

Identification of new FLT3 inhibitors that potently inhibit AML cell lines, via an azo click-it/staple-it approach

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Experimental section

Chemistry. Quizartinib (>99.5% purity) and crenolanib (>99.5% purity) were purchased from Chemietek.com. Unless otherwise noted, all materials were obtained from commercial suppliers and used as obtained. Solvents were purchased from Sigma and used directly without purification. NMR spectra were recorded on a Bruker 400, 500 or 600 MHz spectrometer at ambient temperature. Chemical shifts are reported in parts per million (δ) and are calibrated using residual undeuterated solvent as an internal reference. Data for ¹H NMR spectra are reported as follows: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, or combinations thereof.

Procedures for the synthesis

General procedure I for compounds **HSW971**, **HSW972** and **HSW310-4**.

Aromatic amine (1.0 mmol) was added to a stirred solution of 12 N HCl (0.23 mL) and water (0.67 mL) in a 10 mL flask at 0 °C for 15 min. The resultant stirring mixture was treated dropwise with a cooled NaNO₂ solution (69 mg in 1.33 mL water) for 15 min. Then a cooled aromatic amine solution (1.0 mmol in 2.93 mL acetonitrile) was added dropwise to the above solution for stirring another 10 hour at 0 °C. The resultant precipitate was filtered by a Buchner funnel and washed with a mixture of water and acetonitrile from the ratio 3:1 to 6:1 until the filtrate became clear. Then the solid was collected and dried at room temperature.

General procedure (II) for the rest of compounds in Figure **S1** (except **4ABM** and **HS001**).

Aminobenzamidinium dihydrochloride (212 mg, 1.0 mmol) was added to a stirred solution of 12 N HCl (0.27 mL) and water (1.5 mL) in a 10 mL flask at 0 °C for 15 min. The resultant stirring mixture was treated dropwise with a cooled NaNO₂ solution (76 mg in 0.27 mL water) for 15 min followed by the addition of a cooled NaOAc solution (328 mg in 1.5 mL water) to adjust the pH = 6.0 for 15 min. Then a cooled aromatic amine solution (1.0 mmol in 1.0 mL methanol) was added dropwise to the above solution for stirring another 1 hour at 0 °C. After the reaction was finished, the solvent was removed

under reduced pressure. The residue was treated with a large amount of water (100 mL) and followed by the extract of dichloromethane (2 x 15 mL). The aqueous layer was treated with 2.5% NaOH solution to make the pH > 10.0 followed by the extraction of large amount of ethyl acetate (2 x 100 mL). Then, the organic layer was washed with brine and dried by sodium sulfate. Finally, the solvent was removed under reduced pressure and the product was obtained.

Synthesis procedure (III) for **HS001**.

HSW630-1 (15 mg 0.04 mmol) was added to a solution of sodium dithionite (0.5 g) in water (10 ml) and the pH was adjusted to 10 using NaOH solution. The mixture was stirred at 65 °C until the solid disappeared, and then for a further 15 min. The solution was cooled, and organic portion was extracted by ethyl acetate (3×15 ml). The combined organic layer was dried over Na₂SO₄ and filtered. The crude product obtained after evaporation of solvent was purified by column chromatography on silica gel by 1:1 ethyl acetate hexane mixture. Orange powder pure product was obtained after removing the solvent in reduced pressure.

General procedure (IV) for compounds with alkyne as stable linker in **Table 1**

6-chloroisoquinolin-3-amine (3.2 mmol) was iodinated by N-Iodosuccinimide (675 mg) and purified by flash column (part 1). 4-ethynylbenzaldehyde (1 eq) was dissolved in tert-butyl alcohol and the corresponding diamine (1 eq) was added and stirred for 1 hour at room temperature. 1.1 eq of iodine and 3 eq of potassium carbonate were added and the mixture was reflux overnight. Crude product was subject to column purification (part 2). Pure part 1 (50 mg), 10 mol% Bis(triphenylphosphine)palladium(II) dichloride, 10 mol% triphenylphosphine were added, purged with nitrogen three times before anhydrous DMF (5 mL) was added. The reaction mixture was stirred at 45 °C for another 5 minutes before the corresponding pure part 2 (1.1 eq) was added via syringe in 1ml anhydrous DMF in 2 minutes. DIPEA (2 mL) was subsequently added and stirred overnight. Crude product was subject to column purification.

