ClN_4O_4S_2^+$ [M + H^+]: 453.0, found: 453.0.

Compound 43: methyl 3-((5-amino-3-((4-chlorobenzyl)thio)-1*H*-1,2,4-triazol-1-yl)sulfonyl)-2-methylbenzoate



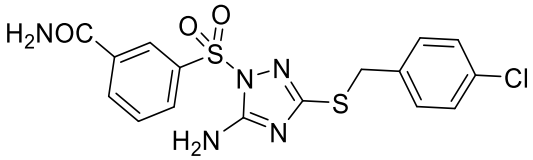
Compound **43** was prepared from methyl 3-(chlorosulfonyl)-2-methylbenzoate (1.2 equiv, 0.25 mmol, 62 mg) using the general procedure. Flash chromatography (SiO_2, 30% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.24 (d, J = 8.2 Hz, 1H), 8.08 (d, J = 7.9 Hz, 1H), 7.48 (t, J = 8.1 Hz, 1H), 7.13 (m, 4H), 5.79 (s, 2H), 4.11 (s, 2H), 3.95 (s, 3H), 2.70 (s, 3H). LRMS-ESI *m/z* calculated for $C_{18}H_{18}ClN_4O_4S_2^+$ [M + H$^+$]: 453.0, found: 453.0.

Compound 44: 1-(3-((5-amino-3-((4-chlorobenzyl)thio)-1*H*-1,2,4-triazol-1-yl)sulfonyl)-phenyl)ethan-1-one



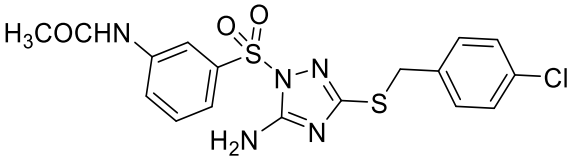
Compound **44** was prepared from 3-acetylphenylsulfonyl chloride (1.2 equiv, 0.25 mmol, 55 mg) using the general procedure. Flash chromatography (SiO_2) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.51 (t, J = 1.7 Hz, 1H), 8.27 (dt, J = 7.7, 1.6 Hz, 1H), 8.08 (d, J = 8.1 Hz,1H), 7.67 (t, J = 7.9 Hz, 1H), 7.25 (d, J = 8.5 Hz, 2H), 7.18 (d, J = 8.5 Hz, 2H), 5.96 (s, 2H), 4.19 (s, 2H), 2.66 (s, 3H). LRMS-ESI *m/z* calculated for $C_{17}H_{16}ClN_4O_3S_2^+$ [M + H$^+$]: 423.0, found: 423.0.

Compound 45: 3-((5-amino-3-((4-chlorobenzyl)thio)-1*H*-1,2,4-triazol-1-yl)sulfonyl)-benzamide



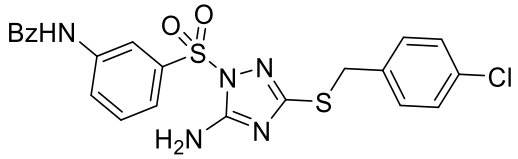
Compound **45** was prepared from 3-carbamoylbenzenesulfonyl chloride (1.2 equiv, 0.25 mmol, 55 mg) using the general procedure. Flash chromatography (SiO_2, 50% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.47 (s, 1H), 8.30 (d, J = 8.2 Hz, 2H), 8.06 (d, J = 8.7 Hz,1H), 7.76 (m, 2H), 7.55 (s, 2H), 7.29 (m, 4H), 4.19 (s, 2H). LRMS-ESI *m/z* calculated for $C_{16}H_{15}ClN_5O_3S_2^+$ [M + H$^+$]: 424.0, found: 424.0.

Compound 46: N-(3-((5-amino-3-((4-chlorobenzyl)thio)-1*H*-1,2,4-triazol-1-yl)sulfonyl)-phenyl)acetamide



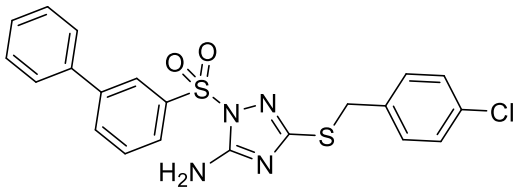
Compound **46** was prepared from 3-acetamidophenylsulfonyl chloride (1.2 equiv, 0.25 mmol, 58 mg) using the general procedure. Flash chromatography (SiO_2, 60% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.13 (s, 1H), 7.87 (d, J = 7.4 Hz, 1H), 7.65 (d, J = 7.9 Hz, 1H), 7.48 (m, 2H), 7.24 (d, J = 8.4 Hz, 2H), 7.18 (d, J = 8.4 Hz, 2H), 5.71 (s, 2H), 4.18 (s, 2H), 2.22 (s, 3H). LRMS-ESI *m/z* calculated for $C_{17}H_{17}ClN_5O_3S_2^+$ [M + H$^+$]: 438.0, found: 438.0.

Compound 10: 5-amino-1-((3-(benzoylamino)phenylsulfonyl)-3-((4-chlorobenzyl)thio)-1*H*-1,2,4-triazole



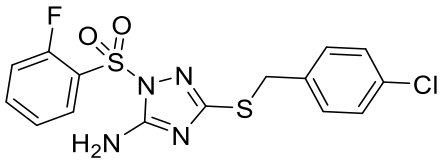
A stirred solution 1-((3-aminophenyl)sulfonyl)-3-((4-chlorobenzyl)thio)-5-amino-1*H*-1,2,4-triazole in dichloromethane was treated with pyridine at 0°C. Benzoyl chloride was added and the mixture stirred at 0°C for 30 min and then overnight at room temperature. The solvent was evaporated and the crude mixture purified by flash chromatography (SiO_2, 25% EtOAc/hexanes) to yield the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.33 (s, 1H), 8.47 (d, J = 8.2 Hz, 1H), 7.96 (s, 1H), 7.87 (d, J = 7.7 Hz, 2H), 7.69 (m, 1H), 7.62–7.50 (m, 4H), 7.25 (d, J =8.2 Hz, 2H), 7.17 (d, J =8.2 Hz, 2H), 5.81 (s, 2H), 4.21 (s, 2H). LRMS-ESI *m/z* calculated for $C_{22}H_{19}ClN_5O_3S_2^+$ [M + H$^+$]: 500.0, found: 500.0.

