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Supplementary Materials for

Cell-based screen identifies a new potent and highly selective CK2 inhibitor for modulation of circadian rhythms and cancer cell growth

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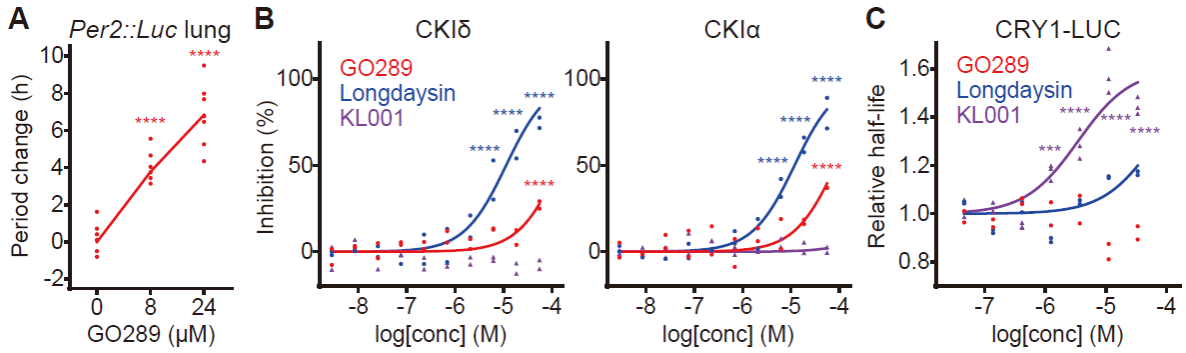
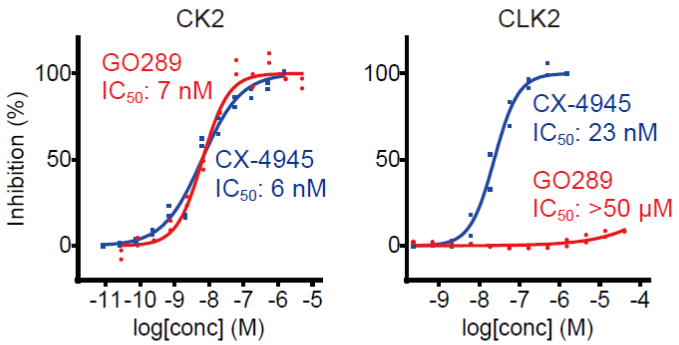


Fig. S1. Effect of GO289 on circadian period, CKI activity, and CRY stability. (**A**) Effect of GO289 on circadian rhythms in *Per2::Luc* knock-in lung explants. Luminescence rhythms were monitored in the presence of various concentrations of GO289, and period changes compared to a DMSO control are plotted (n = 8-11). (**B**) Effect of GO289 on CKIδ and CKIα activity *in vitro*. Kinase activity was analyzed in the presence of each compound at various concentrations (n = 2). (**C**) Effect of GO289 on CRY1 stability in HEK293 cells. Reporter cells expressing CRY1-luciferase fusion protein (CRY1-LUC) or LUC alone were treated with each compound at various concentrations for 24 h, and then cycloheximide was added to measure the half-life of the luminescence signal. Half-life of CRY1-LUC relative to LUC is plotted by setting the DMSO control to 1 (n = 2-3). ****$p < 0.0001$ and ***$p < 0.001$ against the DMSO control.

A

	TBB		DMAT		CX-4945		GO289	
	IC_{50} (nM)	To CK2	IC_{50} (nM)	To CK2	IC_{50} (nM)	To CK2	IC_{50} (nM)	To CK2
CK2	150	1.0	130	1.0	1	1.0	7	1.0
PIM1	1040	6.9	148	1.1	46	46.0	ND	-
PIM2	4300	28.7	1600	12.3	ND	-	13000	1857
PIM3	860	5.7	97	0.7	ND	-	ND	-
Ref	29		29		30		This study	

B



C

	CX-4945		CX-4945		CX-4945		GO289	
	IC_{50} (nM)	To CK2	IC_{50} (nM)	To CK2	IC_{50} (nM)	To CK2	IC_{50} (nM)	To CK2
CK2	1	1.0	15	1.0	6	1.0	7	1.0
CLK2	ND	-	4	0.3	23	3.8	>50000	>7000
CLK3	41	41.0	90	6.0	ND	-	ND	-
Ref	30		31		This study		This study	

Fig. S2. Effect of GO289 on kinase activity. (**A**) Effect of CK2 inhibitors on PIM family kinases. GO289 is compared to published values for CK2 inhibitors TBB, DMAT, and CX-4945. (**B**) CK2 and CLK2 activities were analyzed in the presence of GO289 and CX-4945 at various concentrations (n = 2). The data for GO289 against CK2 are reproduced from Fig. 3A for comparison. (**C**) GO289 is compared to published values for CX-4945.

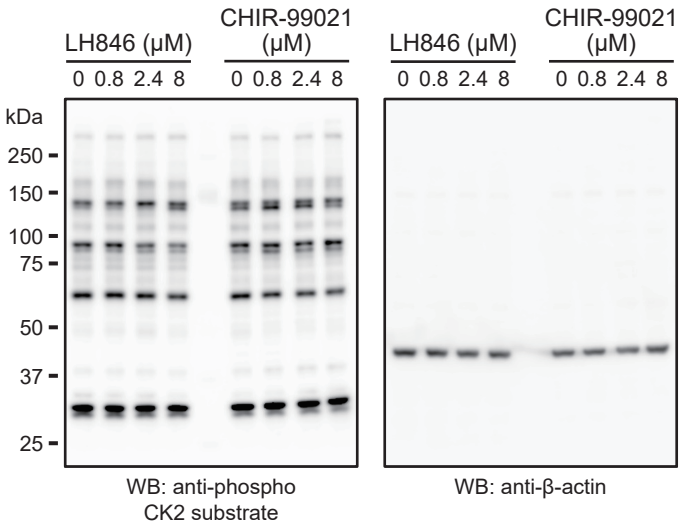


Fig. S3. Effect of CKI inhibitor LH846 and GSK-3 inhibitor CHIR-99021 on the immunoreactivity of an anti-phospho CK2 substrate antibody. HEK293T cells were treated with compounds at various concentrations for 24 h and subjected to immunoblotting with anti-phospho CK2 substrate antibody (the left panel). The membrane was reprobed with anti-β-actin antibody (the right panel).

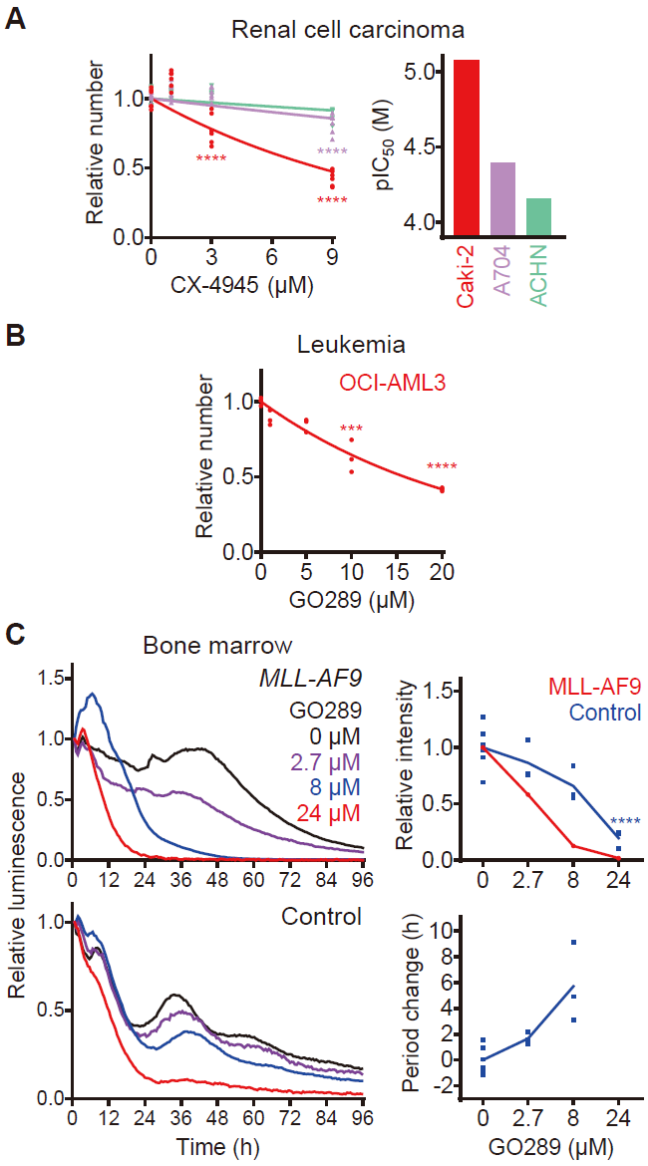


Fig. S4. Effect of CK2 inhibition on the growth and circadian rhythms of cancer cells. (**A**, **B**) Effect of CX-4945 on growth of human renal cell carcinoma lines (A) and GO289 on a human acute myeloid leukemia cell line (B). Cells were grown in the presence of various concentrations of the compounds. Cell numbers are plotted in the left panel by setting the DMSO control to 1 (n = 6 in **A** and n = 3 in **B**). pIC_{50} values are plotted in the right panel of **A**. (C) Effect of GO289 on circadian period and reporter signal intensity in bone marrow explants of *MLL-AF9* mice. Luminescence rhythms of the *Per2::Luc* knock-in reporter were monitored in the presence of various concentrations of GO289 and indicated in the top left (*MLL-AF9* mice, n = 1) and the bottom left (control mice, mean of n = 3-6) panels. Changes in intensity (top right panel) and period (bottom right panel) compared to the DMSO control are plotted. ****$p < 0.0001$ and ***$p < 0.001$ against the DMSO control.

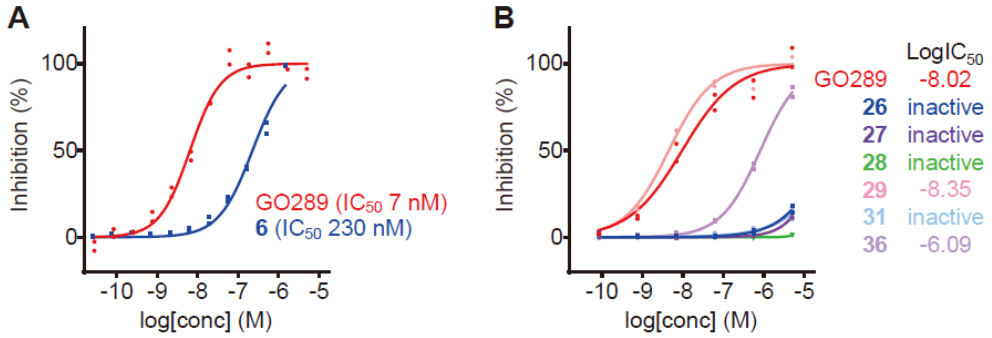


Fig. S5. Effect of GO289 derivatives on CK2 activity. (**A**, **B**) Kinase activity was analyzed in the presence of compounds at various concentrations (n = 2). In **A**, the data for GO289 are reproduced from Fig. 3A for comparison.

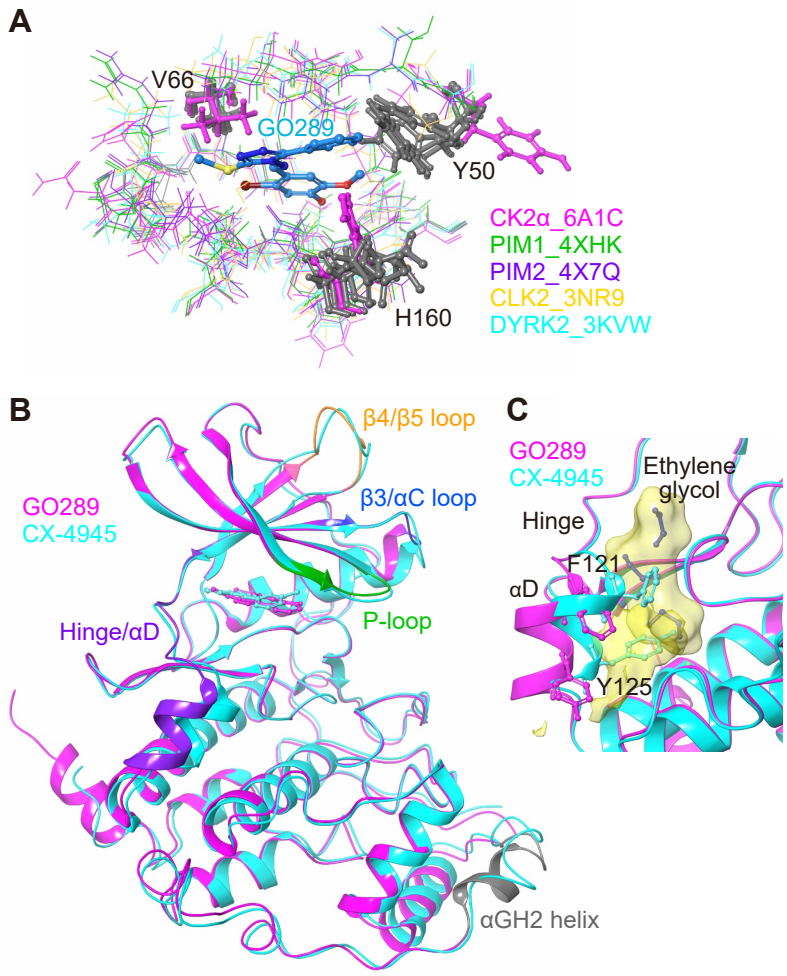


Fig. S6. Structural features of the CK2α-GO289 complex. (**A**) Binding pocket of CK2α aligned with PIM1, PIM2, CLK2, and DYRK2. Three residues showing consistent differences between CK2α and other kinases are shown in the gray CPK model for PIM1, PIM2, CLK2, and DYRK2. (**B**) Overall structure of CK2α in complex with GO289 or CX-4945. Five regions showing conformational variability are highlighted in the CK2α-GO289 complex. (**C**) Different conformations of the hinge/αD region between CK2α-GO289 and CK2-CX-4945. A cavity (yellow surface) containing three ethylene glycol molecules (blue sticks) was formed due to movement of the αD helix in CK2α-GO289. Y125 occupies this cavity in the CX-4945-bound structure. The C-Cα-Cβ-Cγ torsion angle of F121 is 50.75 in CK2-GO289 and -55.18 in CK2α-CX-4945. The C-Cα-Cβ-Cγ torsion angle of Y125 is 56.85 in CK2α-GO289 and 161.38 in CK2α-CX-4945.