Synthesis procedure (V) for **HSM1859**.

Following the same procedure above, 6-chloroisoquinolin-3-amine (3.2 mmol) was iodinated by N-Iodosuccinimide (675 mg) and purified by flash column. To a solution of iodo compound (1 equiv), Pd(PPh₃)₄ (20 mol%) and cesium carbonate (1 equiv) in 1,4-dioxane/water (1 mL) were de-oxygenated using steam of Argon gas. A de-oxygenated solution of 4-vinylbenzonitrile (0.49 mmol, 1.5 equiv) in DMF (3 mL) was added slowly over a period of 15 min to the solution and the reaction temperature was increased to 70 °C and allowed to stir 12 h. The reaction was quenched by addition of water (5 mL) at room temperature and subject to flash column purification. After purification, it was dissolved in 2 mL absolute ethanol and 1 mL of hydroxylamine solution was added and reflux for 6 hours. After TLC showed complete consumption of starting material, ethanol was removed in vacuo and pure product was obtained by flash column chromatography.

Biology

***In vitro* kinase assays**

The Reaction Biology Corporation (www.reactionbiology.com, Malvern, PA) HotSpot assay platform was used to measure kinase/inhibitor interactions exactly as previously described. Kinase and substrate were mixed in a buffer containing 20 mM HEPES pH 7.5, 10 mM MgCl₂, 1 mM EGTA, 0.02% Brij35, 0.02 mg/mL BSA, 0.1 mM Na₃VO₄, 2 mM DTT and 1% DMSO. Single-dose of compounds (500 nM) were then added to each reaction mixture. After 20-minute incubation, ATP (Sigma) and [γ -³³P] ATP (Perkin Elmer) were added at a final total concentration of 100 μ M for addition 2 hours at room temperature, followed by spotting onto P81 ion exchange cellulose chromatography paper (Whatman, Inc.). Filter paper was washed in 0.75% phosphoric acid to remove unincorporated ATP. Percent remaining kinase activity of a vehicle (DMSO) containing kinase reaction was calculated for each kinase/inhibitor pair using Prism 5 (GraphPad).

Docking method for FLT3.

The docking package CANDOCK¹ was used to identify docking poses of HSW630-1 in the binding site of FLT3 kinases. The structure of FLT3 was obtained from the protein databank (PDB# 4XUF). The entire protein was considered flexible. No binding site was provided to CANDOCK and the location of this site was determined by the program automatically. CANDOCK used default parameters with interactive flexibility with a maximum iteration of 10 and only the top 2% of the top seed values were used to generate full ligands.

Cell lines and culturing

Molm-14 cells were the kind gift of Dr. Mark Levis from Johns Hopkins University. THP-1, MV4-11, K-562 and MRC-5A cells were purchased from ATCC (ATCC, Manassas, VA). All cell lines were grown in 37°C with 5% CO₂ atmosphere with RPMI 1640 (Life technologies, Carlsbad, CA) supplemented with heat-inactivated 10% (V/V) fetal bovine serum. Cell lines were grown and maintained according to ATCC recommendations.

IC₅₀ proliferation assay

Cell lines were seeded into 96-well plates the afternoon prior to treatment. Approximately 18 hours later, compounds were semi-serially diluted in dimethyl sulfoxide (DMSO) and then growth medium, and added to cells. Plates were incubated for 72 hours prior to addition of Alamar Blue (Life Technologies, Carlsbad, CA). Plates were read after 4 additional hours of incubation at 37°C using a Bio-Tek Synergy HT plate reader (Bio-Tek, Winooski, VT). Data was analyzed and graphed using GraphPad Prism Software (Graphpad, La Jolla, CA).