Compound 47: 1-([1,1'-biphenyl]-3-ylsulfonyl)-3-((4-chlorobenzyl)thio)-5-amino-1*H*-1,2,4-triazole



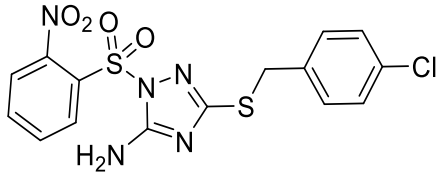
Compound **47** was prepared from 3-phenylbenzenesulfonyl chloride (1.2 equiv, 0.25 mmol, 63 mg) using the general procedure. Flash chromatography (SiO_2, 30% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.17 (t, J = 2.0 Hz, 1H), 7.90 (m, 2H), 7.61 (m, 3H), 7.46 (m, 3H), 7.21 (d, J = 8.4 Hz, 2H), 7.12 (d, J = 8.4 Hz, 2H), 5.93 (s, 2H), 4.18 (s, 2H). LRMS-ESI *m/z* calculated for $C_{21}H_{18}ClN_4O_2S_2^+$ [M + H$^+$]: 457.0, found: 457.0.

Compound 48: 3-((4-chlorobenzyl)thio)-1-((2-fluorophenyl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



Compound **48** was prepared from 2-fluorophenylsulfonyl chloride (1.2 equiv, 0.25 mmol, 33 μL) using the general procedure. Flash chromatography (SiO_2, 50% EtOAc/hexanes) yielded the desired product as a white solid (47 mg, 60%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.06 (m, 1H), 7.74 (m, 1H), 7.39 (m, 1H), 7.22 (m,1H), 7.17 (d, J = 8.6 Hz, 2H), 7.10 (d, J = 8.6 Hz, 2H), 5.86 (s, 2H), 4.12 (s, 2H). LRMS-ESI *m/z* calculated for $C_{15}H_{13}ClFN_4O_2S_2^+$ [M + H$^+$]: 399.0, found: 399.0.

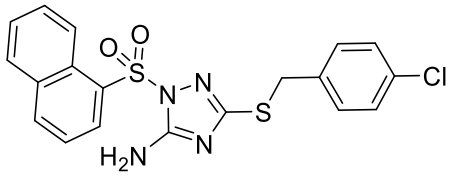
Compound 49: 3-((4-chlorobenzyl)thio)-1-((2-nitrophenyl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



Compound **49** was prepared from 2-nitrophenylsulfonyl chloride (1.2 equiv, 0.25 mmol, 35 μL) using the general procedure. Flash chromatography (SiO_2, 50% EtOAc/hexanes) yielded the

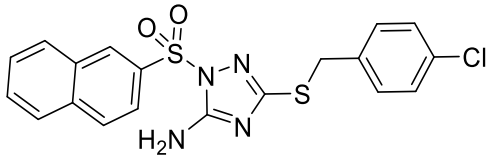
desired product as a white solid (83 mg, 78%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.27 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.94–7.76 (m, 3H), 7.22 (d, *J* = 8.7 Hz, 2H), 7.14 (d, *J* = 8.7 Hz, 2H), 5.76 (s, 2H), 4.15 (s, 2H). LRMS-ESI *m/z* calculated for $C_{15}H_{13}ClN_5O_4S_2^+$ [M + H$^+$]: 426.0, found: 426.0.

Compound 50: 3-((4-chlorobenzyl)thio)-1-(naphth-1-ylsulfonyl)-5-amino-1*H*-1,2,4-triazole



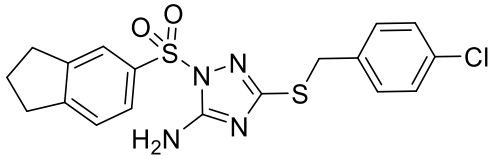
Compound **50** was prepared from napthalene-1-sulfonyl chloride (1.2 equiv, 0.25 mmol, 35 μL) using the general procedure. Flash chromatography (SiO_2, 40% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.68 (d, *J* = 7.9 Hz, 1H), 8.42 (dd, *J* = 7.5, 1.0 Hz, 1H), 8.23 (d, *J* = 8.1 Hz, 1H), 8.00 (m, 1H), 7.65 (m, 3H), 7.01 (m, 4H), 5.83 (s, 2H), 4.06 (s, 2H). HRMS-ESI-TOF *m/z* calculated for $C_{19}H_{16}ClN_4O_2S_2^+$ [M + H$^+$]: 431.0398, found: 431.0403.

Compound 51: 3-((4-chlorobenzyl)thio)-1-(naphth-2-ylsulfonyl)-5-amino-1*H*-1,2,4-triazole



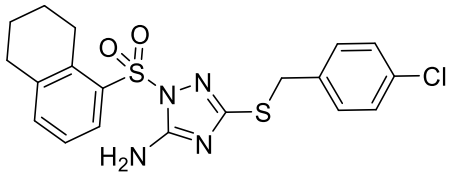
Compound **51** was prepared from napthalene-2-sulfonyl chloride (1.2 equiv, 0.25 mmol, 35 μL) using the general procedure. Flash chromatography (SiO_2, 40% EtOAc/hexanes) yielded the desired product as a white solid (53 mg, 59%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.58 (s, 1H), 7.99 (m, 3H), 7.81 (dd, *J* = 8.7, 1.7 Hz, 1H), 7.71 (m, 2H), 7.16 (d, *J* = 8.4 Hz, 2H), 6.99 (d, *J* = 8.4 Hz, 2H), 5.83 (s, 2H), 4.15 (s, 2H). HRMS-ESI-TOF *m/z* calculated for $C_{19}H_{16}ClN_4O_2S_2^+$ [M + H$^+$]: 431.0398, found: 431.0400.

Compound 52: 3-((4-chlorobenzyl)thio)-1-((2,3-dihydro-1*H*-inden-5-yl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



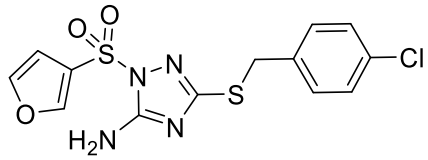
Compound **52** was prepared from indane-5-sulfonyl chloride (1.2 equiv, 0.25 mmol, 54 mg) using the general procedure. Flash chromatography (SiO_2, 30% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.75 (s, 1H), 7.72 (d, *J* = 8.1 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 8.3 Hz, 2H), 7.16 (d, *J* = 8.3 Hz, 2H), 5.84 (s, 2H), 4.18 (s, 2H), 2.99 (dt, *J* = 16.6, 7.5 Hz, 4H), 2.17 (p, *J* = 7.6 Hz, 2H). LRMS-ESI *m/z* calculated for $C_{18}H_{18}ClN_4O_2S_2^+$ [M + H$^+$]: 421.1, found: 421.0.