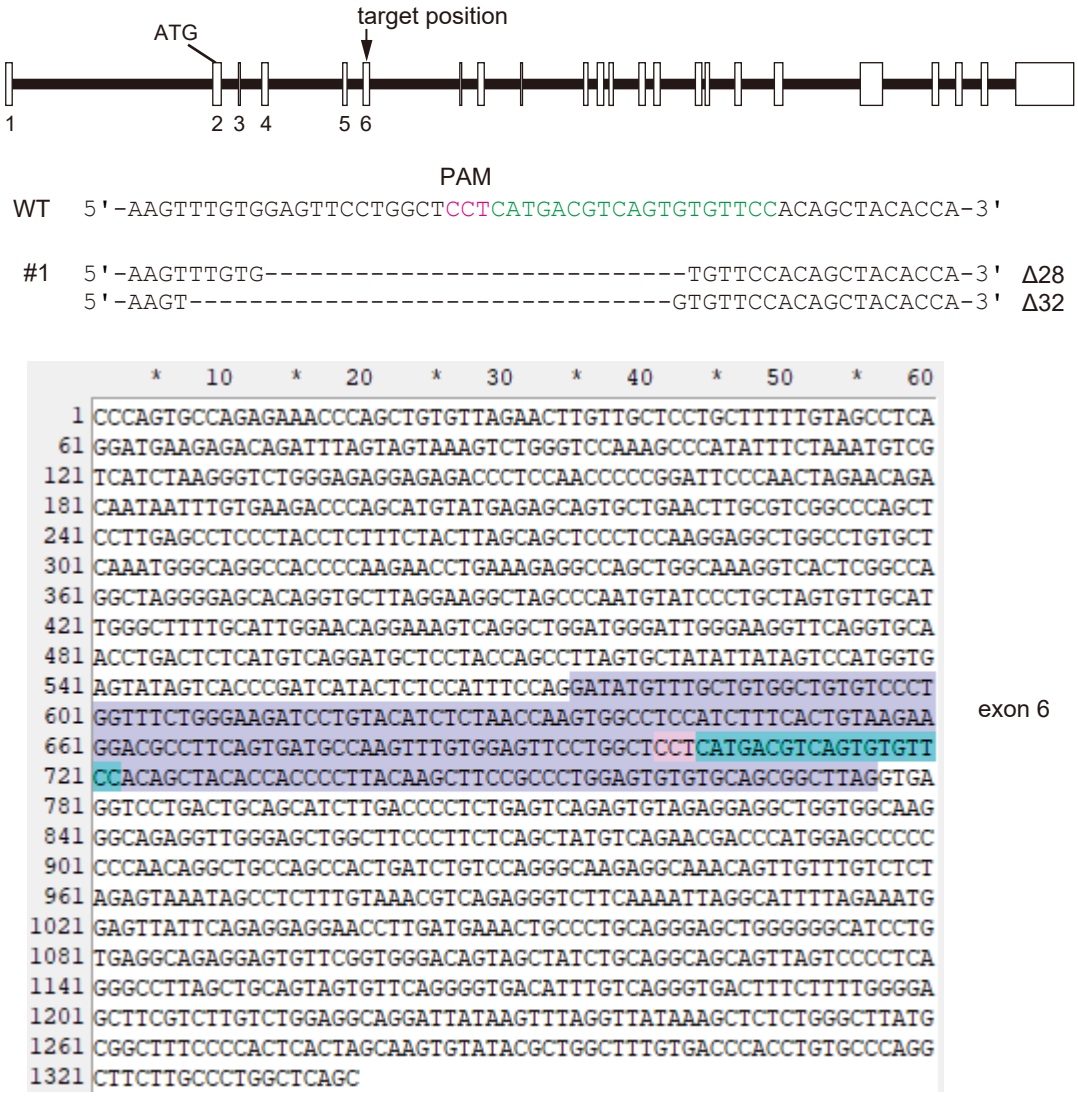


Fig. S7. Mouse *Per2* gene knockout with CRISPR-Cas9.

Table S1. Effect of GO289 on the phosphorylation of PER2 residues previously reported to be phosphorylated by CK2. Flag-tagged PER2, CRY1, CLOCK, and BMAL1 were co-expressed with CK2α in HEK293T cells, and cells were treated with 8 μM GO289 for 24 h. Cell lysates were subjected to immunoprecipitation with anti-Flag antibody and then LC-MS/MS analysis. Phosphorylation level was calculated by dividing MS spectra number of phosphorylated peptides with total peptides (Ratio). Effect of GO289 was defined by dividing the ratio of the GO289-treated sample with the control sample (GO289/Control).

	Control			GO289			GO289/
Residue	Phos	Total	Ratio	Phos	Total	Ratio	Control
T12	7	49	0.14	6	39	0.15	1.08
S13	7	49	0.14	11	39	0.28	1.97
T15	10	49	0.20	12	39	0.31	1.51
S53	3	17	0.18	10	25	0.40	2.27

Table S2. Data collection and refinement statistics.

Data collection	
Space group	$P2_12_12_1$
Unit cell (Å)	*a* = 51.657
	b = 78.831
	c = 79.403
Observations	273866
Unique reflections	37267
Resolutions (Å)	55.95– 1.68
	(1.71-1.68)
Completeness (%)	99.6 (99.3)
R_{merge} (%)[a]	5.6 (46.2)
I/σ	30.6 (5.7)
Refinement statistics	
Resolution (Å)	55.95-1.68 (1.73-1.68)
Reflections	35376
Total atoms	3136
R-factor (%)	13.8 (14.2)
R_{free} (%)	19.6 (22.4)
R.m.s. deviations	
Bond length (Å)	0.018
Bond angle (°)	1.9

Values in parentheses are for the highest-resolution shell.
[a]$R_{merge}=\Sigma_h\Sigma_j|I_{hj}-\langle I_h\rangle|/\Sigma_h\Sigma_j|I_{hj}|$, where *h* represents a unique reflection and *j* represents symmetry-equivalent indices. *I* is the observed intensity and <*I*> is the mean value of *I*.

Table S3. Statistical analysis of Figs. 3E and 5A. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ against the DMSO control.

Fig. 3E

Period

log[conc] (M)	GO289	TBB	DMAT	CX-4945
-4.62	****	ns	-	-
-5.10	****	ns	****	-
-5.58	****	ns	**	****
-6.05	****	ns	ns	****
-6.53	****	ns	ns	****
-7.01	ns	ns	ns	***
-7.49	ns	ns	ns	ns
-7.96	ns	ns	ns	ns
-8.44	ns	ns	ns	ns
-8.92	ns	ns	ns	ns
-9.39	ns	ns	ns	ns

Intensity

log[conc] (M)	GO289	TBB	DMAT	CX-4945
-4.62	****	***	****	****
-5.10	****	ns	****	****
-5.58	****	ns	****	****
-6.05	ns	ns	ns	****
-6.53	ns	ns	ns	****
-7.01	ns	ns	ns	ns
-7.49	ns	ns	ns	ns
-7.96	ns	ns	ns	ns
-8.44	ns	ns	ns	ns
-8.92	ns	ns	ns	ns
-9.39	ns	ns	ns	ns

Fig. 5A

GO289 (μM)	Caki-2	A498	769-P	786-O	RCC4+ vec	RCC4+ VHL	A704	ACHN
1	ns	**	ns	ns	ns	ns	ns	ns
3	****	****	ns	ns	ns	**	ns	ns
9	****	****	****	****	ns	****	****	*

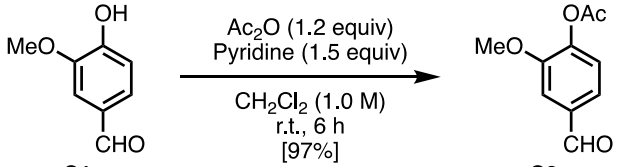
Data file S1. Compound synthesis.

1. General

Unless otherwise noted, all reactants or reagents including dry solvents were obtained from commercial suppliers and used as received. Unless otherwise noted, all reactions were performed with dry solvents under an atmosphere of argon in dried glassware using standard vacuum-line techniques. All work-up and purification procedures were carried out with reagent-grade solvents in air. Analytical thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F_{254} precoated plates (0.25 mm). The developed chromatogram was analyzed by UV lamp (254 nm). Flash column chromatography was performed with E. Merck silica gel 60 (230–400 mesh) or Biotage Isolera® instrument equipped with a Biotage SNAP Ultra 10 g cartridge. Reversed-phase column chromatography was performed with Biotage Isolera® instrument equipped with a KP-C18-HS 12 g cartridge. Preparative thin-layer chromatography (PTLC) was performed using Wakogel B5-F silica coated plates (0.75 mm) prepared in our laboratory. Preparative recycling gel permeation chromatography (GPC) was performed with JAI LC-9204 instrument equipped with JAIGEL-2H/JAIGEL-2H columns using chloroform as an eluent. LCMS analysis was conducted on an Agilent 6100 instrument equipped with Poroshell 120 EC-C18 column (2.1x100 mm, 2.7 μm) using acetonitrile/5 mM $HCOONH_4$ in water as an eluent. The high-resolution mass spectra were conducted on Thermo Fisher Scientific™ Exactive™. Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL JNM-ECA-400 (^{1}H 400 MHz, ^{13}C 100 MHz), JEOL JNM-ECA-500II (^{1}H 500 MHz, ^{13}C 125 MHz) and JEOL JMN-ECA-600II with Ultra COOL™ probe (^{1}H 600 MHz, ^{13}C 150 MHz) spectrometer. Chemical shifts for ^{1}H NMR are expressed in parts per million (ppm) relative to tetramethylsilane (δ 0.00 ppm) or residual peak of DMSO-d_6 (δ 2.49 ppm). Chemical shift for ^{13}C NMR are expressed in ppm relative to $CDCl_3$ (δ 77.0 ppm) or DMSO-d_6 (δ 39.5 ppm. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublets of doublets, t = triplet, q = quartet, m = multiplet, br = broad signal), coupling constant (Hz), and integration.

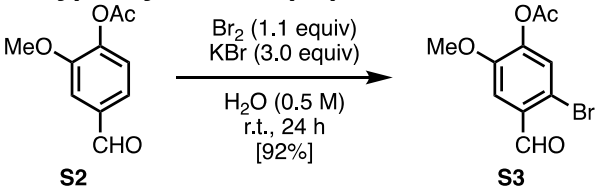
2. Synthesis of GO289 derivatives

4-Formyl-2-methoxyphenyl acetate (S2)



To a solution of vanillin (**S1**: 3.04 g, 20.0 mmol) in CH_2Cl_2 (20 mL) were added pyridine (1.95 mL, 24.2 mmol, 1.2 equiv) and Ac_2O (2.30 mL, 24.3 mmol, 1.2 equiv) at 0 °C. After stirring the mixture at room temperature for 6 h, the reaction was quenched by the addition of 1.0 M HCl. The reaction mixture was extracted with CH_2Cl_2, washed with brine, dried over Na_2SO_4, and then filtrated. The removal of solvents *in vacuo* afforded **S2** (3.77 g, 97% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.95 (s, 1H), 7.50 (d, *J* = 2.0 Hz, 1H), 7.48 (dd, *J* = 7.5, 2.0 Hz, 1H), 7.22 (d, *J* = 7.5 Hz, 1H), 3.91 (s, 3H), 2.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 191.0, 168.3, 151.9, 144.9, 135.2, 124.7, 123.4, 110.8, 56.1, 20.6; HRMS (ESI) *m/z* calcd for $C_{11}H_{14}O_5Na$ $[M+MeOH+Na]^+$: 249.0733 found 249.0732.

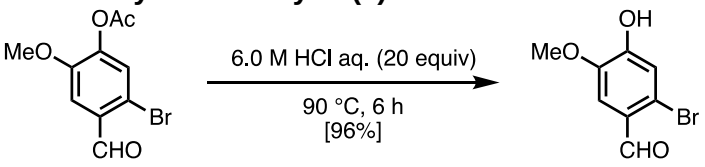
5-Bromo-4-formyl-2-methoxyphenyl acetate (S3)



To a suspension of **S2** (3.77 g, 19.4 mmol) in H_2O (40 mL) were added potassium bromide (6.90 g, 57.9 mmol, 3.0 equiv) and bromine (1.10 mL, 21.3 mmol, 1.1 equiv) at room temperature. After stirring the mixture at room temperature for 24 h, the precipitated solid was filtrated, washed with

H_2O and dissolved in CH_2Cl_2. The solution was washed with sat. $Na_2S_2O_3$ aq. and brine, dried over Na_2SO_4, and then filtrated. The removal of solvents *in vacuo* afforded **S3** (4.87 g, 92% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.27 (s, 1H), 7.51 (s, 1H), 7.36 (s, 1H), 3.88 (s, 3H), 2.34 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.8, 167.9, 151.2, 144.9, 131.6, 128.0, 117.9, 112.1, 56.3, 20.5; HRMS (ESI) *m/z* calcd for $C_{11}H_{13}BrO_5Na$ [M+MeOH+Na]$^+$: 326.9839 found 326.9838.

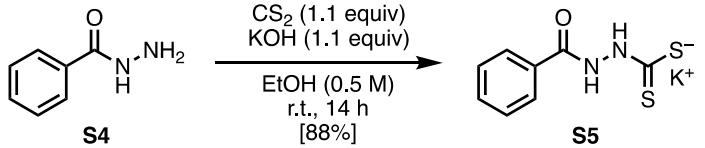
2-Bromo-4-hydroxy-5-methoxybenzaldehyde (1)



S3 **1**

A suspension of **S3** (4.81 g, 17.6 mmol) in 6.0 M HCl (60 mL) was heated at 90 °C for 4 h. The precipitated solid was filtrated and washed with H_2O to afford **1** (3.92 g, 96% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.18 (s, 1H), 7.43 (s, 1H), 7.18 (s, 1H), 6.20 (br, 1H), 3.95 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.9, 151.8, 146.4, 126.3, 120.8, 119.1, 110.3, 56.3; HRMS (ESI) *m/z* calcd for $C_8H_6BrO_3$ [M–H]$^-$: 228.9495 found 228.9503.

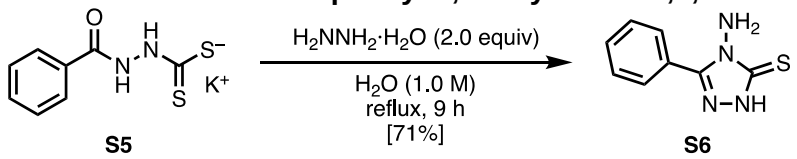
Representative procedure A: Potassium 2-benzoylhydrazine-1-carbodithioate (S5)



S4 **S5**

To a solution of benzohydrazide (**S4**: 1.41 g, 10.3 mmol) in EtOH (20 mL) were added potassium hydroxide (663 mg, 11.8 mmol, 1.1 equiv) and carbon disulfide (0.67 mL, 11.1 mmol, 1.1 equiv) at 0 °C. After stirring the mixture at room temperature for 14 h, the salts were filtrated and washed with EtOH to afford **S5** (2.27 g, 88% yield) as a white solid. This compound was used in the next step without further purification.

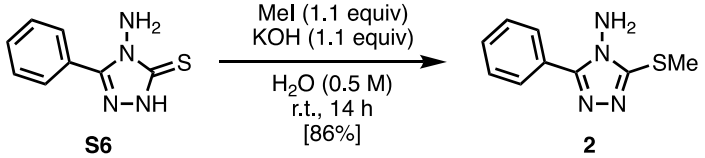
Representative procedure B: 4-Amino-5-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (S6)



S5 **S6**

To a solution of **S5** (3.85 g, 15.4 mmol) in H_2O (15 mL) was added hydrazine hydrate (1.5 mL, 30.9 mmol, 2.0 equiv) at 0 °C. After stirring the mixture at 100 °C for 9 h, the reaction was quenched by the addition of cold distilled water and then the mixture was acidified with 6.0 M HCl. The precipitated solid was filtrated and washed with H_2O to afford **S6** (2.09 g, 71% yield) as a white solid. This compound was used in the next step without further purification.