Western blotting

Effect of HSW630-1 on protein expression was tested in the MV4-11 leukemia cell line. Total protein extracts were prepared using RIPA buffer (SIGMA) supplemented with Complete Mini™ protease inhibitor and PHOStop™ phosphatase inhibitors (Roche). Equal amounts of proteins (up to 25 μ g) were separated on 4-12% NuPAGE gels in 1X MOPS or 1x MES buffer (Invitrogen) and transferred onto PVDF membranes (Millipore). The membrane was blocked with 5% dry milk in 1X TBS/0.1% Tween 20 (TBST) for at least 1 h at room temperature and incubated with human specific primary

antibodies: FLT3, Phospho-FLT3, c-Myc (Cell Signaling Technologies), KRAS (Santa Cruz), or mouse anti- β -actin (SIGMA) overnight at 4°C. The membrane was washed 3 times in TBST and incubated with a horseradish peroxidase-conjugated secondary anti-rabbit or anti-mouse antibody (Cell Signaling Technologies) for 1 h at room temperature. Blots were again washed and the signal was detected with SuperSignal West Femto Chemiluminescent Substrate (Pierce) and exposed to HyBlot CL® autoradiography film (Denville).

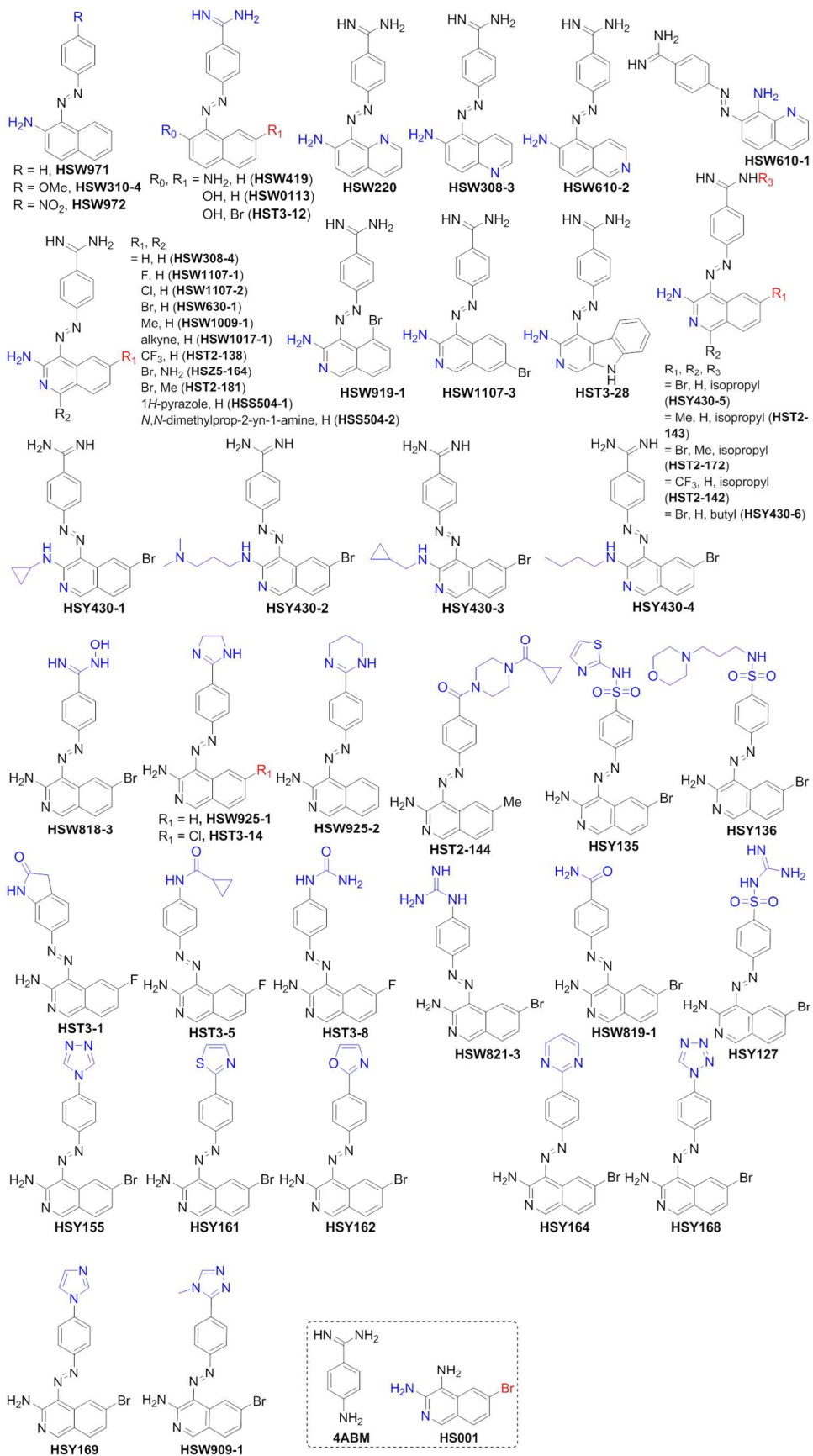
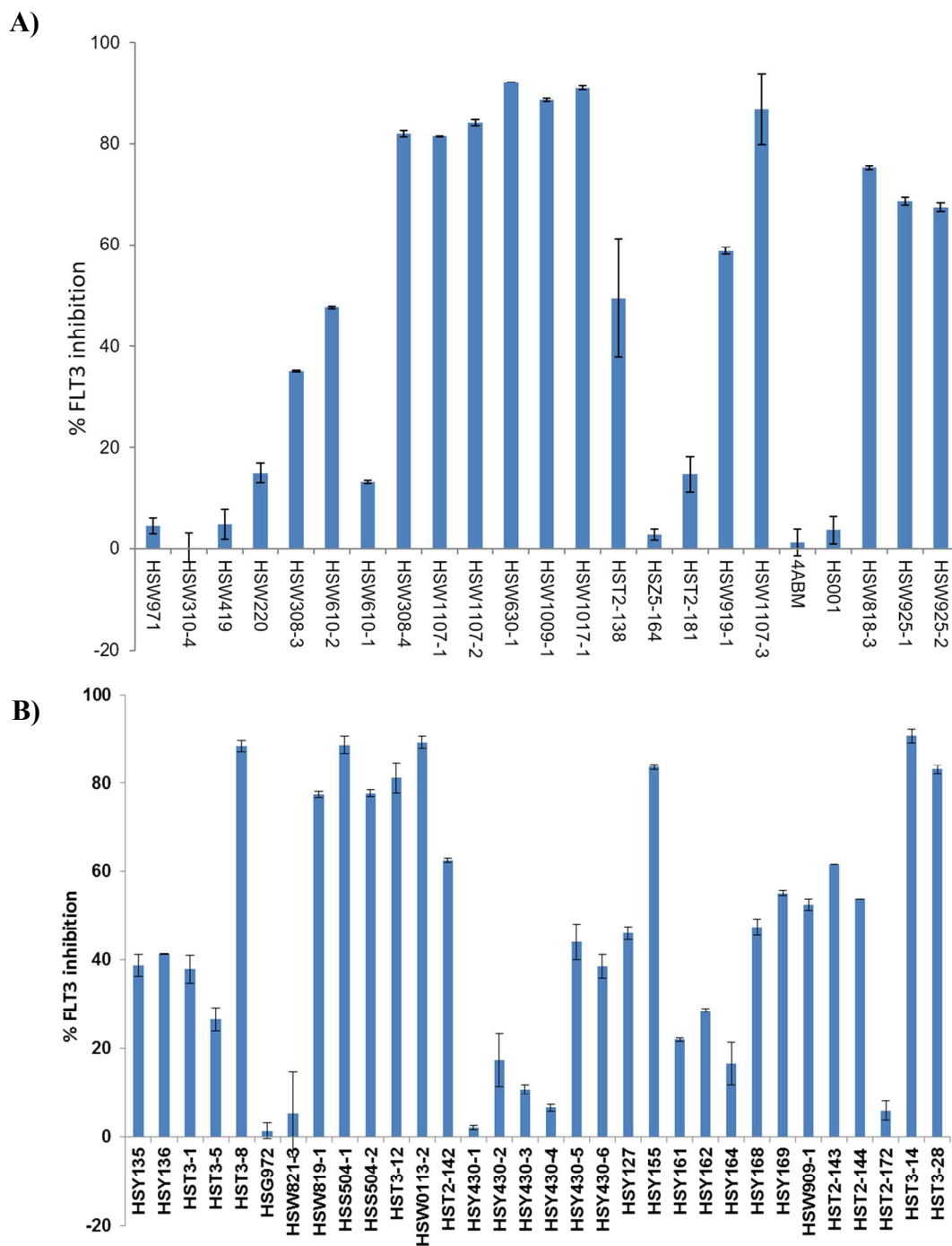