Compound 53: 3-((4-chlorobenzyl)thio)-1-((5,6,7,8-tetrahydronaphthalen-1-yl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



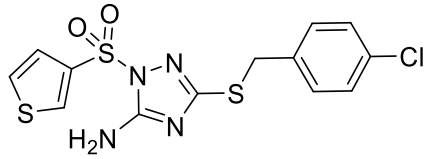
Compound **53** was prepared from 5,6,7,8-tetrahydronapthalene-1-sulfonyl chloride (1.2 equiv, 0.25 mmol, 34.6 mg) using the general procedure. Flash chromatography (SiO_2, 30% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.62 (m, 2H), 7.22 (m, 3H), 7.16 (d, *J* = 8.4 Hz, 2H), 5.86 (s, 2H), 4.18 (s, 2H), 2.86 (m, 2H), 2.80 (m, 2H), 1.83 (m, 4H). LRMS-ESI *m/z* calculated for $C_{19}H_{20}ClN_4O_2S_2^+$ [M + H^+]: 435.1, found: 435.0.

Compound 54: 3-((4-chlorobenzyl)thio)-1-(furan-3-ylsulfonyl)-5-amino-1*H*-1,2,4-triazole



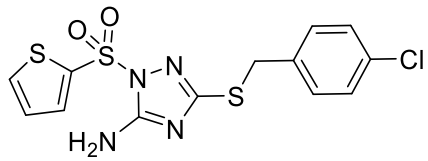
Compound **54** was prepared from furan-3-sulfonyl chloride (1.2 equiv, 0.25 mmol, 27 μL) using the general procedure. Flash chromatography (SiO_2, 40% EtOAc/hexanes) yielded the desired product as a white solid (36 mg, 47%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.08 (s, 1H), 7.50 (t, *J* =1.6 Hz, 1H), 7.30 (d, *J* = 8.5 Hz, 2H), 7.23 (d, *J* = 8.5 Hz, 2H), 6.59 (s, 1H), 5.79 (s, 2H), 4.22 (s, 2H). LRMS-ESI *m/z* calculated for $C_{13}H_{12}ClN_4O_3S_2^+$ [M + H^+]: 370.0, found: 370.0.

Compound 55: 3-((4-chlorobenzyl)thio)-1-(thiophen-3-ylsulfonyl)-5-amino-1*H*-1,2,4-triazole



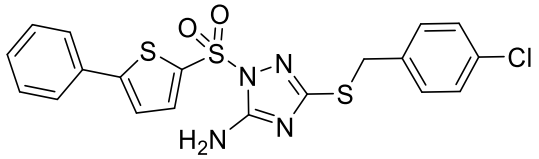
Compound **55** was prepared from thiophene-3-sulfonyl chloride (1.2 equiv, 0.25 mmol, 30 μL) using the general procedure. Flash chromatography (SiO_2, 35% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.16 (m, 1H), 7.44 (dd, *J* = 5.2, 3.1 Hz, 1H), 7.32 (d, *J* = 5.2 Hz, 1H), 7.29 (d, *J* = 8.3 Hz, 2H), 7.23 (d, *J* = 8.3 Hz, 2H), 5.95 (s, 2H), 4.22 (s, 2H). HRMS-ESI-TOF *m/z* calculated for $C_{13}H_{12}ClN_4O_2S_3^+$ [M + H^+]: 386.9805, found: 386.9805.

Compound 56: 3-((4-chlorobenzyl)thio)-1-(thiophen-2-ylsulfonyl)-5-amino-1*H*-1,2,4-triazole



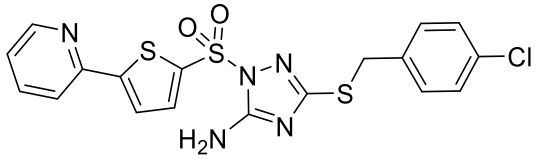
Compound **56** was purchased from Ambinter (AMB2745086).

Compound 57: 3-((4-chlorobenzyl)thio)-1-((5-phenylthiophen-2-yl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



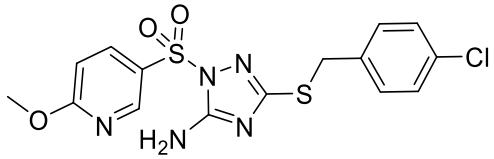
Compound **57** was prepared from 5-phenyl-2-thiophenesulfonyl chloride (1.2 equiv, 0.25 mmol, 64.5 mg) using the general procedure. Flash chromatography (SiO_2, 50% EtOAc/hexanes) yielded the desired product as a white solid (52 mg, 45%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.71 (d, J = 4.0 Hz, 1H), 7.59 (m, 2H), 7.45 (m, 3H), 7.30 (d, J = 8.3 Hz, 2H), 7.28 (d, J = 4.0 Hz, 1H), 7.19 (d, J = 8.3 Hz, 2H), 5.82 (s, 2H), 4.22 (s, 2H). HRMS-ESI-TOF *m/z* calculated for $C_{19}H_{16}ClN_4O_2S_3^+$ [M + H$^+$]: 463.0118, found: 463.0121.

Compound 58: 3-((4-chlorobenzyl)thio)-1-((5-(pyridin-2-yl)thiophen-2-yl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



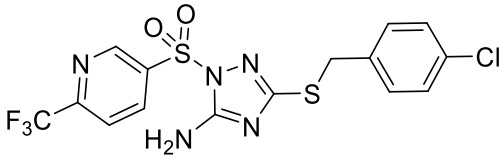
Compound **58** was purchased from Ambinter (AMB2745089).