Representative procedure C: 3-(Methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-amine (2)

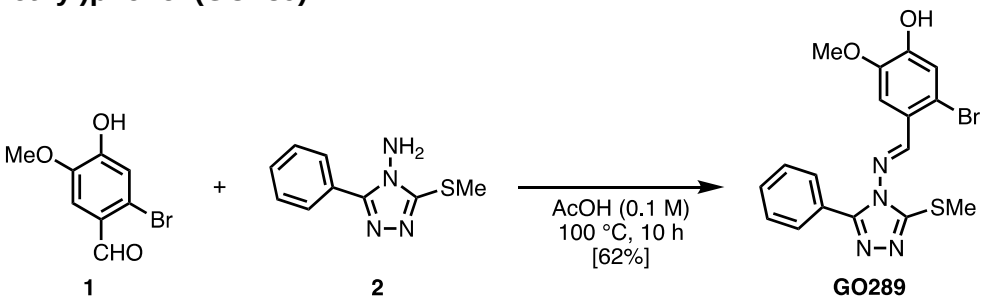


S6 **2**

To a suspension of **S6** (2.09 g, 10.9 mmol) in H_2O (20 mL) were added potassium hydroxide (709 mg, 12.6 mmol, 1.1 equiv) and iodomethane (0.75 mL, 12.0 mmol, 1.1 equiv) at room temperature. After stirring the mixture at room temperature for 14 h, the precipitated solid was filtrated and washed with H_2O to afford **2** (1.92 g, 86% yield) as a yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.01-7.95 (m, 2H), 7.54-7.46 (m, 3H), 6.13 (s, 2H), 2.61 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 154.5, 154.1, 129.5, 128.5, 127.7, 126.9, 13.7; HRMS (ESI) *m/z* calcd for

$C_9H_9N_4S$ $[M–H]^-$: 205.0542 found 205.0548.

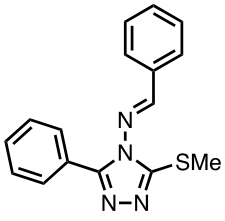
Representative procedure D:
(*E*)-5-Bromo-2-methoxy-4-(((3-(methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)imino)methyl)phenol (GO289)



1 **2** **GO289**

A solution of **2** (206 mg, 1.00 mmol) and **1** (232 mg, 1.01 mmol, 1.0 equiv) in AcOH (10 mL) was heated at 100 °C for 10 h. After the removal of solvents *in vacuo*, the precipitated solid was filtrated and washed with $CHCl_3$ to afford **GO289** (260 mg, 62% yield) as a pale yellow solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.73 (br, 1H), 8.81 (s, 1H), 7.83 (dd, *J* = 7.8, 1.8 Hz, 2H), 7.54 (s, 1H), 7.54-7.47 (m, 3H), 7.11 (s, 1H), 3.81 (s, 3H), 2.69 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 164.0, 152.8, 151.3, 148.0, 147.7, 129.9, 128.7, 128.1, 126.5, 120.8, 119.3, 118.2, 109.9, 55.7, 15.2; HRMS (ESI) *m/z* calcd for $C_{17}H_{14}BrN_4O_2S$ $[M–H]^-$: 417.0015 found 417.0034.

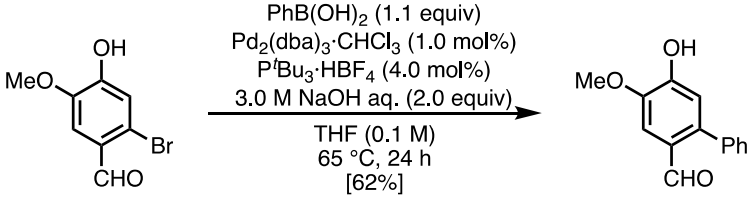
(*E*)-*N*-(3-(Methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)-1-phenylmethanimine (3)



3

Following representative procedure D, **3** was prepared as a white solid (41.4 mg, 69%) from **2** (41.8 mg, 0.20 mmol) following purification by PTLC (MeOH/$CHCl_3$ = 1:40). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.48 (s, 1H), 7.90 (dd, *J* = 7.8, 1.8 Hz, 2H), 7.83 (dd, *J* = 7.8, 1.2 Hz, 2H), 7.58 (t, *J* = 7.8 Hz, 1H), 7.51 (t, *J* = 7.8 Hz, 2H), 7.47-7.42 (m, 3H), 2.78 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 164.7, 152.1, 148.7, 133.1, 131.6, 129.9, 129.2, 129.0, 128.7, 128.3, 126.6, 15.3; HRMS (ESI) *m/z* calcd for $C_{16}H_{14}N_4SNa$ $[M+Na]^+$: 317.0831 found 317.0830.

5-Hydroxy-4-methoxy-[1,1'-biphenyl]-2-carbaldehyde (S7)

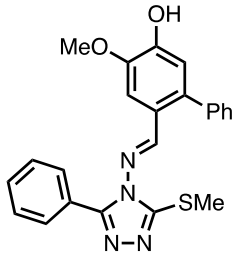


1 **S7**

A Schlenk tube containing a magnetic stirring bar was dried with a heat gun under reduced pressure. After cooling to room temperature, the tube was charged with nitrogen. Then, the tube was charged with **1** (114 mg, 0.49 mmol), phenylboronic acid (68.2 mg, 0.56 mmol, 1.1 equiv), $Pd_2(dba)_3{\cdot}CHCl_3$ (5.2 mg, 5.02 μmol, 1.0 mol%) and $P^tBu_3{\cdot}HBF_4$ (7.0 mg, 24.1 μmol, 4.0 mol%) under a gentle flow of nitrogen. Then THF (5.0 mL) and an aqueous solution of NaOH (0.33 mL of a 3.0 M solution) were added. After stirring the mixture at 65 °C for 24 h, the mixture was passed through Celite® with EtOAc as an eluent. The resulted solution was washed with water, and the organic layer was dried over Na_2SO_4, filtrated, and concentrated *in vacuo*. The residue was purified by GPC to afford **S7** (69.6 mg, 62% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.81 (s, 1H), 7.55 (s, 1H), 7.47-7.39 (m, 3H), 7.38-7.34 (m, 2H), 6.98 (s, 1H), 6.32 (br,

1H), 4.00 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 191.2, 150.6, 146.4, 142.2, 137.3, 130.2, 128.3, 127.9, 126.7, 116.3, 108.5, 56.2; HRMS (ESI) *m/z* calcd for $C_{14}H_{11}O_3$ $[M–H]^–$: 227.0703 found 227.0713.

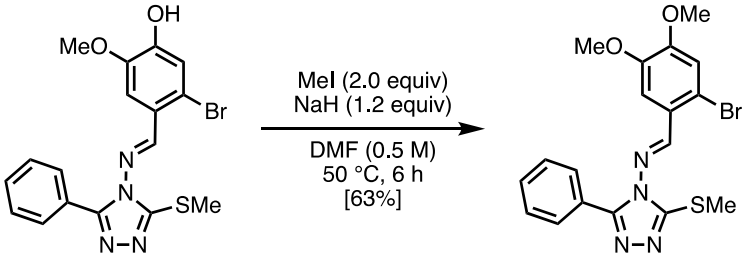
(*E*)-4-Methoxy-6-(((3-(methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)imino)methyl)-[1,1'-biphenyl]-3-ol (4)



4

Following representative procedure D, **4** was prepared as a white solid (48.4 mg, 57%) from **2** (42.4 mg, 0.21 mmol) following purification by PTLC (MeOH/$CHCl_3$ = 1:40). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.39 (br, 1H), 8.26 (s, 1H), 7.75 (d, *J* = 7.2 Hz, 2H), 7.69 (s, 1H), 7.56-7.46 (m, 3H), 7.38-7.29 (m, 3H), 7.12 (d, *J* = 7.2 Hz, 2H), 6.83 (s, 1H), 3.88 (s, 3H), 2.60 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 165.1, 151.6, 151.0, 147.8, 147.8, 139.9, 137.6, 129.8, 129.5, 128.8, 128.4, 127.9, 127.6, 126.5, 119.4, 116.9, 108.6, 55.7, 14.9; HRMS (ESI) *m/z* calcd for $C_{23}H_{19}N_4O_2S$ $[M–H]^–$: 415.1223 found 415.1237.

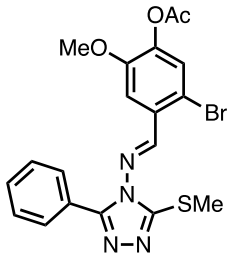
Representative procedure E:
(*E*)-1-(2-Bromo-4,5-dimethoxyphenyl)-*N*-(3-(methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)methanimine (5)



GO289 **5**

To a solution of **GO289** (42.5 mg, 0.10 mmol) in DMF (0.2 mL) was slowly added NaH (60% dispersion in mineral oil, 4.8 mg, 0.12 mmol, 1.2 equiv) at 0 °C. After stirring for 30 min at room temperature, iodomethane (13 µL, 0.21 mmol, 2.0 equiv) was slowly added to the reaction mixture at 0 °C. After stirring the mixture for 6 h at 50 °C, the reaction was quenched by the addition of saturated aqueous NH_4Cl. The reaction mixture was extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtrated, and then concentrated *in vacuo*. The crude mixture was purified by PTLC (MeOH/$CHCl_3$ = 1:40) to afford **5** (27.6 mg, 63% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.83 (s, 1H), 7.87 (dd, *J* = 8.0, 2.0 Hz, 2H), 7.63 (s, 1H), 7.49-7.44 (m, 3H), 7.05 (s, 1H), 3.95 (s, 3H), 3.93 (s, 3H), 2.81 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 162.3, 153.6, 152.2, 149.0, 148.8, 129.9, 128.7, 128.5, 126.7, 123.0, 119.0, 115.3, 109.2, 56.4, 56.1, 15.3; HRMS (ESI) *m/z* calcd for $C_{18}H_{17}BrN_4O_2SNa$ $[M+Na]^+$: 455.0148 found 455.0144.

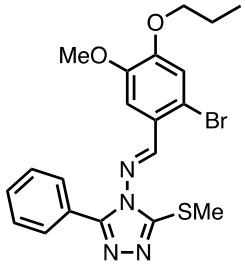
(*E*)-5-Bromo-2-methoxy-4-(((3-(methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)imino)methyl)phenyl acetate (6)



6

Following representative procedure D, **6** was prepared as a white solid (43.4 mg, 46%) from **2** (41.9 mg, 0.20 mmol) following purification by PTLC (MeOH/$CHCl_3$ = 1:40). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.89 (s, 1H), 7.84 (dd, *J* = 7.8, 2.4 Hz, 2H), 7.72 (s, 1H), 7.49-7.45 (m, 3H), 7.34 (s, 1H), 3.88 (s, 3H), 2.82 (s, 3H), 2.34 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 168.2, 161.0, 152.4, 151.2, 148.9, 144.0, 130.1, 129.1, 128.7, 128.5, 127.7, 126.6, 116.9, 110.8, 56.2, 20.5, 15.4; HRMS (ESI) *m/z* calcd for $C_{19}H_{17}BrN_4O_3SNa$ $[M+Na]^+$: 483.0097 found 483.0099.

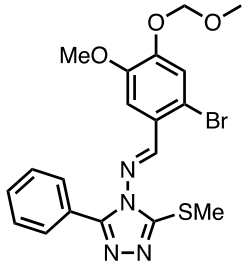
(*E*)-1-(2-Bromo-5-methoxy-4-propoxyphenyl)-*N*-(3-(methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)methanimine (7)



7

Following representative procedure E, **7** was prepared as a white solid (30.5 mg, 66%) from **GO289** (42.0 mg, 0.10 mmol) following purification by PTLC (MeOH/$CHCl_3$ = 1:40). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.82 (s, 1H), 7.87 (dd, *J* = 7.8, 1.8 Hz, 2H), 7.62 (s, 1H), 7.48-7.43 (m, 3H), 7.04 (s, 1H), 4.03 (t, *J* = 7.2 Hz, 2H), 3.91 (s, 3H), 2.80 (s, 3H), 1.95-1.87 (m, 2H), 1.07 (t, *J* = 7.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 162.5, 153.3, 152.2, 149.2, 148.8, 129.9, 128.6, 128.4, 126.8, 122.7, 119.0, 116.2, 109.4, 70.9, 56.2, 22.2, 15.3, 10.3; HRMS (ESI) *m/z* calcd for $C_{20}H_{21}BrN_4O_2SNa$ $[M+Na]^+$: 483.0461 found 483.0465.

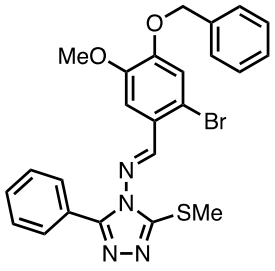
(*E*)-1-(2-Bromo-5-methoxy-4-(methoxymethoxy)phenyl)-*N*-(3-(methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)methanimine (8)



8

Following representative procedure E, **8** was prepared as a white solid (42.6 mg, 91%) from **GO289** (42.2 mg, 0.10 mmol) following purification by PTLC (MeOH/$CHCl_3$ = 1:40). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.84 (s, 1H), 7.86 (dd, *J* = 6.6, 2.4 Hz, 2H), 7.66 (s, 1H), 7.48-7.43 (m, 3H), 7.38 (s, 1H), 5.30 (s, 2H), 3.93 (s, 3H), 3.53 (s, 3H), 2.81 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 162.2, 152.2, 151.1, 149.4, 148.8, 130.0, 128.7, 128.5, 126.7, 124.2, 119.4, 118.6, 109.7, 95.2, 56.7, 56.2, 15.3; HRMS (ESI) *m/z* calcd for $C_{19}H_{19}BrN_4O_3SNa$ $[M+Na]^+$: 485.0253 found 485.0255.

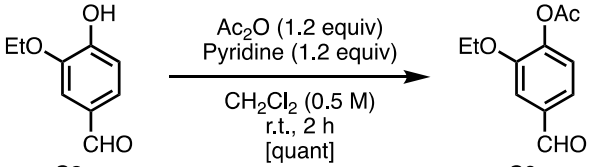
(*E*)-1-(4-(Benzyloxy)-2-bromo-5-methoxyphenyl)-*N*-(3-(methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)methanimine (9)



9

Following representative procedure E, **9** was prepared as a white solid (32.1 mg, 63%) from **GO289** (42.0 mg, 0.10 mmol) following purification by PTLC (MeOH/$CHCl_3$ = 1:40). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.81 (s, 1H), 7.87 (dd, *J* = 7.8, 1.8 Hz, 2H), 7.64 (s, 1H), 7.48-7.42 (m, 5H), 7.40 (dd, *J* = 7.8, 6.6 Hz, 2H), 7.35 (t, *J* = 6.6 Hz, 1H), 7.09 (s, 1H), 5.19 (s, 2H), 3.92 (s, 3H), 2.80 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 162.2, 152.8, 152.2, 149.4, 148.7, 135.3, 129.9, 128.7, 128.6, 128.4, 127.3, 126.7, 123.2, 118.7, 117.1, 109.6, 71.1, 56.1, 15.3 (one carbon signal is missing because of overlap); HRMS (ESI) *m/z* calcd for $C_{24}H_{21}BrN_4O_2SNa$ $[M+Na]^+$: 531.0461 found 531.0462.