Figure S1. Azo compounds designed, synthesized and screened in this paper. The Azo Click-it-staple-it approach facilitated the rapid synthesis of diverse amino isoquinoline/quinoline compounds, which were then tested for FLT3 inhibition (see Figure S2).



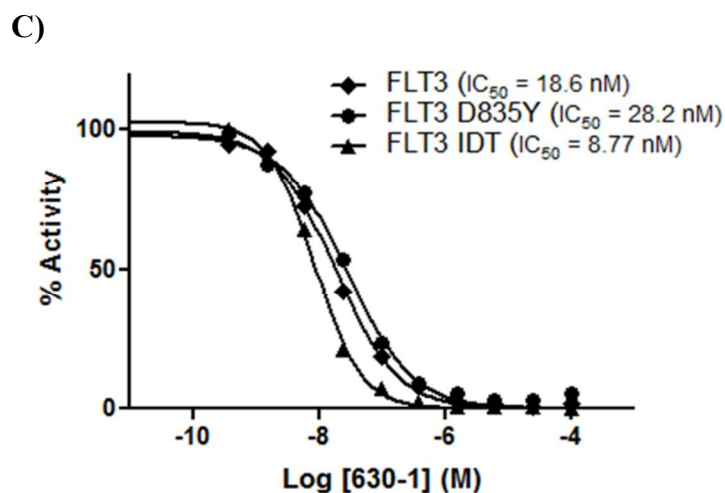


Figure S2. A and B) % inhibition of FLT3 activities by azo compounds at concentration of 500 nM, in the presence of 100 μM radiolabeled ATP. C) IC_{50} values of HSW630-1 against FLT3 and important FLT3 mutants (FLT3 D835Y and FLT3 ITD).