Compound 59: 3-((4-chlorobenzyl)thio)-1-((6-methoxypyridin-3-yl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



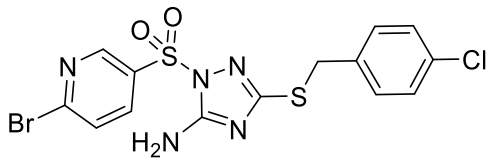
Compound **59** was prepared from 6-methoxypyridine-3-sulfonyl chloride (1.2 equiv, 0.25 mmol, 52 mg) using the general procedure. Flash chromatography (SiO_2, 50% EtOAc/hexanes) yielded the desired product as a white solid (52 mg, 60%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.75 (d, J = 2.4 Hz, 1H), 7.93 (dd, J = 8.9, 2.6 Hz, 1H), 7.27 (d, J = 8.6 Hz, 2H), 7.22 (d, J = 8.6 Hz, 2H), 6.81 (d, J = 8.9 Hz, 1H), 5.80 (s, 2H), 4.18 (s, 2H), 4.05 (s, 3H). LRMS-ESI *m/z* calculated for $C_{15}H_{15}ClN_5O_3S_2^+$ [M + H$^+$]: 412.0, found: 412.1.

Compound 60: 3-((4-chlorobenzyl)thio)-1-((6-(trifluoromethyl)pyridin-3-yl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



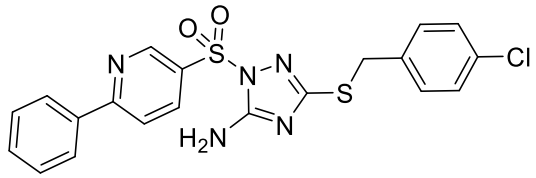
Compound **60** was prepared from 6-trifluoromethylpyridine-3-sulfonyl chloride (1.2 equiv, 0.25 mmol, 61 mg) using the general procedure. Flash chromatography (SiO_2) yielded the desired product as a white solid (61 mg, 65%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.31 (d, J = 2.1 Hz, 1H), 8.61 (dd, J = 8.3, 2.1 Hz, 1H), 8.23 (d, J = 8.3 Hz, 1H), 7.67 (s, 2H), 7.34 (d, J = 8.6 Hz, 2H), 7.29 (d, J = 8.6 Hz, 2H), 4.22 (s, 2H). LRMS-ESI *m/z* calculated for $C_{15}H_{12}ClF_3N_5O_2S_2^+$ [M + H$^+$]: 450.0, found: 450.0.

Compound 61: 1-((6-bromopyridin-2-yl)sulfonyl)-3-((4-chlorobenzyl)thio)-5-amino-1*H*-1,2,4-triazole



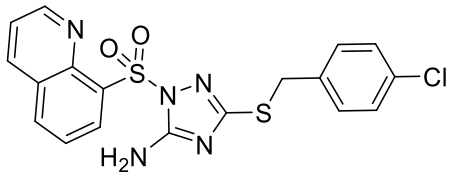
Compound **61** was prepared from 6-bromopyridine-3-sulfonyl chloride (1.2 equiv, 0.25 mmol, 63 mg) using the general procedure. Flash chromatography (SiO_2, 45% EtOAc/hexanes) yielded the desired product as a white solid (45 mg, 46%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.91 (d, J = 2.6 Hz, 1H), 7.93 (dd, J = 8.4, 2.6 Hz, 1H), 7.65 (d, J = 8.4 Hz, 1H), 7.27 (m, 4H), 5.76 (s, 2H), 4.18 (s, 2H). HRMS-ESI-TOF *m/z* calculated for $C_{14}H_{12}BrClN_5O_2S_2^+$ [M + H$^+$]: 459.9299, found: 459.9298.

Compound 62: 3-((4-chlorobenzyl)thio)-1-((6-phenylpyridin-3-yl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



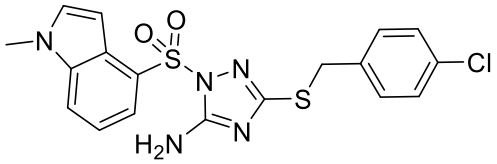
Compound **62** was prepared from 6-phenylpyridine-3-sulfonyl chloride (1.2 equiv, 0.25 mmol, 63 mg) using the general procedure. Flash chromatography (SiO_2, 35% EtOAc/hexanes) yielded the desired product as a white solid (78 mg, 81%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.19 (d, J = 2.4 Hz, 1H), 8.15 (dd, J = 8.5, 2.4 Hz, 1H), 8.10 (d, J = 3.5 Hz, 1H), 8.09 (d, J = 2.4 Hz, 1H), 7.87 (d, J = 8.5 Hz, 1H), 7.54 (m, 3H), 7.28 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 8.5 Hz, 2H), 5.90 (s, 2H), 4.20 (s, 2H). HRMS-ESI-TOF *m/z* calculated for $C_{20}H_{17}ClN_5O_2S_2^+$ [M + H$^+$]: 458.0507, found: 458.0511.

Compound 63: 3-((4-chlorobenzyl)thio)-1-(quinolin-8-ylsulfonyl)-5-amino-1*H*-1,2,4-triazole



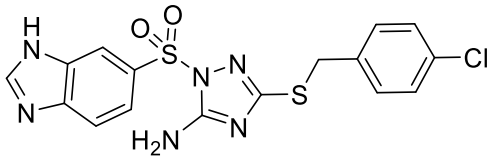
Compound **63** was prepared from quinoline-8-sulfonyl chloride (1.2 equiv, 0.25 mmol, 57 mg) using the general procedure. Flash chromatography (SiO_2, 60% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.90 (dd, J = 4.3, 1.8 Hz, 1H), 8.60 (dd, J = 8.4, 1.8 Hz, 1H), 8.57 (dd, J = 7.5, 1.5 Hz, 1H), 8.50 (dd, J = 8.3, 1.5 Hz, 1H), 7.89 (t, J = 7.8 Hz, 1H), 7.72 (dd, J = 8.4, 4.3 Hz, 1H), 7.39 (s, 2H), 7.02 (m, 4H), 4.03 (s, 2H). HRMS-ESI-TOF *m/z* calculated for $C_{18}H_{15}ClN_5O_2S_2^+$ [M + H$^+$]: 431.0350, found: 431.0355.