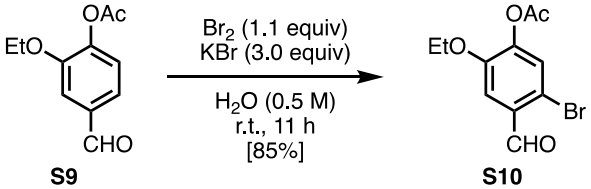
2-Ethoxy-4-formylphenyl acetate (S9)



S8 **S9**

To a solution of 3-ethoxy-4-hydroxybenzaldehyde (**S8**: 831 mg, 5.00 mmol) in CH_2Cl_2 (10 mL) were added pyridine (485 µL, 6.01 mmol, 1.2 equiv) and Ac_2O (570 µL, 6.03 mmol, 1.2 equiv) at 0 °C. After stirring the mixture at room temperature for 2 h, the reaction was quenched by the addition of 1.0 M HCl. The reaction mixture was extracted with CH_2Cl_2, washed with brine, dried over Na_2SO_4, and then filtrated. The removal of solvents *in vacuo* afforded **S9** (1.05 g, quant) as colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.94 (s, 1H), 7.48 (d, *J* = 1.5 Hz, 1H), 7.46 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 1H), 4.14 (q, *J* = 7.0 Hz, 2H), 2.34 (s, 3H), 1.42 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 191.1, 168.4, 151.3, 145.2, 135.1, 124.5, 123.3, 111.7, 64.6, 20.6, 14.5; HRMS (ESI) *m/z* calcd for $C_{12}H_{16}O_5Na$ $[M+MeOH+Na]^+$: 263.0890 found 263.0887.

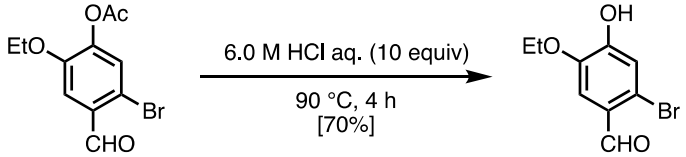
5-Bromo-2-ethoxy-4-formylphenyl acetate (S10)



S9 **S10**

To a suspension of **S9** (1.05 g, 5.00 mmol) in H_2O (10 mL) were added potassium bromide (1.02 g, 15.0 mmol, 3.0 equiv) and bromine (290 µL, 5.62 mmol, 1.1 equiv) at room temperature. After stirring the mixture at room temperature for 11 h, the precipitated solid was filtrated, washed with H_2O and dissolved in CH_2Cl_2. The solution was washed with sat. $Na_2S_2O_3$ aq. and brine, dried over Na_2SO_4, and then filtrated. The removal of solvents *in vacuo* afforded **S10** (1.22 g, 85% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.26 (s, 1H), 7.49 (s, 1H), 7.35 (s, 1H), 4.11 (q, *J* = 7.0 Hz, 2H), 2.33 (s, 3H), 1.41 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.9, 167.9, 150.5, 145.2, 131.5, 127.9, 117.6, 112.9, 64.8, 20.4, 14.4; HRMS (ESI) *m/z* calcd for $C_{12}H_{15}BrO_5Na$ $[M+MeOH+Na]^+$: 340.9995 found 340.9995.

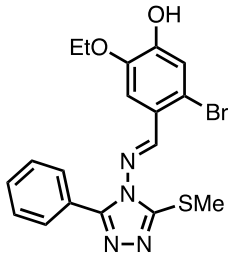
2-Bromo-5-ethoxy-4-hydroxybenzaldehyde (S11)



S10 **S11**

A suspension of **S10** (1.22 g, 4.27 mmol) in 6.0 M HCl (8.0 mL) was heated at 90 °C for 4 h. After cooling to room temperature, the precipitated solid was filtrated and purified by flash column chromatography (hexane/EtOAc = 3:1) to afford **S11** (732 mg, 70% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.17 (s, 1H), 7.41 (s, 1H), 7.17 (s, 1H), 6.36 (s, 1H), 4.18 (q, *J* = 7.0 Hz, 2H), 1.47 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.9, 151.9, 145.7, 126.2, 120.6, 119.0, 111.0, 65.0, 14.6; HRMS (ESI) *m/z* calcd for $C_9H_8BrO_3$ $[M–H]^–$: 242.9651 found 242.9662.

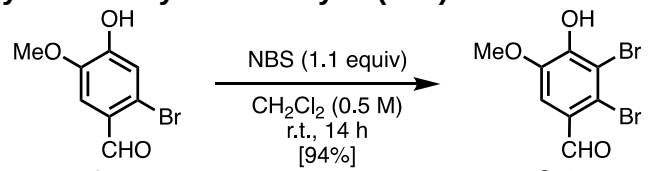
(*E*)-5-Bromo-2-ethoxy-4-(((3-(methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)imino)methyl)phenol (10)



10

Following representative procedure D, **10** was prepared as a white solid (43.4 mg, 46%) from **2** (41.9 mg, 0.20 mmol) following purification by PTLC (MeOH/$CHCl_3$ = 1:40). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.68 (br, 1H), 8.80 (s, 1H), 7.84-7.79 (m, 2H), 7.54-7.47 (m, 4H), 7.12 (s, 1H), 4.05 (q, *J* = 7.0 Hz, 2H), 2.68 (s, 3H), 1.34 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 164.0, 153.0, 151.3, 147.7, 147.1, 129.9, 128.7, 128.0, 126.6, 120.8, 119.3, 118.1, 110.9, 64.1, 15.2, 14.4; HRMS (ESI) *m/z* calcd for $C_{18}H_{16}BrN_4O_2S$ $[M–H]^–$: 431.0172 found 431.0178.

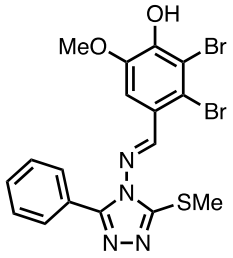
2,3-Dibromo-4-hydroxy-5-methoxybenzaldehyde (S12)



1 **S12**

To a solution of **1** (115 mg, 0.50 mmol) in CH_2Cl_2 (1.0 mL) was added *N*-bromosuccinimide (NBS; 99.1 mg, 0.56 mmol, 1.1 equiv). After stirring the mixture at room temperature for 14 h, the reaction was quenched with saturated aqueous $Na_2S_2O_3$. The mixture was extracted with CH_2Cl_2, washed with brine, dried over Na_2SO_4 and filtrated. The removal of solvents *in vacuo* afforded 2,3-dibromo-4-hydroxy-5-methoxybenzaldehyde (**S12**: 146 mg, 94% yield) as a white solid without further purification.

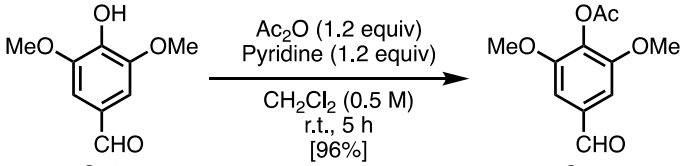
(*E*)-2,3-Dibromo-6-methoxy-4-(((3-(methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)imino)methyl)phenol (11)



11

Following representative procedure D, **11** was prepared as a white solid (43.3 mg, 43%) from **2** (41.6 mg, 0.20 mmol) following purification by PTLC (MeOH/$CHCl_3$ = 1:40). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.85 (s, 1H), 7.83 (dd, *J* = 7.5, 1.5 Hz, 2H), 7.55-7.46 (m, 4H), 3.83 (s, 3H), 2.68 (s, 3H), (one proton signal is missing); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 165.9, 151.9, 148.8, 148.3, 130.4, 129.3, 128.5, 127.1, 114.9, 108.8, 56.6, 15.6, (three carbon signals are missing); HRMS (ESI) *m/z* calcd for $C_{17}H_{13}Br_2N_4O_2S$ $[M–H]^-$: 494.9120 found 494.9133.

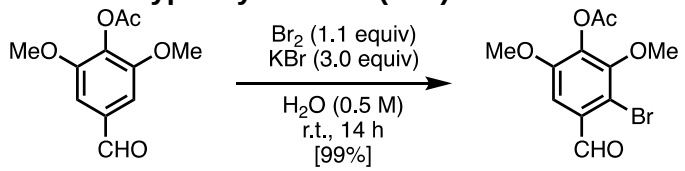
4-Formyl-2,6-dimethoxyphenyl acetate (S14)



S13 **S14**

To a solution of 4-hydroxy-3,5-dimethoxybenzaldehyde (**S13**: 909 mg, 4.99 mmol) in CH_2Cl_2 (10 mL) were added pyridine (485 µL, 6.01 mmol, 1.2 equiv) and Ac_2O (570 µL, 6.03 mmol, 1.2 equiv) at 0 °C. After stirring the mixture at room temperature for 5 h, the reaction was quenched by the addition of 1.0 M HCl. The reaction mixture was extracted with CH_2Cl_2, washed with brine, dried over Na_2SO_4, and then filtrated. The removal of solvents *in vacuo* afforded **S14** (1.08 g, 96% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.91 (s, 1H), 7.15 (s, 2H), 3.90 (s, 6H), 2.37 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 191.0, 168.0, 152.8, 134.3, 133.7, 106.0, 56.3, 20.4; HRMS (ESI) *m/z* calcd for $C_{12}H_{16}O_6Na$ $[M+MeOH+Na]^+$: 279.0839 found 279.0838.

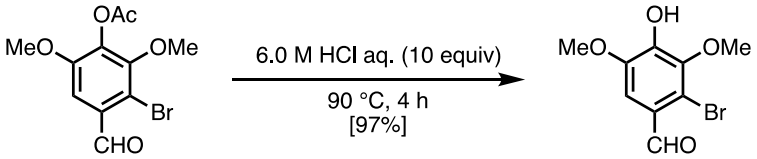
3-Bromo-4-formyl-2,6-dimethoxyphenyl acetate (S15)



S14 **S15**

To a suspension of **S14** (1.08 g, 4.82 mmol) in H_2O (10 mL) were added potassium bromide (1.79 g, 15.0 mmol, 3.0 equiv) and bromine (290 µL, 5.62 mmol, 1.1 equiv) at room temperature. After stirring the mixture at room temperature for 14 h, the precipitated solid was filtrated, washed with H_2O and dissolved in CH_2Cl_2. The solution was washed with sat. $Na_2S_2O_3$ aq. and brine, dried over Na_2SO_4, and then filtrated. The removal of solvents *in vacuo* afforded **S15** (1.45 g, 99% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.34 (s, 1H), 7.35 (s, 1H), 3.89 (s, 6H), 2.39 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.9, 167.7, 152.1, 150.7, 139.3, 131.4, 114.7, 107.4, 61.4, 56.4, 20.4; HRMS (ESI) *m/z* calcd for $C_{12}H_{15}BrO_6Na$ $[M+MeOH+Na]^+$: 356.9944 found 356.9938.

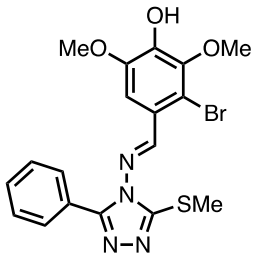
2-Bromo-4-hydroxy-3,5-dimethoxybenzaldehyde (S16)



S15 **S16**

A suspension of **S15** (1.45 g, 4.78 mmol) in 6.0 M HCl (8.0 mL) was heated at 90 °C for 4 h. The precipitated solid was filtrated and washed with H_2O to afford **S16** (1.21 g, 97% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.26 (s, 1H), 7.32 (s, 1H), 6.17 (s, 1H), 3.96 (s, 3H), 3.96 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.8, 147.1, 145.4, 144.1, 125.6, 116.4, 106.6, 60.9, 56.5; HRMS (ESI) *m/z* calcd for $C_9H_8BrO_4$ [M–H]$^-$: 258.9600 found 258.9611.

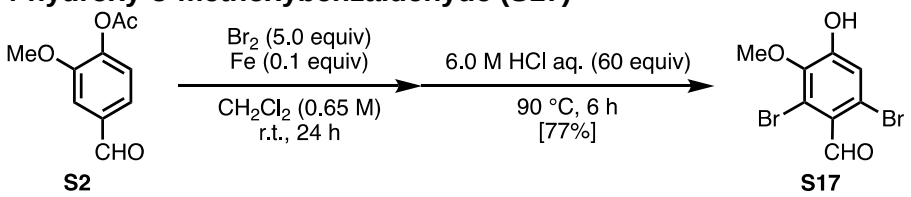
(*E*)-3-Bromo-2,6-dimethoxy-4-(((3-(methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)imino)methyl)phenol (12)



12

Following representative procedure D, **12** was prepared as a white solid (56.5 mg, 62%) from **2** (41.7 mg, 0.20 mmol) following purification by PTLC (MeOH/$CHCl_3$ = 1:40). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.37 (br, 1H), 8.91 (s, 1H), 7.86-7.80 (m, 2H), 7.55-7.47 (m, 3H), 7.43 (s, 1H), 3.84 (s, 3H), 3.75 (s, 3H), 2.69 (s, 3H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 164.3, 151.4, 148.6, 147.7, 145.8, 145.1, 130.0, 128.8, 128.1, 126.5, 120.3, 114.5, 105.7, 60.1, 56.1, 15.2; HRMS (ESI) *m/z* calcd for $C_{18}H_{16}BrN_4O_3S$ [M–H]$^-$: 447.0121 found 447.0128.

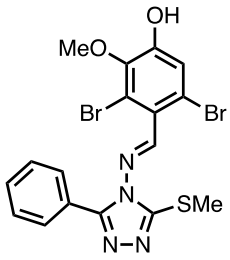
2,6-Dibromo-4-hydroxy-3-methoxybenzaldehyde (S17)



S2 **S17**

To a solution of **S2** (193 mg, 0.99 mmol) in CH_2Cl_2 (1.0 mL) were added a dispersion of iron powder (6.7 mg, 0.12 mmol, 0.1 equiv) and Br_2 (260 µL, 5.04 mmol, 5.0 equiv) in CH_2Cl_2 (0.55 mL). After stirring the mixture at room temperature for 24 h, the reaction was quenched with saturated aqueous $Na_2S_2O_3$. The mixture was extracted with CH_2Cl_2, washed with brine, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The residue was added 10 mL of 6.0 M HCl. After stirring the mixture at 90 °C for 4 h, the precipitated solid was filtrated and washed with H_2O to afford **S17** (269 mg, 77% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.24 (s, 1H), 7.46 (s, 1H), 6.64 (br, 1H), 3.99 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.4, 149.8, 146.3, 127.8, 123.6, 112.9, 109.6, 56.7; HRMS (ESI) *m/z* calcd for $C_8H_5Br_2O_3$ [M–H]$^-$: 360.8600 found 360.8612.