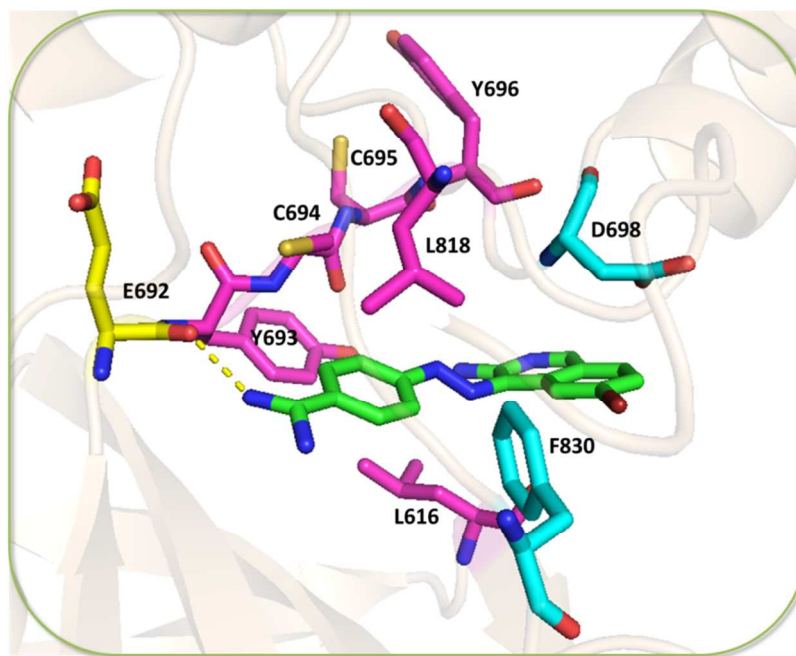


Figure S3. Docking of HSW630-1 against FLT3 active site. HSW630-1 is shown in sticks and colored by atom (bromine in red, nitrogen in blue, carbon in green). FLT3 is shown as ribbon. FLT3 used was taken from PDB# 4XUF. Residues in pink (Y693, C694, C695, L818 and L616) indicate FLT3's hydrophobic pocket; residue in yellow (E692) indicates Hydrogen bonding; Residues in blue (D698 and F830) represent potential C-H or N-H pi-pi interaction.

Table S1. Anti-proliferative activities of azo analogs of HSW630-1 against different leukemia cancer cell lines.

Entry	Compound Name	Anti-proliferative activities against MV4-11 (μM)
1	HSW971	> 5
2	HSW310-4	> 5
3	HSW419	> 5
4	HSW220	> 5
5	HSW308-3	> 5
6	HSW610-2	> 5
7	HSW610-1	> 5
8	HSW308-4	0.9 \pm 0.6
9	HSW1107-1	0.3 \pm 0.1
10	HSW1107-2	0.06 \pm 0.04
11	HSW1009-1	0.3 \pm 0.2
12	HSW1017-1	0.5 \pm 0.4
13	HST2-138	0.2 \pm 0.05
14	HSZ5-164	> 5
15	HST2-181	2.3 \pm 0.1
16	HSW919-1	> 5
17	HSW1107-3	0.4 \pm 1.1
18	4ABM	> 5
19	HS001	> 5
20	HSW818-3	0.17 \pm 0.00
21	HSW925-1	0.24 \pm 0.07
22	HSW925-2	NA
23	HSY135	> 5
24	HSY136	0.5 \pm 0.5
25	HST3-1	> 5
26	HST3-5	0.2 \pm 0.1
27	HST3-8	0.2 \pm 0.3
28	HSW972	> 5
29	HSW821-3	0.9 \pm 0.6
30	HSW819-1	0.1 \pm 0.2
31	HSS504-1	0.3 \pm 0.3
32	HSS504-2	0.2 \pm 0.06
33	HST3-12	> 5
34	HSW0113-2	0.9 \pm 0.2
35	HST2-142	NA
36	HSY430-1	> 5
37	HSY430-2	> 5

Entry	Compound Name	Anti-proliferative activities against MV4-11 (μM)
38	HSY430-3	2.5 \pm 0.4
39	HSY430-4	> 5
40	HSY430-5	0.3 \pm 0.1
41	HSY430-6	0.3 \pm 0.1
42	HSY127	NA
43	HSY155	NA
44	HSY161	> 5
45	HSY162	> 5
46	HSY164	> 5
47	HSY168	NA
48	HSY169	NA
49	HSW909-1	0.5 \pm 0.3
50	HST2-143	0.2 \pm 0.1
51	HST2-144	0.2 \pm 0.1
52	HST2-172	1.9 \pm 1.1
53	HST3-14	0.07 \pm 0.06
54	HST3-28	> 5