Compound 64: 3-((4-chlorobenzyl)thio)-1-((1-methyl-1*H*-indol-4-yl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



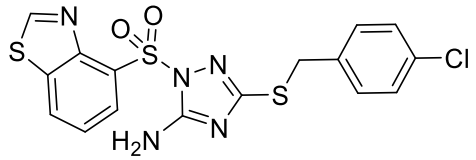
Compound **64** was prepared from 1-methyl-1*H*-indole-4-sulfonyl chloride (1.2 equiv, 0.25 mmol, 57 mg) using the general procedure. Flash chromatography (SiO_2, 40% EtOAc/hexanes) yielded the desired product as a white solid (13 mg, 15%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.88 (d, *J* = 7.7 Hz, 1H), 7.70 (d, *J* = 7.7 Hz, 1H), 7.33 (t, *J* = 7.6 Hz, 1H), 7.25 (m, 1H), 7.05 (m, 4H), 6.93 (d, *J* = 3.5 Hz, 1H), 6.01 (s, 2H), 4.10 (s, 2H), 3.90 (s, 3H). LRMS-ESI *m/z* calculated for $C_{18}H_{17}ClN_5O_2S_2^+$ [M + H^+]: 434.1, found: 434.4.

Compound 65: 1-((1*H*-benzo[*d*]imidazol-6-yl)sulfonyl)-3-((4-chlorobenzyl)thio)-5-amino-1*H*-1,2,4-triazole



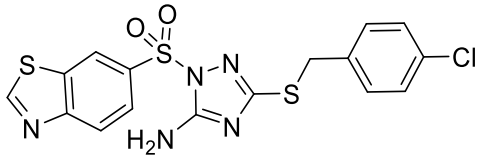
Compound **65** was prepared from 1*H*-benzimidazole-5-sulfonyl chloride (1.2 equiv, 0.25 mmol, 52 mg) using the general procedure. Flash chromatography (SiO_2, 100% EtOAc) yielded the desired product as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.58 (s, 1H), 8.31 (s, 1H), 7.83 (d, *J* = 8.9 Hz, 1H), 7.73 (d, *J* = 8.9 Hz, 1H), 7.49 (s, 2H), 7.26 (d, *J* = 7.9 Hz, 2H), 7.17 (d, *J* = 7.9 Hz, 2H), 4.18 (s, 2H). LRMS-ESI *m/z* calculated for $C_{16}H_{14}ClN_6O_2S_2^+$ [M + H^+]: 421.0, found: 421.0.

Compound 67 1-(benzo[*d*]thiazol-4-ylsulfonyl)-3-((4-chlorobenzyl)thio)-5-amino-1*H*-1,2,4-triazole



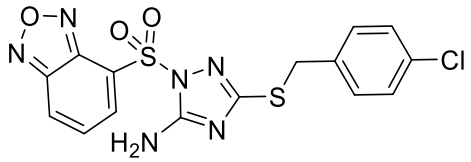
Compound **67** was prepared from 1,3-benzthiazole-4-sulfonyl chloride (1.2 equiv, 0.25 mmol, 49 mg) using the general procedure. The white residue was recrystallized in minimum volume of hexanes to yield the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.11 (s, 1H), 8.35 (m, 2H), 7.68 (t, *J* = 8.1 Hz, 1H), 7.06 (d, *J* = 8.6 Hz, 2H), 7.00 (d, *J* = 8.6 Hz, 2H), 6.39 (s, 2H), 4.08 (s, 2H). LRMS-ESI *m/z* calculated for $C_{16}H_{13}ClN_5O_2S_3^+$ [M + H^+]: 438.0, found: 438.0.

Compound 11: 1-(benzo[*d*]thiazol-6-ylsulfonyl)-3-((4-chlorobenzyl)thio)-5-amino-1*H*-1,2,4-triazole



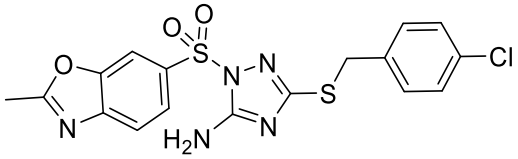
Compound **11** was prepared from 1,3-benzthiazole-6-sulfonyl chloride (1.2 equiv, 0.25 mmol, 58 mg) using the general procedure. Flash chromatography (SiO_2) yielded the desired product as a white solid (73 mg, 79%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.30 (s, 1H), 8.64 (d, *J* = 1.5 Hz, 1H), 8.28 (d, *J* = 8.6 Hz, 1H), 8.03 (dd, *J* = 8.6, 1.5 Hz, 1H), 7.22 (d, *J* = 8.3 Hz, 2H), 7.13 (d, *J* = 8.3 Hz, 2H), 5.86 (s, 2H), 4.17 (s, 2H). HRMS-ESI-TOF *m/z* calculated for $C_{16}H_{13}ClN_5O_2S_3^+$ [M + H^+]: 437.9914, found: 437.9915.

Compound 68: 1-(benzo[*c*][1,2,5]oxadiazol-4-ylsulfonyl)-3-((4-chlorobenzyl)thio)-5-amino-1*H*-1,2,4-triazole



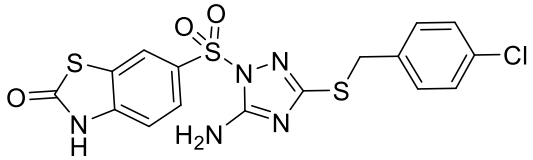
Compound **68** was purchased from Ambinter (AMB2745088).

Compound 13: 3-((4-chlorobenzyl)thio)-1-((2-methylbenzo[*d*]oxazol-6-yl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



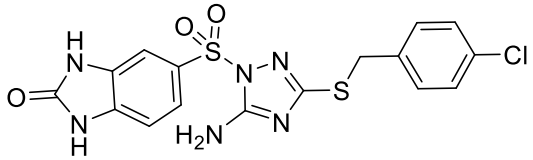
Compound **13** was prepared from 2-methyl-1,3-benzoxazole-6-sulfonyl chloride (1.2 equiv, 0.25 mmol, 58 mg) using the general procedure. Flash chromatography (SiO_2, 30% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.09 (d, J = 1.8 Hz, 1H), 7.89 (dd, J = 8.4, 1.7 Hz, 1H), 7.77 (d, J = 8.4 Hz, 1H), 7.25 (d, J = 8.4 Hz, 2H), 7.16 (d, J = 8.4 Hz, 2H), 5.88 (s, 2H), 4.17 (s, 2H), 2.74 (s, 3H). LRMS-ESI *m/z* calculated for $C_{17}H_{15}ClN_5O_3S_2^+$ [M + H$^+$]: 436.0, found: 436.0.