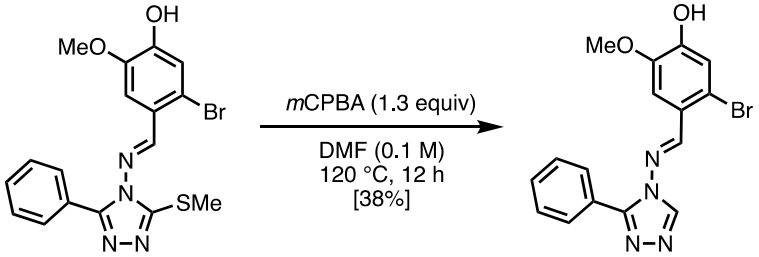
(*E*)-3,5-Dibromo-2-methoxy-4-(((3-(methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)imino)methyl)phenol (13)



13

Following representative procedure D, **13** was prepared as a white solid (35.2 mg, 35%) from **2** (41.8 mg, 0.20 mmol). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 11.12 (br, 1H), 8.95 (s, 1H), 7.83 (d, J = 7.2 Hz, 2H), 7.60 (s, 1H), 7.55-7.48 (m, 3H), 3.88 (s, 3H), 2.70 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 164.7, 151.4, 150.1, 147.6, 147.5, 129.9, 128.7, 128.1, 126.5, 122.5, 121.0, 113.6, 108.7, 56.3, 15.2; HRMS (ESI) *m/z* calcd for $C_{17}H_{13}Br_2N_4O_2S$ [M–H]$^-$: 494.9120 found 494.9131.

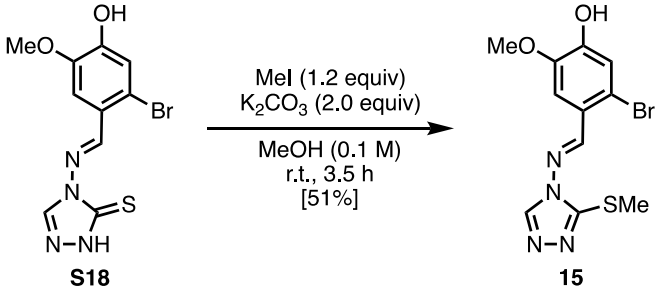
(*E*)-5-Bromo-2-methoxy-4-(((3-phenyl-4*H*-1,2,4-triazol-4-yl)imino)methyl)phenol (14)



GO289 **14**

To a solution of **GO289** (70.9 mg, 0.17 mmol) in DMF (1.7 mL) was slowly added *m*CPBA (containing ca. 60% water, 50.3 mg, 0.22 mmol, 1.3 equiv) at 0 °C. After stirring the mixture for 10 h at 120 °C, the reaction was quenched by the addition of saturated aqueous $NaHCO_3$. The reaction mixture was extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtrated, and concentrated *in vacuo*. The crude mixture was purified by PTLC (MeOH/$CHCl_3$ = 1:10) to afford **14** (23.8 mg, 38% yield) as a white solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.57 (br, 1H), 9.41 (s, 1H), 8.94 (s, 1H), 8.00 (dd, J = 7.8, 1.2 Hz, 2H), 7.56-7.49 (m, 4H), 7.12 (s, 1H), 3.80 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 157.2, 152.0, 150.1, 147.8, 137.8, 129.9, 128.5, 128.5, 126.4, 121.7, 119.3, 117.2, 110.5, 55.6; HRMS (ESI) *m/z* calcd for $C_{16}H_{12}BrN_4O_2$ [M–H]$^-$: 371.0138 found 371.0141.

(*E*)-5-Bromo-2-methoxy-4-(((3-(methylthio)-4*H*-1,2,4-triazol-4-yl)imino)methyl)phenol (15)

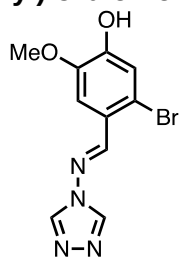


S18 **15**

Following representative procedure D, **S18** was prepared as a white solid (303 mg, 92%) from 4-amino-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (116 mg, 1.00 mmol) without further purification. To a solution of **S18** (31.8 mg, 0.10 mmol) in MeOH (1.0 mL) was slowly added K_2CO_3 (23.8 mg, 0.17 mmol, 1.2 equiv) at 0 °C. After stirring for 1 h at room temperature, iodomethane (10 µL, 0.16 mmol, 1.2 equiv) was slowly added to the reaction mixture at 0 °C. After stirring the mixture for 2 h at 50 °C, the reaction was quenched by the addition of 1.0 M HCl. The precipitated solid was filtrated and washed with H_2O to afford **15** (17.0 mg, 51% yield) as a white solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.57 (br, 1H), 9.36 (s, 1H), 8.85 (s, 1H), 7.48 (s, 1H), 7.11 (s, 1H), 3.83

(s, 3H), 2.65 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 155.6, 152.0, 150.2, 147.8, 138.0, 121.5, 119.3, 117.1, 110.2, 55.7, 13.4; HRMS (ESI) *m/z* calcd for $C_{11}H_{10}BrN_4O_2S$ $[M–H]^-$: 340.9702 found 340.9718.

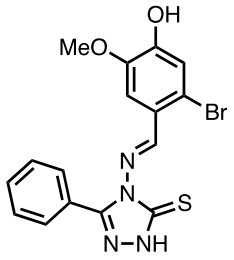
(*E*)-4-(((4*H*-1,2,4-Triazol-4-yl)imino)methyl)-5-bromo-2-methoxyphenol (16)



16

Following representative procedure D, **16** was prepared as a white solid (48.3 mg, 78%) from 4*H*-1,2,4-triazol-4-amine (17.6 mg, 0.21 mmol). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.54 (br, 1H), 9.16 (s, 2H), 8.92 (s, 1H), 7.51 (s, 1H), 7.11 (s, 1H), 3.83 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 156.3, 151.9, 147.8, 139.0, 121.5, 119.2, 117.2, 110.1, 55.8; HRMS (ESI) *m/z* calcd for $C_{10}H_8BrN_4O_2$ $[M–H]^-$: 294.9825 found 294.9833.

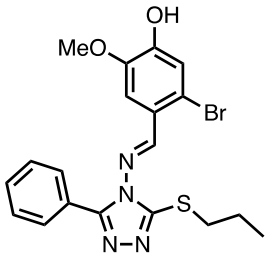
(*E*)-4-((2-Bromo-4-hydroxy-5-methoxybenzylidene)amino)-5-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (33)



33

Following representative procedure D, **33** was prepared as a white solid (109 mg, 53%) from **S6** (96.7 mg, 0.50 mmol). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 14.22 (br, 1H), 10.54 (br, 1H), 10.16 (s, 1H), 7.92-7.87 (m, 2H), 7.56-7.52 (m, 3H), 7.52 (s, 1H), 7.12 (s, 1H), 3.77 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 162.4, 162.0, 152.2, 148.8, 147.9, 130.7, 128.6, 128.6, 125.5, 121.7, 119.2, 118.0, 109.5, 55.6; HRMS (ESI) *m/z* calcd for $C_{16}H_{12}BrN_4O_2S$ $[M–H]^-$: 402.9859 found 402.9874.

Representative procedure F:
(*E*)-5-Bromo-2-methoxy-4-(((3-phenyl-5-(propylthio)-4*H*-1,2,4-triazol-4-yl)imino)methyl)phenol (17)

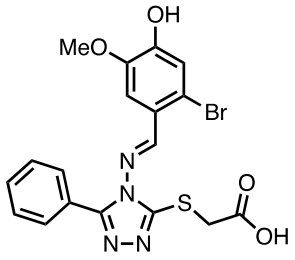


17

To a solution of **33** (40.5 mg, 0.10 mmol) in DMF (0.6 mL) was slowly added NaH (60% dispersion in mineral oil, 6.4 mg, 0.16 mmol, 1.2 equiv) at 0 °C. After stirring for 30 min at room temperature, a solution of propyl bromide (11 µL, 0.12 mmol, 1.2 equiv) in DMF (0.4 mL) was slowly added into the reaction mixture at 0 °C. After stirring the mixture for 11 h at room temperature, the reaction was quenched by the addition of saturated aqueous NH_4Cl. The reaction mixture was extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtrated, and

concentrated *in vacuo*. The crude mixture was purified by PTLC (MeOH/$CHCl_3$ = 1:40) to afford **17** (28.9 mg, 65% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.85 (s, 1H), 7.90-7.85 (m, 2H), 7.64 (s, 1H), 7.48-7.42 (m, 3H), 7.20 (s, 1H), 6.28 (br, 1H), 3.96 (s, 3H), 3.30 (t, *J* = 7.2 Hz, 2H), 1.88-1.80 (m, 2H), 1.05 (t, *J* = 7.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 163.3, 152.0, 150.8, 148.0, 146.6, 129.9, 128.6, 128.4, 126.8, 122.6, 119.5, 119.0, 109.0, 56.3, 35.3, 22.8, 13.3; HRMS (ESI) *m/z* calcd for $C_{19}H_{18}BrN_4O_2S$ $[M–H]^–$: 445.0328 found 445.0347.

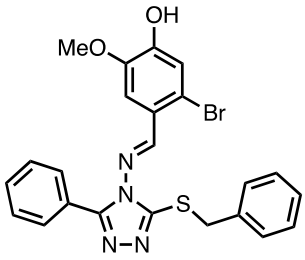
(*E*)-2-((4-((2-Bromo-4-hydroxy-5-methoxybenzylidene)amino)-5-phenyl-4*H*-1,2,4-triazol-3-yl)thio)acetic acid (18)



18

Following representative procedure F, **18** was prepared as a pale yellow solid (17.7 mg, 38%) from **33** (40.4 mg, 0.10 mmol). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.77 (br, 1H), 8.80 (s, 1H), 7.82 (dd, *J* = 8.4, 1.8 Hz, 2H), 7.55 (s, 1H), 7.54-7.48 (m, 3H), 7.11 (s, 1H), 4.09 (s, 2H), 3.82 (s, 3H), (one proton signal is missing); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 169.2, 164.2, 152.8, 151.2, 148.0, 146.4, 130.0, 128.8, 128.0, 126.4, 120.8, 119.2, 118.2, 110.0, 55.7, 34.7; HRMS (ESI) *m/z* calcd for $C_{18}H_{14}BrN_4O_4S$ $[M–H]^–$: 460.9914 found 460.9926.

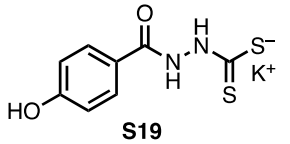
(*E*)-4-(((3-(Benzylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)imino)methyl)-5-bromo-2-methoxyphenol (19)



19

Following representative procedure F, **19** was prepared as a pale yellow solid (20.9 mg, 43%) from **33** (40.0 mg, 0.10 mmol) following purification by PTLC (MeOH/$CHCl_3$ = 1:40). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.73 (s, 1H), 7.87-7.83 (m, 2H), 7.57 (s, 1H), 7.46-7.43 (m, 3H), 7.40 (d, *J* = 6.6 Hz, 2H), 7.31-7.23 (m, 3H), 7.18 (s, 1H), 6.41 (br, 1H), 4.54 (s, 2H), 3.94 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 163.4, 152.0, 150.9, 147.6, 146.6, 136.2, 130.0, 129.3, 128.7, 128.7, 128.4, 127.8, 126.6, 122.5, 119.6, 119.0, 109.0, 56.3, 37.8; HRMS (ESI) *m/z* calcd for $C_{23}H_{18}BrN_4O_2S$ $[M–H]^–$: 493.0328 found 493.0343.

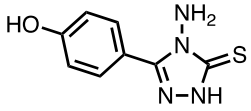
Potassium 2-(4-hydroxybenzoyl)hydrazine-1-carbodithioate (S19)



S19

Following representative procedure A, **S19** was prepared as a white solid (2.53 g, 95% yield) from 4-hydroxybenzohydrazide (1.52 g, 10.0 mmol). This compound was used without further purification.

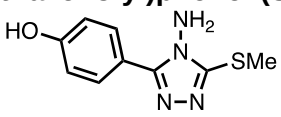
4-Amino-5-(4-hydroxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (S20)



S20

Following representative procedure B, **S20** was prepared as a white solid (2.53 g, 65% yield) from **S19** (2.50 g, 9.39 mmol). This compound was used without further purification.

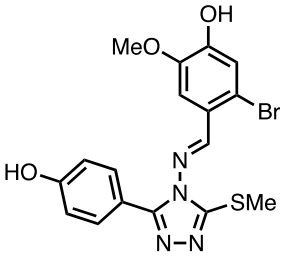
4-(4-Amino-5-(methylthio)-4*H*-1,2,4-triazol-3-yl)phenol (S21)



S21

Following representative procedure C, **S21** was prepared as a white solid (901 mg, 94% yield) from **S20** (894 mg, 4.29 mmol). This compound was used without further purification.

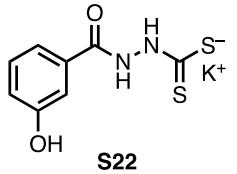
(*E*)-5-Bromo-4-(((3-(4-hydroxyphenyl)-5-(methylthio)-4*H*-1,2,4-triazol-4-yl)imino)methyl)-2-methoxyphenol (20)



20

Following representative procedure D, **20** was prepared as a white solid (256 mg, 54% yield) from **S21** (221 mg, 0.99 mmol). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.70 (br, 1H), 9.92 (br, 1H), 8.80 (s, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.54 (s, 1H), 7.12 (s, 1H), 6.87 (d, J = 8.4 Hz, 2H), 3.82 (s, 3H), 2.66 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 163.6, 158.9, 152.7, 151.5, 148.0, 146.9, 129.7, 120.9, 119.2, 118.1, 117.2, 115.5, 109.9, 55.7, 15.2; HRMS (ESI) *m/z* calcd for $C_{17}H_{14}BrN_4O_3S$ $[M–H]^-$: 432.9965 found 432.9971.

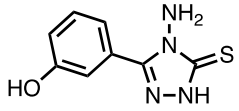
Potassium 2-(3-hydroxybenzoyl)hydrazine-1-carbodithioate (S22)



S22

Following representative procedure A, **S22** was prepared as a white solid (2.59 g, 97% yield) from 3-hydroxybenzohydrazide (1.52 g, 10.0 mmol) without further purification.

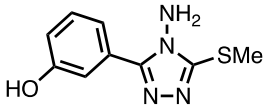
4-Amino-5-(3-hydroxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (S23)



S23

Following representative procedure B, **S23** was prepared as a white solid (964 mg, 50% yield) from **S22** (2.47 g, 9.28 mmol) and used without further purification.

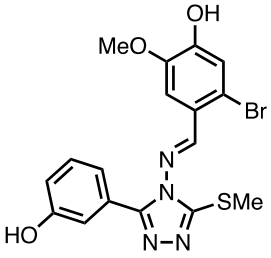
3-(4-Amino-5-(methylthio)-4*H*-1,2,4-triazol-3-yl)phenol (S24)



S24

Following representative procedure C, **S24** was prepared as a white solid (987 mg, 96% yield) from **S23** (964 mg, 4.63 mmol). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.66 (br, 1H), 7.43-7.37 (m, 2H), 7.28 (t, J = 8.0 Hz, 1H), 6.87 (ddd, J = 8.0, 2.5, 1.0 Hz, 1H), 6.07 (br, 2H), 2.59 (s, 3H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 157.3, 154.4, 154.0, 129.5, 128.0, 118.3, 116.6, 114.6, 13.7; HRMS (ESI) *m/z* calcd for $C_9H_9N_4OS$ [M–H]$^-$: 221.0492 found 211.0499.