Compound 14: 6-((5-amino-3-((4-chlorobenzyl)thio)-1*H*-1,2,4-triazol-1-yl)sulfonyl)benzo-[*d*]thiazol-2(3*H*)-one



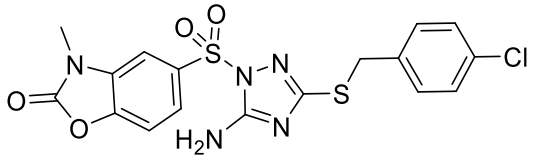
Compound **14** was prepared from 2-oxo-2,3-dihydrobenzothiazole-6-sulfonyl chloride (1.2 equiv, 0.25 mmol, 62 mg) using the general procedure. Flash chromatography (SiO_2) yielded the desired product as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.36 (s, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.46 (s, 2H), 7.35–7.22 (m, 6H), 4.18 (s, 2H). LRMS-ESI *m/z* calculated for $C_{16}H_{13}ClN_5O_3S_3^+$ [M + H$^+$]: 454.0, found: 454.0.

Compound 69: 5-((5-amino-3-((4-chlorobenzyl)thio)-1*H*-1,2,4-triazol-1-yl)sulfonyl)-1,3-dihydro-2*H*-benzo[*d*]imidazol-2-one



Compound **69** was prepared from 2-oxo-2,3-dihydro-1*H*-1,3-benzodiazole-5-sulfonyl chloride (1.2 equiv, 0.25 mmol, 58 mg) using the general procedure. Flash chromatography (SiO_2) yielded the desired product as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.41 (s, 1H), 11.18 (s, 1H), 7.58 (d, J = 7.6 Hz, 1H), 7.46 (s, 2H), 7.43 (s, 1H), 7.30 (d, J = 8.5 Hz, 2H), 7.24 (d, J = 8.5 Hz, 2H), 4.18 (s, 2H). LRMS-ESI *m/z* calculated for $C_{16}H_{14}ClN_6O_3S_2^+$ [M + H$^+$]: 437.0, found: 437.0.

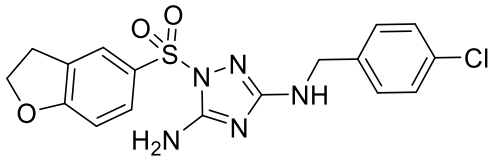
Compound 70: 5-((5-amino-3-((4-chlorobenzyl)thio)-1*H*-1,2,4-triazol-1-yl)sulfonyl)-3-methylbenzo[*d*]oxazol-2(3*H*)-one



Compound **70** was prepared from 3-methyl-2-oxo-2.3-dihydrobenzoxazole-5-sulfonyl chloride (1.2 equiv, 0.25 mmol, 61 mg) using the general procedure. Flash chromatography (SiO_2, 50% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.80 (d, J = 8.3 Hz, 1H), 7.75 (d, J = 1.6 Hz, 1H), 7.28 (d, J = 8.3 Hz, 2H), 7.21 (d, J = 8.3 Hz, 2H), 7.05 (d, J = 8.3 Hz, 1H), 6.00 (s, 2H), 4.19 (s, 2H), 3.48 (s, 3H). LRMS-ESI *m/z* calculated for $C_{17}H_{15}ClN_5O_4S_2^+$ [M + H$^+$]: 452.0, found: 452.0.

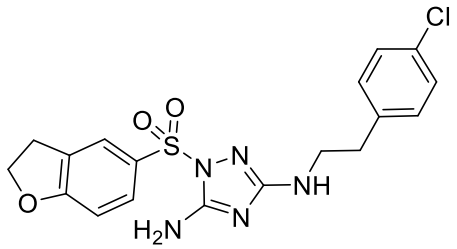
<u>Characterization for representative inactive compounds</u>

Compound 15: 3-(4-chlorobenzyl)amino-1-((2,3-dihydrobenzofuran-5-yl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



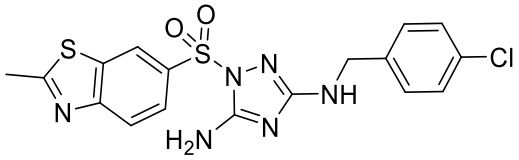
Compound **15** was prepared from 2,3-dihydrobenzofuran-5-sulfonyl chloride (1.2 equiv, 0.25 mmol, 59 mg) using the general procedure. Flash chromatography (SiO_2, 60% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68–7.64 (m, 2H), 7.33 (d, J = 8.4 Hz, 2H), 7.25 (d, J = 8.4 Hz, 2H), 6.82 (d, J = 8.3 Hz, 1H), 6.67 (t, J = 5.5 Hz, 1H), 4.70 (t, J = 8.7 Hz, 2H), 4.55 (d, J = 6.2 Hz, 2H), 4.12 (s, 2H), 3.24 (t, J = 8.7 Hz, 2H). LRMS-ESI *m/z* calculated for $C_{17}H_{17}ClN_5O_3S^+$ [M + H$^+$]: 406.1, found: 406.0.

Compound 16: 3-(4-chlorophenethyl)amino-1-((2,3-dihydrobenzofuran-5-yl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



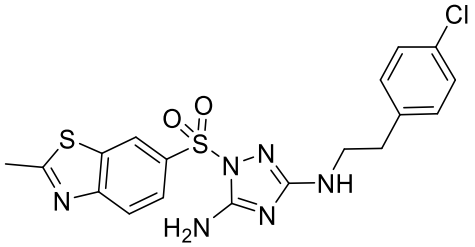
Compound **16** was prepared from 2,3-dihydrobenzofuran-5-sulfonyl chloride (1.2 equiv, 0.25 mmol, 58 mg) using the general procedure. Flash chromatography (SiO_2, 40% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.80–7.75 (m, 2H), 7.22 (d, J = 8.1 Hz, 2H), 7.03 (d, J = 8.1 Hz, 2H), 6.85 (d, J = 8.3 Hz, 1H), 6.23 (t, J = 6.0Hz, 1H), 5.89 (s, 2H), 4.69 (m, 2H), 3.40 (m, 2H), 3.24 (t, J = 8.8 Hz, 2H), 2.77 (t, J = 6.7 Hz, 2H). LRMS-ESI *m/z* calculated for $C_{18}H_{19}ClN_5O_3S^+$ [M + H$^+$]: 420.1, found: 420.0.

Compound 17: 1-((2-methylbenzo[*d*]thiazol-6-yl)sulfonyl)-3-(4-chlorobenzyl)amino-5-amino-1*H*-1,2,4-triazole



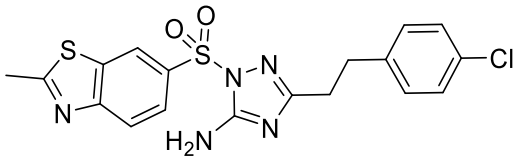
Compound **17** was prepared from 2-methyl-1,3-benzothiazole-6-sulfonyl chloride (1.2 equiv, 0.25 mmol, 65 mg) using the general procedure. Flash chromatography (SiO_2, 50% EtOAc/hexanes) yielded the desired product as a white solid (61 mg, 64%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.43 (d, *J* = 1.3 Hz, 1H), 8.02 (d, *J* = 8.6 Hz, 1H), 7.92 (dd, *J* = 8.6, 1.3 Hz, 1H), 7.14 (d, *J* = 8.4 Hz, 2H), 7.09 (d, *J* = 8.4 Hz, 2H), 5.80 (s, 2H), 4.52 (s, 1H), 4.32 (s, 2H), 2.92 (s, 3H). HRMS-ESI-TOF *m/z* calculated for $C_{17}H_{16}ClN_6O_2S_2^+$ [M + H^+]: 435.0459, found: 435.0470.

Compound 18: 3-(4-chlorophenethyl)amino-1-((2-methylbenzo[*d*]thiazol-6-yl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



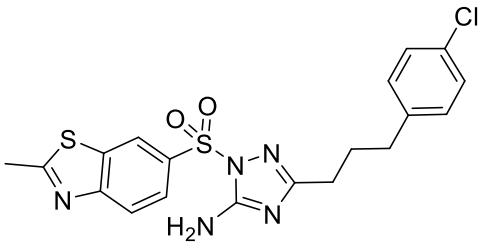
Compound **18** was prepared from 2-methyl-1,3-benzothiazole-6-sulfonyl chloride (1.2 equiv, 0.25 mmol, 62 mg) using the general procedure. Flash chromatography (SiO_2) yielded the desired product as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.73 (s, 1H), 8.12 (d, *J* = 8.7 Hz, 1H), 7.90 (d, *J* = 9.2 Hz, 1H), 7.23 (s, 2H), 7.19 (d, *J* = 8.1 Hz, 2H), 6.98 (d, *J* = 8.1 Hz, 2H), 6.29 (t, *J* = 6.0 Hz, 1H), 3.12 (q, *J* = 7.0 Hz, 2H), 2.84 (s, 3H), 2.62 (t, *J* = 7.1 Hz, 2H). LRMS-ESI *m/z* calculated for $C_{18}H_{18}ClN_6O_2S_2^+$ [M + H^+]: 449.1, found: 449.0.

Compound 19: 3-(4-chlorophenethyl)-1-((2-methylbenzo[*d*]thiazol-6-yl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



Compound **19** was prepared from 2-methyl-1,3-benzothiazole-6-sulfonyl chloride (1.2 equiv, 0.25 mmol, 62 mg) using the general procedure. Flash chromatography (SiO_2, 50% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.51 (d, *J* = 1.8 Hz, 1H), 8.08 (d, *J* = 8.7 Hz, 1H), 7.98 (dd, *J* = 8.7, 1.8 Hz, 1H), 7.06 (d, *J* = 8.4 Hz, 2H), 6.98 (d, *J* = 8.4 Hz, 2H), 5.90 (s, 2H), 2.93 (s, 3H), 2.90 (m, 2H), 2.75 (t, *J* = 7.3 Hz, 2H). LRMS-ESI *m/z* calculated for $C_{18}H_{17}ClN_5O_2S_2^+$ [M + H^+]: 434.1, found: 434.0.

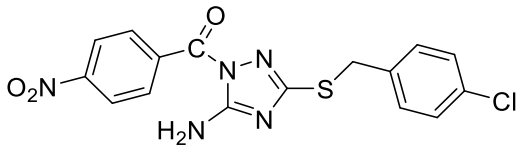
Compound 20: 3-(3-(4-chlorophenyl)propyl)-1-((2-methylbenzo[*d*]thiazol-6-yl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



Compound **20** was prepared from 2-methyl-1,3-benzothiazole-6-sulfonyl chloride (1.2 equiv, 0.25 mmol, 62 mg) using the general procedure. Flash chromatography (SiO_2, 50% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR (CD_2Cl_2, 400 MHz) δ 8.55 (d. *J* = 1.7 Hz,

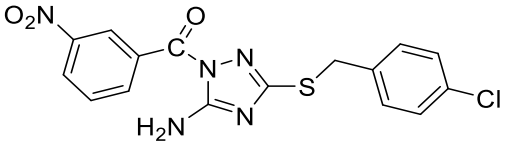
1H), 8.06 (d, *J* = 8.8 Hz, 1H), 8.00 (dd, *J* = 8.8, 1.6 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 2H), 7.02 (d, *J* = 8.4 Hz, 2H) 6.31 (s, 2H), 2.87 (s, 3H), 2.48 (m, 4H), 1.89 (m, 2H). LRMS-ESI *m/z* calculated for $C_{19}H_{19}ClN_5O_2S_2^+$ [M + H$^+$]: 448.1, found: 448.0.

Compound 72: (5-amino-3-((4-chlorobenzyl)thio)-1*H*-1,2,4-triazol-1-yl)(4-nitrophenyl)-methanone



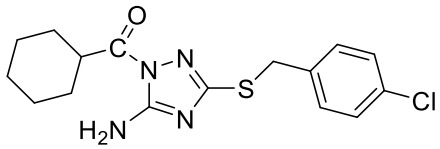
Compound **72** was prepared from 4-nitrobenzoyl chloride (1.2 equiv, 0.25 mmol, 46 mg) using the general procedure. Flash chromatography (SiO_2) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.31 (d, *J* = 9.0 Hz, 2H), 8.25 (d, *J* = 9.0 Hz, 2H), 7.32–7.26 (m, 4H), 6.56 (s, 2H), 4.23 (s, 2H). HRMS-ESI-TOF *m/z* calculated for $C_{16}H_{13}ClN_5O_3S^+$ [M + H$^+$]: 390.0422, found: 390.0420.