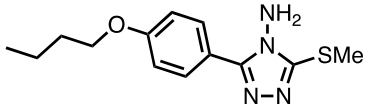
(*E*)-5-Bromo-4-(((3-(3-hydroxyphenyl)-5-(methylthio)-4*H*-1,2,4-triazol-4-yl)imino)methyl)-2-methoxyphenol (21)



21

Following representative procedure D, **21** was prepared as a white solid (279 mg, 59% yield) from **S24** (222 mg, 1.00 mmol). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.74 (br, 1H), 9.74 (br, 1H), 8.79 (s, 1H), 7.55 (s, 1H), 7.30 (dd, J = 8.0, 7.0 Hz, 1H), 7.26-7.22 (m, 2H), 7.11 (s, 1H), 6.87 (ddd, J = 8.0, 2.5, 1.0 Hz, 1H), 3.82 (s, 3H), 2.67 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 163.9, 157.4, 152.7, 151.2, 148.0, 147.6, 129.8, 127.6, 120.9, 119.2, 118.7, 118.1, 116.9, 114.7, 110.1, 55.7, 15.1; HRMS (ESI) *m/z* calcd for $C_{17}H_{14}BrN_4O_3S$ [M–H]$^-$: 432.9965 found 432.9974.

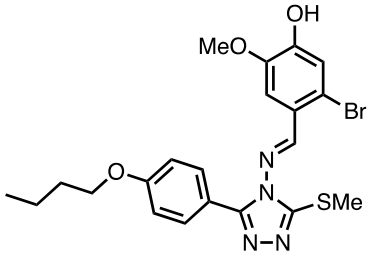
3-(4-Butoxyphenyl)-5-(methylthio)-4*H*-1,2,4-triazol-4-amine (S25)



S25

Following representative procedure E, **S25** was prepared as a pale yellow solid (69.0 mg, 58% yield) from **S21** (95.1 mg, 0.43 mmol). This compound was used in the next step without further purification.

(*E*)-5-Bromo-4-(((3-(4-butoxyphenyl)-5-(methylthio)-4*H*-1,2,4-triazol-4-yl)imino)methyl)-2-methoxyphenol (22)

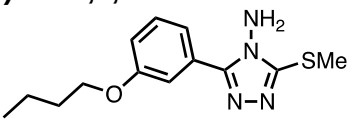


22

Following representative procedure D, **22** was prepared as a white solid (90.4 mg, 90% yield) from **S25** (56.6 mg, 0.20 mmol) following purification by PTLC (MeOH/$CHCl_3$ = 1:40). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.79 (s, 1H), 7.76 (d, J = 9.0 Hz, 2H), 7.54 (s, 1H), 7.10 (s, 1H), 7.04 (d, J = 9.0 Hz, 2H), 3.99 (t, J = 6.0 Hz, 2H), 3.82 (s, 3H), 2.66 (s, 3H), 1.71-1.37 (m, 2H), 1.45-1.37 (m, 2H), 0.91 (t, J = 7.2 Hz, 3H), (one proton signal is missing); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 163.8, 159.9, 153.4, 151.2, 148.1, 147.0, 129.5, 120.4, 119.3, 118.7, 118.3, 114.6, 109.8, 67.3,

55.7, 30.6, 18.7, 15.2, 13.6; HRMS (ESI) *m/z* calcd for $C_{21}H_{22}BrN_4O_3S$ $[M–H]^–$: 489.0591 found 489.0604.

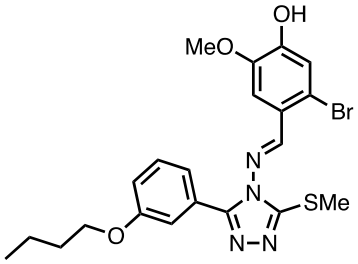
3-(3-Butoxyphenyl)-5-(methylthio)-4*H*-1,2,4-triazol-4-amine (S26)



S26

Following representative procedure E, **S26** was prepared as a pale yellow solid (21.6 mg, 20% yield) from **S24** (87.3 mg, 0.39 mmol). This compound was used in the next step without further purification.

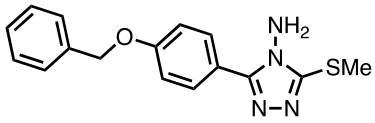
(*E*)-5-Bromo-4-(((3-(3-butoxyphenyl)-5-(methylthio)-4*H*-1,2,4-triazol-4-yl)imino)methyl)-2-methoxyphenol (23)



23

Following representative procedure D, **23** was prepared as a white solid (39.5 mg, quant) from **S26** (21.6 mg, 0.08 mmol) following purification by PTLC ($MeOH/CHCl_3$ = 1:40). ^1H NMR (DMSO-d_6, 600 MHz) δ 8.76 (s, 1H), 7.54 (s, 1H), 7.43-7.35 (m, 3H), 7.06 (s, 1H), 7.06-7.01 (m, 1H), 3.95 (t, *J* = 6.6 Hz, 2H), 3.80 (s, 3H), 2.68 (s, 3H), 1.67-1.60 (m, 2H), 1.40-1.31 (m, 2H), 0.86 (t, *J* = 7.2 Hz, 3H), (one proton signal is missing); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 164.4, 158.6, 154.3, 150.9, 148.3, 147.8, 129.9, 127.7, 120.1, 119.6, 119.4, 118.7, 116.2, 113.7, 109.7, 67.3, 55.6, 30.5, 18.6, 15.0, 13.5; HRMS (ESI) *m/z* calcd for $C_{21}H_{22}BrN_4O_3S$ $[M–H]^–$: 489.0591 found 489.0600.

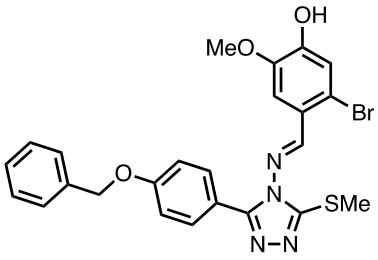
3-(4-(Benzyloxy)phenyl)-5-(methylthio)-4*H*-1,2,4-triazol-4-amine (S27)



S27

Following representative procedure E, **S27** was prepared as a white solid (78.1 mg, 58% yield) from **S21** (429 mg, 0.43 mmol). This compound was used in the next step without further purification.

(*E*)-4-(((3-(4-(Benzyloxy)phenyl)-5-(methylthio)-4*H*-1,2,4-triazol-4-yl)imino)methyl)-5-bromo-2-methoxyphenol (24)

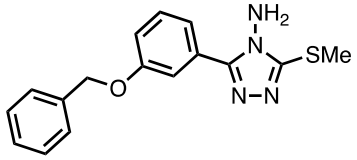


24

Following representative procedure D, **24** was prepared as a white solid (88.1 mg, 84% yield) from **S27** (62.4 mg, 0.20 mmol) following purification by PTLC ($MeOH/CHCl_3$ = 1:40). ^1H NMR (DMSO-d_6, 600 MHz) δ 10.77 (br, 1H), 8.81 (s, 1H), 7.77 (d, *J* = 9.0 Hz, 2H), 7.53 (s, 1H), 7.44 (d,

J = 7.2 Hz, 2H), 7.38 (t, *J* = 7.2 Hz, 2H), 7.32 (t, *J* = 7.2 Hz, 1H), 7.14 (d, *J* = 9.0 Hz, 2H), 7.11 (s, 1H), 5.16 (s, 2H), 3.80 (s, 3H), 2.67 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 163.7, 159.5, 152.9, 151.2, 148.0, 147.1, 136.7, 129.6, 128.4, 127.9, 127.7, 120.7, 119.3, 119.0, 118.2, 115.0, 109.9, 69.3, 55.7, 15.2; HRMS (ESI) *m/z* calcd for $C_{24}H_{20}BrN_4O_3S$ $[M–H]^-$: 523.0434 found 523.0442.

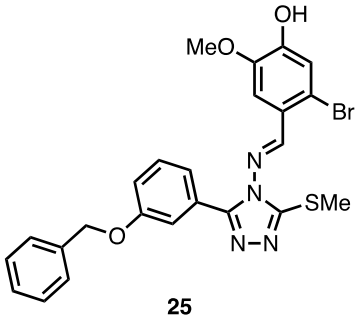
3-(3-(Benzyloxy)phenyl)-5-(methylthio)-4*H*-1,2,4-triazol-4-amine (S28)



S28

Following representative procedure E, **S28** was prepared as a white solid (16.4 mg, 13% yield) from **S24** (87.4 mg, 0.39 mmol). This compound was used in the next step without further purification.

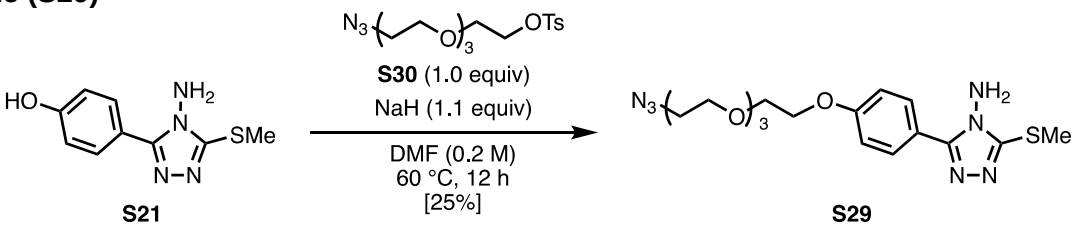
(*E*)-4-(((3-(3-(Benzyloxy)phenyl)-5-(methylthio)-4*H*-1,2,4-triazol-4-yl)imino)methyl)-5-bromo-2-methoxyphenol (25)



25

Following representative procedure D, **25** was prepared as a white solid (31.8 mg, quant) from **S28** (16.4 mg, 0.05 mmol) following purification by PTLC (MeOH/$CHCl_3$ = 1:40). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.70 (s, 1H), 7.48 (s, 2H), 7.42-7.25 (m, 7H), 7.12-7.08 (m, 1H), 6.98 (s, 1H), 5.07 (s, 2H), 3.68 (s, 3H), 2.64 (s, 3H), (one proton signal is missing); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 164.4, 158.3, 150.9, 148.5, 147.8, 136.7, 130.0, 128.4, 127.9, 127.8, 127.6, 120.4, 119.5, 119.1, 118.8, 116.3, 114.2, 109.5, 69.4, 55.5, 15.1, (one carbon signal is missing because of overlap); HRMS (ESI) *m/z* calcd for $C_{24}H_{20}BrN_4O_3S$ $[M–H]^-$: 523.0434 found 523.0442.

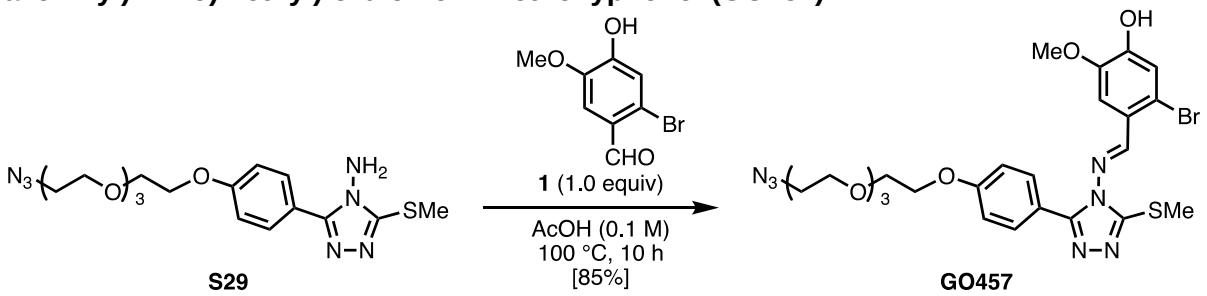
3-(4-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethoxy)phenyl)-5-(methylthio)-4*H*-1,2,4-triazol-4-amine (S29)



S21 **S29**

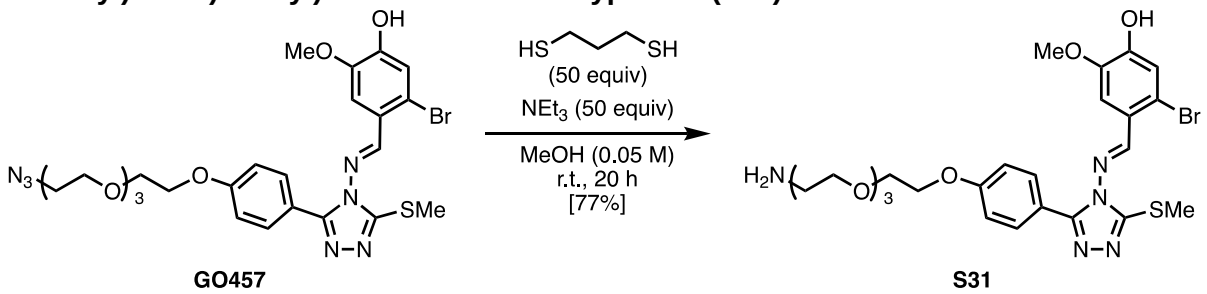
To a solution of **S21** (225 mg, 1.01 mmol) in DMF (3.5 mL) was slowly added NaH (60% dispersion in mineral oil, 46.6 mg, 1.17 mmol, 1.1 equiv) at 0 °C. After stirring for 30 min at room temperature, a solution of **S30** (369 mg, 0.99 mmol, 1.0 equiv) in DMF (1.5 mL) was slowly added to the reaction mixture at 0 °C. After stirring the mixture for 10 h at 60 °C, the reaction was quenched by the addition of saturated aqueous NH_4Cl. The reaction mixture was extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtrated, and then concentrated *in vacuo*. The crude mixture was roughly purified by PTLC (MeOH/$CHCl_3$ = 1:20) to afford **S29** (106 mg, 25% yield) as pale yellow viscous oil, which was used in next step without further purification.

(*E*)-4-(((3-(4-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethoxy)phenyl)-5-(methylthio)-4*H*-1,2,4-triazol-4-yl)imino)methyl)-5-bromo-2-methoxyphenol (GO457)



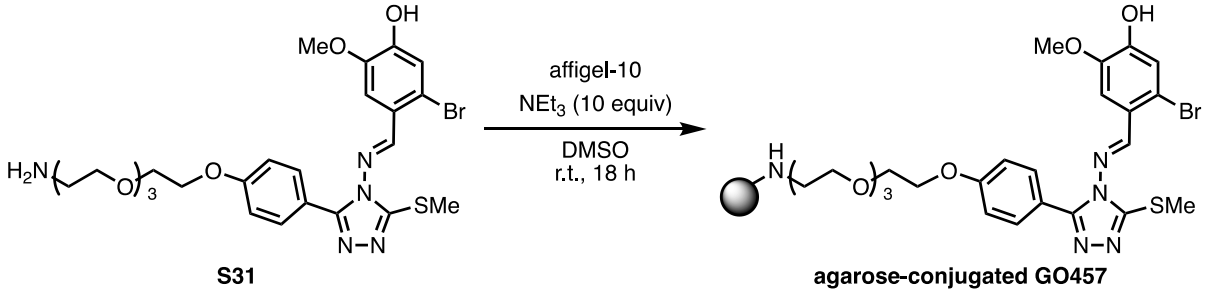
Following representative procedure D, **GO457** was prepared as pale yellow viscous oil (121 mg, 85%) from **S29** (94.8 mg, 0.22 mmol) following purification by PTLC ($MeOH/CHCl_3$ = 1:40). ^1H NMR ($CDCl_3$, 600 MHz) δ 8.85 (s, 1H), 7.80 (d, *J* = 9.0 Hz, 2H), 7.63 (s, 1H), 7.23 (s, 1H), 6.98 (d, *J* = 9.0 Hz, 2H), 6.74 (br, 1H), 4.17 (t, *J* = 4.8 Hz, 2H), 3.95 (s, 3H), 3.87 (t, *J* = 4.8 Hz, 2H), 3.75-3.72 (m, 2H), 3.71-3.64 (m, 8H), 3.37 (t, *J* = 4.8 Hz, 2H), 2.78 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 162.9, 160.1, 152.1, 151.1, 148.3, 146.8, 129.9, 122.5, 119.5, 119.2, 119.1, 114.8, 109.0, 70.9, 70.7, 70.7, 70.0, 69.6, 67.5, 56.3, 50.7, 15.4, (one carbon signal is missing because of overlap); HRMS (ESI) *m/z* calcd for $C_{25}H_{29}BrN_7O_6S$ $[M–H]^–$: 634.1078 found 634.0194.