Compound 75: (5-amino-3-((4-chlorobenzyl)thio)-1*H*-1,2,4-triazol-1-yl)(3-nitrophenyl)-methanone



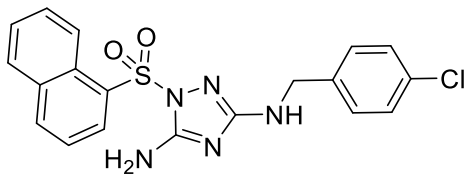
Compound **75** was prepared from 3-nitrobenzoyl chloride (1.2 equiv, 0.25 mmol, 46 mg) using the general procedure. Flash chromatography (SiO_2, 40% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR (DMSO-*d*$_6$, 500 MHz) δ 8.87 (t, *J* = 1.9 Hz, 1H), 8.49 (ddd, *J* = 8.4, 2.3, 1.0 Hz, 1H), 8.40 (dt, *J* = 8.0, 1.0 Hz, 1H), 7.94 (br s, 2H), 7.85 (t, *J* = 8.0 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 2H), 7.33 (d, *J* = 8.5 Hz, 2H), 4.27 (s, 2H). HRMS-ESI-TOF *m/z* calculated for $C_{16}H_{13}ClN_5O_3S^+$ [M + H$^+$]: 390.0422, found: 390.0427.

Compound 76: (5-amino-3-((4-chlorobenzyl)thio)-1*H*-1,2,4-triazol-1-yl)(cyclohexyl)-methanone



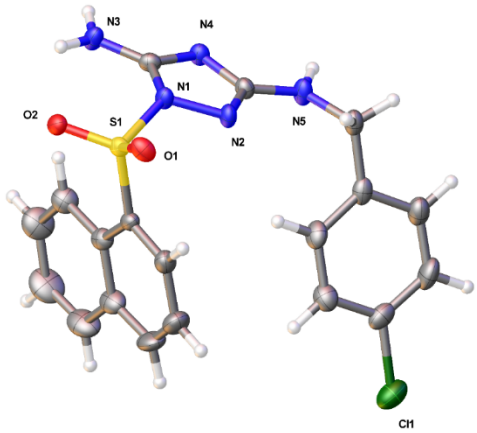
Compound **76** was prepared from cyclohexylbenzoyl chloride (1.2 equiv, 0.25 mmol, 30 μL) using the general procedure. Flash chromatography (SiO_2) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36 (d, *J* = 8.5 Hz, 2H), 7.27 (d, *J* = 8.5 Hz, 2H), 6.48 (br s, 2H), 4.25 (s, 2H), 3.29 (tt, *J* = 11.3, 3.4 Hz, 1H), 2.08–1.79 (m, 6H), 1.61–1.30 (m, 4H). LRMS-ESI *m/z* calculated for $C_{16}H_{20}ClN_4OS^+$ [M + H$^+$]: 351.1, found: 351.3.

Compound 78: 3-(4-chlorobenzyl)amino-1-(naphth-1-sulfonyl)-5-amino-1*H*-1,2,4-triazole

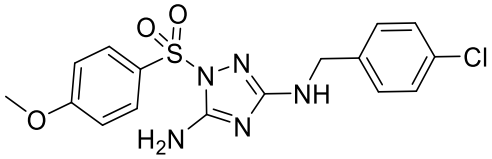


Compound **78** was prepared from naphthalene-1-sulfonyl chloride (1.2 equiv, 0.25 mmol, 61 mg) using the general procedure. Flash chromatography (SiO_2, 60% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.72 (d, J = 8.5 Hz, 1H), 8.37 (d, J = 8.2 Hz, 1H), 8.30 (d, J = 7.5 Hz, 1H), 8.12 (d, J = 8.2 Hz, 1H), 7.77–7.57 (m, 3H), 7.26 (s, 2H), 7.16 (d, J = 8.3 Hz, 2H), 7.04 (d, J = 8.3 Hz, 2H), 6.72 (t, J = 6.4 Hz, 1H), 4.06 (d, J = 6.3 Hz, 2H). LRMS-ESI *m/z* calculated for $C_{19}H_{17}ClN_5O_2S^+$ [M + H$^+$]: 414.1, found: 414.0.

The structure of **78** was confirmed with an X-ray crystal structure (CDCC 1825323; **Supplementary Table 8**).

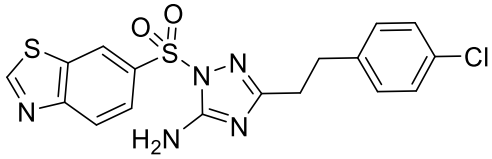


Compound 80: 3-(4-chlorobenzyl)amino-1-((4-methoxyphenyl)sulfonyl)-5-amino-1*H*-1,2,4-triazole



Compound **80** was prepared from 4-methoxyphenylsulfonyl chloride (1.2 equiv, 0.25 mmol, 58 mg) using the general procedure. Flash chromatography (SiO_2) yielded the desired product as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.78 (d, J = 9.1 Hz, 2H), 7.32 (d, J = 8.3 Hz, 2H), 7.24 (d, J = 8.3 Hz, 2H), 6.94 (d, J = 9.1 Hz, 2H), 6.56 (t, J = 6.1 Hz, 1H), 4.58 (d, J = 6.1 Hz, 2H), 3.88 (s, 3H). LRMS-ESI *m/z* calculated for $C_{16}H_{17}ClN_5O_3S^+$ [M + H$^+$]: 394.1, found: 394.0.

Compound 88: 1-(benzo[*d*]thiazol-6-ylsulfonyl)-3-(4-chlorophenethyl)-5-amino-1*H*-1,2,4-triazole



Compound **88** was prepared from 1,3-benzothiazole-6-sulfonyl chloride (1.2 equiv, 0.25 mmol, 58 mg) using the general procedure. Flash chromatography (SiO_2, 40% EtOAc/hexanes) yielded the desired product as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.74 (s, 1H), 8.98 (d, J = 1.7 Hz, 1H), 8.34 (d, J = 8.7 Hz, 1H), 7.99 (dd, J = 8.7, 1.7 Hz, 1H), 7.33 (s, 2H), 7.07 (d, J = 8.4 Hz, 2H) 7.03 (d, J = 8.4 Hz, 2H), 2.80 (t, J = 7.4 Hz, 2H), 2.62 (t, J = 7.4 Hz, 2H). LRMS-ESI *m/z* calculated for $C_{17}H_{15}ClN_5O_2S_2^+$ [M + H$^+$]: 420.0, found: 420.0.