(*E*)-4-(((3-(4-(2-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)ethoxy)phenyl)-5-(methylthio)-4*H*-1,2,4-triazol-4-yl)imino)methyl)-5-bromo-2-methoxyphenol (S31)



To a solution of **GO457** (32.2 mg, 0.05 mmol) in MeOH (1.0 mL) were added NEt_3 (0.35 mL, 2.51 mmol, 50 equiv) and propane-1,3-dithiol (0.25 mL, 2.50 mmol, 50 equiv). After stirring for 20 h at room temperature, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in $CHCl_3$ and washed with water. The organic layer was dried over Na_2SO_4, filtrated, and concentrated *in vacuo*. The residue was roughly purified by column chromatography ($MeCN/H_2O$) to afford **S31** (23.8 mg, 77% yield) as a white solid. This compound was used in next step without further purification.

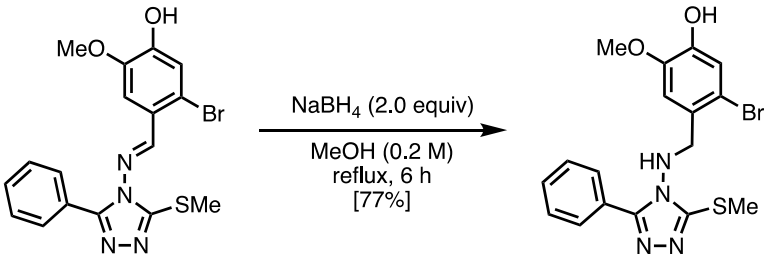
Agarose-conjugated GO457



To a solution of **GO457** (3.05 mg, 5.00 μmol) in DMSO were added triethylamine (6.75 μL, 50.0 μmol, 10.0 equiv) and agarose bead [Affi-gel 10 Gel (153-6046, Bio-Rad), activated by *N*-hydroxysuccinimide (0.01 mmol/mL), (1.65 mL, 3.3 equiv)]. After shaking at room temperature overnight, the reaction was analyzed by LCMS. After 18 h, LCMS indicated that all of the starting material had been consumed, and the reaction mixture was treated with 2-ethanolamine (3.05 mg, 50.0 μmol, 10.0 equiv) and stirred at room temperature overnight. The reaction mixture was filtered through frit. The agarose beads were washed with DMSO (2.0 mL, ×3) and PBS (2.0 mL,

×3). The agarose beads were stored at 4 °C in a PBS solution containing 0.05% NaN_3

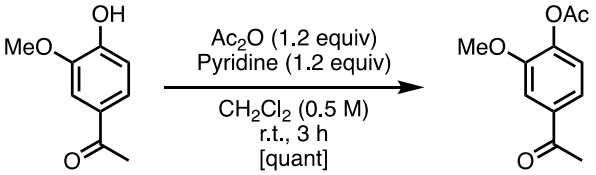
5-Bromo-2-methoxy-4-(((3-(methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)amino)methyl)phenol (26)



GO289 **26**

To a solution of **GO289** (40.8 mg, 0.10 mmol) in MeOH (0.5 mL) was slowly added $NaBH_4$ (7.8 mg, 0.21 mmol, 2.0 equiv). After stirring the mixture at 75 °C for 6 h, the reaction mixture was concentrated *in vacuo*. The reaction mixture was extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtrated, and then concentrated *in vacuo*. The crude mixture was purified by PTLC (MeOH/$CHCl_3$ = 1:40) to afford **26** (31.4 mg, 77% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.81-7.75 (m, 2H), 7.44-7.38 (m, 3H), 6.90 (s, 1H), 6.33 (s, 1H), 5.66 (br, 1H), 5.25 (t, *J* = 6.0 Hz, 1H), 4.09 (d, *J* = 6.0 Hz, 2H), 3.75 (s, 3H), 2.76 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 153.8, 152.9, 146.5, 145.9, 129.9, 128.5, 127.7, 126.4, 125.2, 118.7, 115.7, 113.2, 56.1, 55.7, 15.1; HRMS (ESI) *m/z* calcd for $C_{17}H_{16}BrN_4O_2S$ $[M–H]^-$: 419.0172 found 419.0186.

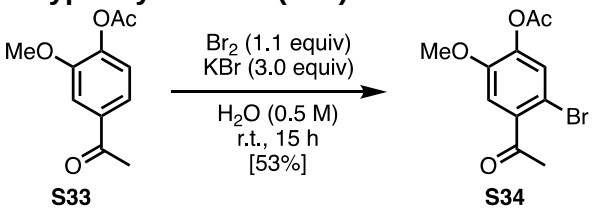
4-Acetyl-2-methoxyphenyl acetate (S33)



S32 **S33**

To a solution of 1-(4-hydroxy-3-methoxyphenyl)ethan-1-one (**S32**: 1.67 g, 10.1 mmol) in CH_2Cl_2 (20 mL) were added pyridine (0.97 mL, 12.0 mmol, 1.2 equiv) and Ac_2O (1.15 mL, 12.2 mmol, 1.2 equiv) at 0 °C. After stirring the mixture at room temperature for 3 h, the reaction was quenched by the addition of 1.0 M HCl. The reaction mixture was extracted with CH_2Cl_2, washed with brine, dried over Na_2SO_4, and then filtrated. The removal of solvents *in vacuo* afforded **S33** (2.20 g, quant) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.60 (d, *J* = 2.0 Hz, 1H), 7.56 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.13 (d, *J* = 8.0 Hz, 1H), 3.89 (s, 3H), 2.60 (s, 3H), 2.34 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 196.9, 168.5, 151.3, 143.8, 135.9, 122.7, 121.9, 111.4, 56.0, 26.5, 20.6; HRMS (ESI) *m/z* calcd for $C_{11}H_{12}O_4Na$ $[M+Na]^+$: 231.0628 found 231.0627.

4-Acetyl-5-bromo-2-methoxyphenyl acetate (S34)

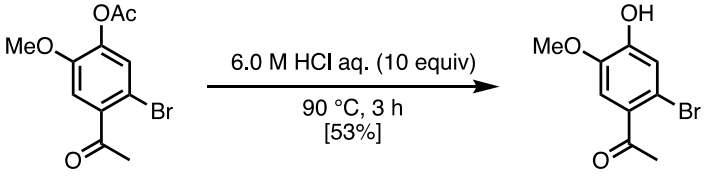


S33 **S34**

To a suspension of **S33** (2.09 g, 10.1 mmol) in H_2O (20 mL) were added potassium bromide (3.62 g, 30.4 mmol, 3.0 equiv) and bromine (0.57 mL, 11.1 mmol, 1.1 equiv) at room temperature. After stirring the mixture at room temperature for 15 h, the precipitated solid was filtrated, washed with H_2O and dissolved in CH_2Cl_2. The solution was washed with sat. $Na_2S_2O_3$ aq. and brine, and then dried over Na_2SO_4, filtrated, and concentrated *in vacuo*. The crude mixture was purified by flash column chromatography (hexane/EtOAc = 1:1) to afford **S34** (1.54 g, 53% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.30 (s, 1H), 7.09 (s, 1H), 3.85 (s, 3H), 2.67 (s, 3H), 2.32 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 200.4, 168.2, 150.6, 141.7, 139.5, 128.0, 112.9,

109.2, 56.2, 30.4, 20.5; HRMS (ESI) *m/z* calcd for $C_{11}H_{11}BrO_4Na$ $[M+Na]^+$: 308.9733 found 308.9731.

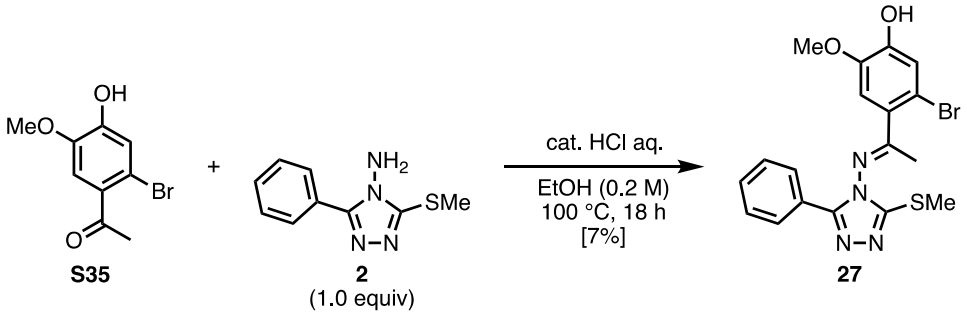
1-(2-Bromo-4-hydroxy-5-methoxyphenyl)ethan-1-one (S35)



S34 **S35**

A suspension of **S34** (1.54 g, 5.36 mmol) in 6.0 M HCl (10 mL) was heated at 90 °C for 3 h. The reaction mixture was extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtrated and concentrate *in vacuo*. The crude mixture was purified by flash column chromatography (hexane/EtOAc = 1:1) and recrystallization ($CHCl_3$) to afford **S35** (692 mg, 53% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.17 (s, 1H), 7.15 (s, 1H), 6.03 (s, 1H), 3.92 (s, 3H), 2.68 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 199.6, 148.8, 145.7, 132.4, 119.8, 112.4, 112.3, 56.2, 30.5; HRMS (ESI) *m/z* calcd for $C_9H_8BrO_3$ $[M–H]^–$: 242.9651 found 242.9662.

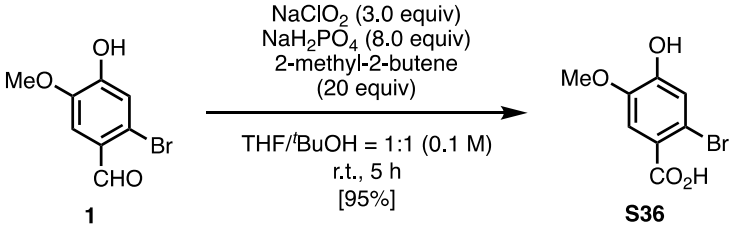
(*E*)-5-Bromo-2-methoxy-4-(1-((3-(methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)imino)ethyl)phenol (27)



S35 **2** (1.0 equiv) **27**

A solution of **2** (43.3 mg, 0.21 mmol), **S35** (52.6 mg, 0.21 mmol, 1.0 equiv) and catalytic amount of 6.0 M HCl in EtOH (1 mL) was heated at 100 °C for 18 h. After the removal of solvents *in vacuo*, the residue was purified by PTLC (MeOH/$CHCl_3$ = 1:20) and then by GPC to afford **27** (6.0 mg, 7% yield) as a yellow solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.75 (dd, J = 8.0, 2.0 Hz, 2H), 7.53-7.46 (m, 3H), 7.04 (s, 1H), 6.91 (s, 1H), 3.77 (s, 3H), 2.67 (s, 3H), 2.09 (s, 3H), (one proton signal is missing); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 182.8, 150.3, 147.6, 147.4, 130.1, 128.9, 127.2, 126.2, 119.9, 113.2, 110.4, 55.8, 21.2, 14.3, (two carbon signals are missing because of overlap); HRMS (ESI) *m/z* calcd for $C_{18}H_{16}BrN_4O_2S$ $[M–H]^–$: 431.0172 found 431.0185.

2-Bromo-4-hydroxy-5-methoxybenzoic acid (S36)

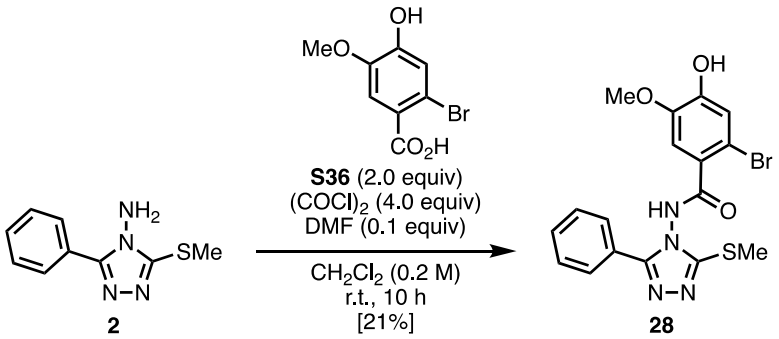


1 **S36**

To a solution of **1** (454 mg, 2.00 mmol) in THF (10 mL) and H_2O (10 mL) were added 2-methyl-2-butene (4.25 mL, 40.1 mmol, 20 equiv), a solution of NaH_2PO_4 (1.93 g, 16.1 mmol, 8.0 equiv) in H_2O (2.5 mL) and a solution of $NaClO_2$ (597 mg, 6.60 mmol, 3.0 equiv) in H_2O (2.5 mL) at room temperature. After stirring the mixture at room temperature for 5 h, the reaction mixture was quenched by the addition of saturated aqueous NH_4Cl. The reaction mixture was extracted with EtOAc, washed with brine, dried over Na_2SO_4, and then filtrated. The removal of solvents *in vacuo* afforded **S36** (462 mg, 95% yield) as a pale yellow solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.88 (br, 1H), 10.22 (br, 1H), 7.38 (s, 1H), 7.04 (s, 1H), 3.78 (s, 3H); ^{13}C NMR (DMSO-d_6, 125

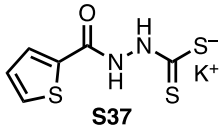
MHz) δ 166.4, 150.4, 146.7, 122.1, 120.4, 114.8, 112.6, 55.8; HRMS (ESI) *m/z* calcd for $C_8H_6BrO_4$ $[M–H]^–$: 244.9444 found 244.9453.

2-Bromo-4-hydroxy-5-methoxy-*N*-(3-(methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)benzamide (28)



To a solution of **S36** (100 mg, 0.40 mmol, 2.0 equiv) in CH_2Cl_2 (2.0 mL) were added DMF (2 drops) and $(COCl)_2$ (70 µL, 0.82 mmol, 4.0 equiv). After stirring the mixture at room temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in DMF (1.2 mL) and added a solution of **2** (43.3 mg, 0.21 mmol) in DMF (0.8 mL). After stirring the mixture at room temperature for 10 h, the reaction mixture was extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude mixture was purified by PTLC ($MeOH/CHCl_3$ = 1:20) to afford **28** (18.9 mg, 21% yield) as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.08 (br, 1H), 10.27 (br, 1H), 7.81-7.74 (m, 2H), 7.58-7.52 (m, 3H), 7.07 (s, 1H), 6.90 (s, 1H), 3.78 (s, 3H), 2.67 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 165.4, 153.9, 153.6, 150.0, 146.8, 130.5, 128.9, 127.4, 125.5, 123.9, 119.9, 112.7, 110.3, 55.9, 14.1; HRMS (ESI) *m/z* calcd for $C_{17}H_{14}BrN_4O_3S$ $[M–H]^–$: 432.9965 found 432.9981.

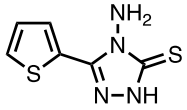
Potassium 2-(thiophene-2-carbonyl)hydrazine-1-carbodithioate (S37)



S37

Following representative procedure A, **S37** was prepared as a white solid (2.36 g, 92% yield) from thiophene-2-carbohydrazide (1.42 g, 9.99 mmol) and used without further purification.

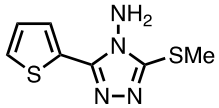
4-Amino-5-(thiophen-2-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (S38)



S38

Following representative procedure B, **S38** was prepared as a white solid (1.15 g, 64% yield) from **S37** (2.32 g, 9.03 mmol) and used without further purification.

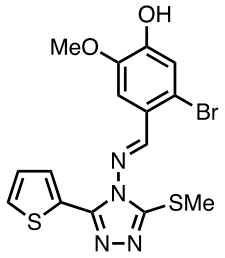
3-(Methylthio)-5-(thiophen-2-yl)-4*H*-1,2,4-triazol-4-amine (S39)



S39

Following representative procedure C, **S39** was prepared as a white solid (947 mg, 81% yield) from **S38** (1.09 g, 5.50 mmol). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.88 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.71 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.20 (dd, *J* = 4.8, 3.6 Hz, 1H), 6.19 (s, 2H), 2.59 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 154.1, 150.4, 128.3, 127.7, 127.6, 127.3, 13.9; HRMS (ESI) *m/z* calcd for $C_7H_7N_4S_2$ $[M–H]^–$: 211.0107 found 211.0116.

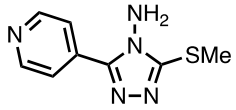
(*E*)-5-Bromo-2-methoxy-4-(((3-(methylthio)-5-(thiophen-2-yl)-4*H*-1,2,4-triazol-4-yl)imino)methyl)phenol (29)



29

Following representative procedure D, **29** was prepared as a white solid (57.5 mg, 68% yield) from **S39** (42.1 mg, 0.20 mmol). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.73 (br, 1H), 8.94 (s, 1H), 7.78 (d, J = 4.8 Hz, 1H), 7.73 (d, J = 3.0 Hz, 1H), 7.70 (s, 1H), 7.21 (dd, J = 4.8, 3.0 Hz, 1H), 7.15 (s, 1H), 3.88 (s, 3H), 2.68 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 163.6, 153.0, 148.1, 147.9, 146.5, 129.4, 128.2, 127.8, 127.1, 120.7, 119.3, 118.4, 110.1, 55.8, 15.6; HRMS (ESI) *m/z* calcd for $C_{15}H_{12}BrN_4O_2S_2$ $[M–H]^–$: 422.9580 found 422.9591.

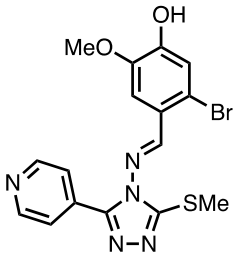
3-(Methylthio)-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-4-amine (S40)



S40

Following representative procedure C, **S40** was prepared as a white solid (40.9 mg, 40% yield) from 4-amino-5-(pyridin-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (95.6 mg, 4.95 mmol). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.72 (dd, J = 4.8, 1.8 Hz, 2H), 7.99 (dd, J = 4.8, 1.8 Hz, 2H), 6.24 (s, 2H), 2.62 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 156.0, 152.0, 150.1, 134.0, 121.3, 13.6; HRMS (ESI) *m/z* calcd for $C_8H_8N_5S$ $[M–H]^–$: 206.0495 found 206.0503.

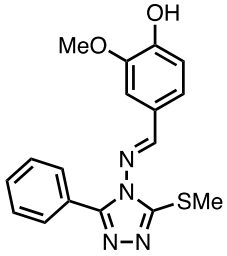
(*E*)-5-Bromo-2-methoxy-4-(((3-(methylthio)-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-4-yl)imino)methyl)phenol (30)



30

Following representative procedure D, **30** was prepared as a white solid (24.0 mg, 53% yield) from **S40** (22.5 mg, 0.11 mmol). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.80 (br, 1H), 8.86 (s, 1H), 8.73 (dd, J = 5.0, 1.5 Hz, 2H), 7.84 (dd, J = 5.0, 1.5 Hz, 2H), 7.58 (s, 1H), 7.12 (s, 1H), 3.84 (s, 3H), 2.71 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 164.8, 153.0, 150.3, 149.5, 148.7, 148.0, 133.6, 121.6, 120.6, 119.3, 118.5, 110.0, 55.8, 15.2; HRMS (ESI) *m/z* calcd for $C_{16}H_{13}BrN_5O_2S$ $[M–H]^–$: 417.9968 found 417.9977.

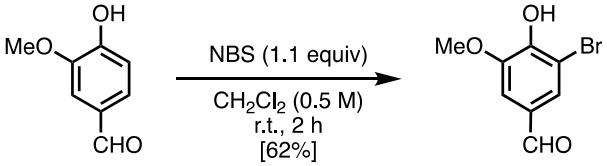
(*E*)-2-Methoxy-4-(((3-(methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)imino)methyl)phenol (31)



31

Following representative procedure D, **31** was prepared as a white solid (46.2 mg, 67%) from **2** (41.6 mg, 0.20 mmol) following purification by PTLC (MeOH/$CHCl_3$ = 1:40). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.34 (s, 1H), 7.89 (dd, *J* = 8.4, 1.8 Hz, 2H), 7.50 (d, *J* = 2.4 Hz, 1H), 7.45-7.41 (m, 3H), 7.21 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 6.13 (br, 1H), 3.99 (s, 3H), 2.77 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 165.5, 151.9, 150.8, 148.8, 147.3, 129.8, 128.6, 128.2, 126.7, 126.0, 124.0, 114.7, 108.6, 56.2, 15.2; HRMS (ESI) *m/z* calcd for $C_{17}H_{15}N_4O_2S$ [M–H]$^-$: 339.0910 found 339.0925.

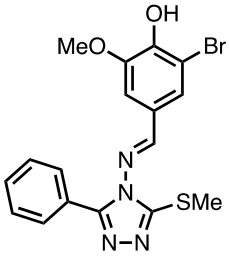
3-Bromo-4-hydroxy-5-methoxybenzaldehyde (S41)



S1 **S41**

To a solution of **S1** (456 mg, 3.00 mmol) in CH_2Cl_2 (6.0 mL) was added *N*-bromosuccinimide (NBS; 588 mg, 3.30 mmol, 1.1 equiv). After stirring the mixture at room temperature for 2 h, the reaction was quenched with saturated aqueous $Na_2S_2O_3$. The mixture was extracted with CH_2Cl_2, washed with brine, dried over Na_2SO_4, filtrated, and concentrated *in vacuo*. The residue was purified by recrystallization ($CHCl_3$) to afford **S41** (428 mg, 62% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.79 (s, 1H), 7.65 (d, *J* = 2.0 Hz, 1H), 7.37 (d, *J* = 2.0 Hz, 1H), 6.52 (s, 1H), 3.99 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 189.7, 148.8, 147.6, 130.1, 130.0, 108.1, 107.9, 56.6; HRMS (ESI) *m/z* calcd for $C_8H_6BrO_3$ [M–H]$^-$: 228.9495 found 228.9499.

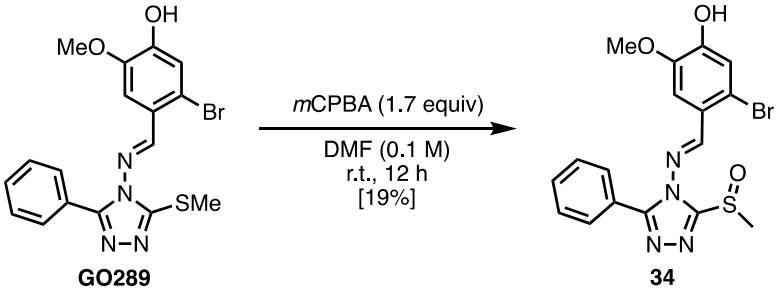
(*E*)-2-Bromo-6-methoxy-4-(((3-(methylthio)-5-phenyl-4*H*-1,2,4-triazol-4-yl)imino)methyl)phenol (32)



32

Following representative procedure D, **32** was prepared as a white solid (37.5 mg, 44%) from **2** (41.8 mg, 0.20 mmol). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.62 (br, 1H), 8.65 (s, 1H), 7.78 (dd, *J* = 7.8, 1.2 Hz, 2H), 7.60 (d, *J* = 1.8 Hz, 1H), 7.48-7.40 (m, 4H), 3.85 (s, 3H), 2.62 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 167.5, 151.0, 148.9, 148.7, 148.0, 129.8, 128.8, 127.7, 127.4, 126.4, 123.4, 109.6, 109.4, 56.4, 15.0; HRMS (ESI) *m/z* calcd for $C_{17}H_{14}BrN_4O_2S$ [M–H]$^-$: 417.0015 found 417.0027.

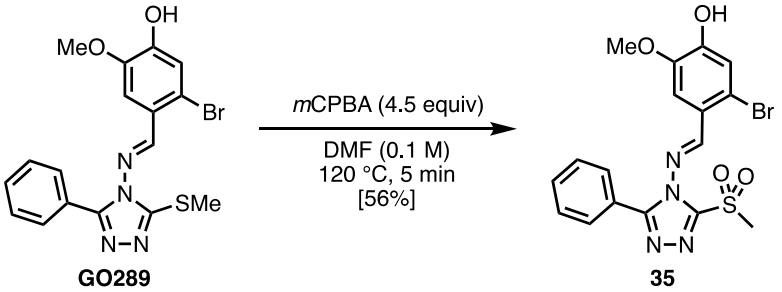
(*E*)-5-Bromo-2-methoxy-4-(((3-(methylsulfinyl)-5-phenyl-4*H*-1,2,4-triazol-4-yl)imino)methyl)phenol (34)



GO289 **34**

To a solution of **GO289** (69.6 mg, 0.17 mmol) in DMF (2.5 mL) was slowly added *m*CPBA (containing ca. 60% water, 62.9 mg, 0.27 mmol, 1.7 equiv) at 0 °C. After stirring for 12 h at room temperature, the reaction was quenched by the addition of saturated aqueous $NaHCO_3$. The reaction mixture was extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtrated, and concentrated *in vacuo*. The crude mixture was purified by PTLC (MeOH/$CHCl_3$ = 1:40) to afford **34** (13.4 mg, 19% yield) as a white solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.82 (br, 1H), 9.01 (s, 1H), 7.89 (dd, *J* = 7.8, 1.8 Hz, 2H), 7.58 (s, 1H), 7.57-7.53 (m, 3H), 7.13 (s, 1H), 3.83 (s, 3H), 3.26 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 167.5, 153.2, 152.8, 151.8, 148.0, 130.5, 128.9, 128.5, 125.6, 120.7, 119.4, 118.7, 110.3, 55.8, 37.3; HRMS (ESI) *m/z* calcd for $C_{17}H_{14}BrN_4O_3S$ $[M–H]^–$: 432.9965 found 432.9982.

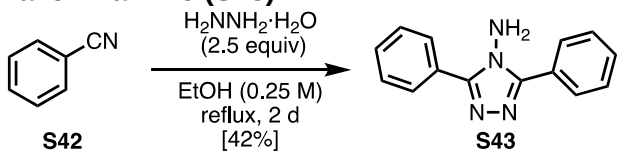
(*E*)-5-Bromo-2-methoxy-4-(((3-(methylsulfonyl)-5-phenyl-4*H*-1,2,4-triazol-4-yl)imino)methyl)phenol (35)



GO289 **35**

To a solution of **GO289** (70.3 mg, 0.17 mmol) in DMF (1.7 mL) was slowly added *m*CPBA (containing ca. 60% water, 173.5 mg, 0.75 mmol, 4.5 equiv) at 0 °C. After stirring for 5 min at 120 °C, the reaction was quenched by the addition of saturated aqueous $NaHCO_3$. The reaction mixture was extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtrated, and concentrated *in vacuo*. The crude mixture was purified by PTLC (MeOH/$CHCl_3$ = 1:10) to afford **35** (42.3 mg, 56% yield) as a pale yellow solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.84 (s, 1H), 8.97 (s, 1H), 7.88-7.84 (m, 2H), 7.59-7.53 (m, 4H), 7.11 (s, 1H), 3.82 (s, 3H), 3.60 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 169.5, 153.4, 151.9, 149.2, 148.0, 130.8, 128.9, 128.8, 125.2, 120.4, 119.4, 118.9, 110.4, 55.8, 43.5; HRMS (ESI) *m/z* calcd for $C_{17}H_{14}BrN_4O_4S$ $[M–H]^–$: 448.9914 found 448.9921.

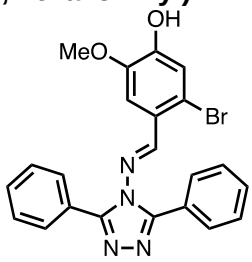
3,5-Diphenyl-4*H*-1,2,4-triazol-4-amine (S43)



S42 **S43**

To a solution of benzonitrile (**S42**: 1.0 mL, 9.70 mmol) in EtOH (0.6 mL) was slowly added hydrazine hydrate (1.25 mL, 25.5 mmol, 2.5 equiv) at 0 °C. After stirring for 2 days at 120 °C, the reaction mixture was filtered and concentrated *in vacuo* to afford **S43** (991 mg, 42% yield) as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.03 (dd, *J* = 8.0, 2.0 Hz, 4H), 7.60-7.49 (m, 6H), 6.28 (s, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 154.2, 129.6, 128.5, 128.3, 127.2; HRMS (ESI) *m/z* calcd for $C_{14}H_{11}N_4$ $[M–H]^–$: 235.0978 found 235.0989.

(*E*)-5-Bromo-4-(((3,5-diphenyl-4*H*-1,2,4-triazol-4-yl)imino)methyl)-2-methoxyphenol (36)



36

Following representative procedure D, **36** was prepared as a white solid (51.2 mg, 55%) from **S43** (49.0 mg, 0.21 mmol). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.68 (br, 1H), 8.49 (s, 1H), 7.82 (dd, J = 8.4, 1.8 Hz, 4H), 7.56 (s, 1H), 7.56-7.48 (m, 6H), 7.00 (s, 1H), 3.83 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 166.4, 152.7, 150.1, 147.9, 129.8, 128.8, 128.5, 126.6, 120.8, 119.1, 117.9, 110.1, 55.7; HRMS (ESI) *m/z* calcd for $C_{22}H_{16}BrN_4O_2$ $[M–H]^-$: 447.0451 found 447.0468.