NA = not effective

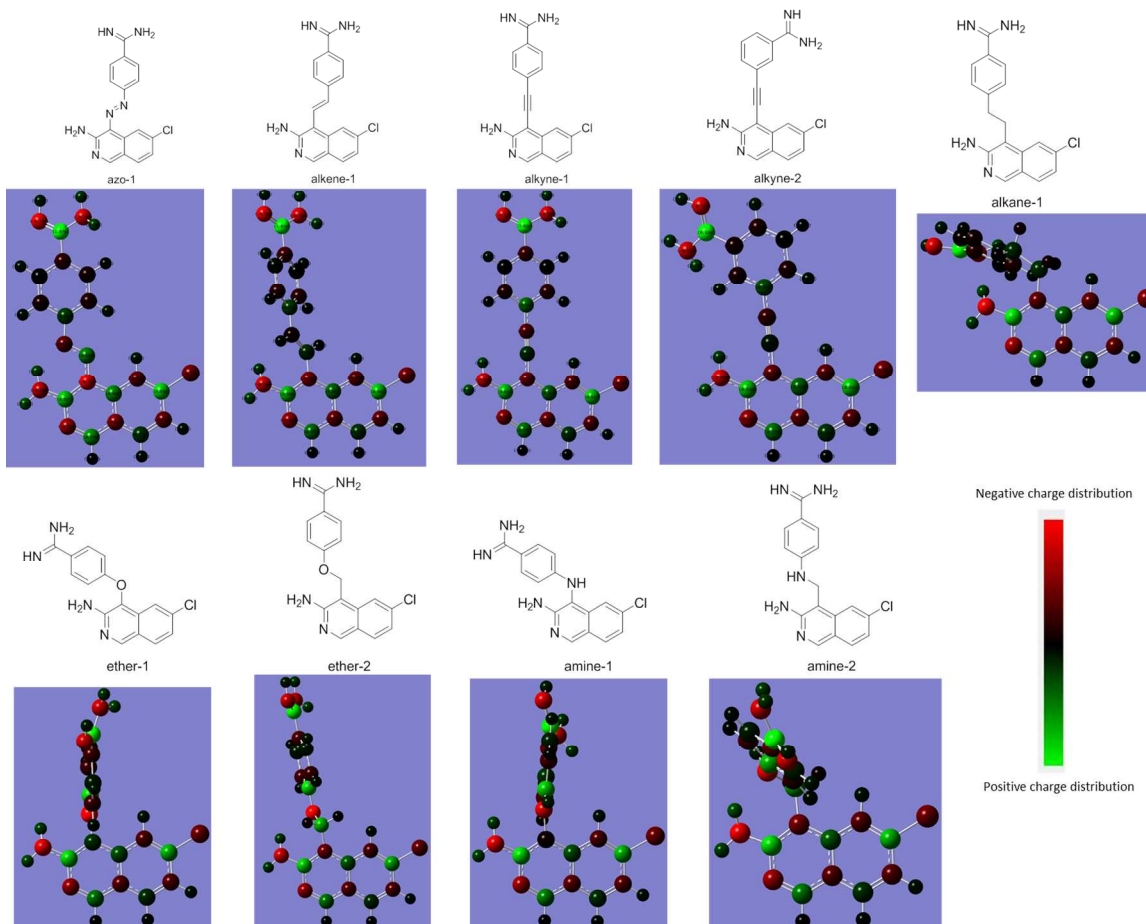


Figure S4: Computational modeling for identifying stable linkers mimicking azo group, using Gaussian09 at B3LYP/6-31+G(d) level of theory. In the azo “hit” compound, the benzimidazole and isoquinoline moieties are co-planar. Only the alkyne analogs could mimic this coplanarity arrangement.

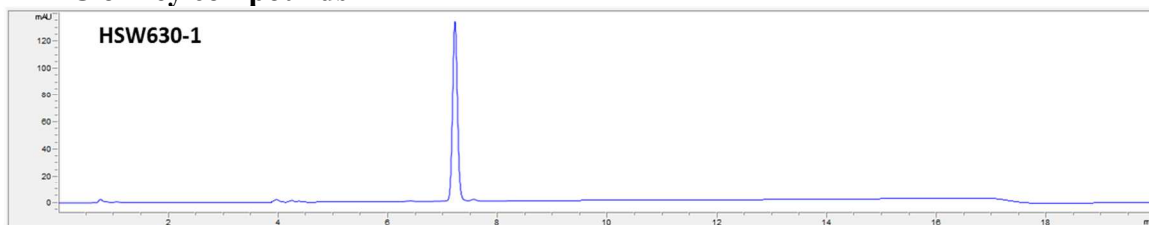
Table S2. % inhibition of TrkC/c-Kit activities by FLT3 inhibitors in clinical trials at concentration of 500 nM, in the presence of 100 μ M radiolabeled ATP.

Entry	Compound name	% inhibition of TrkC at 500 nM	% inhibition of c-Kit at 500 nM
1	Quizartinib	44%	88%
2	Crenolanib	99%	24%

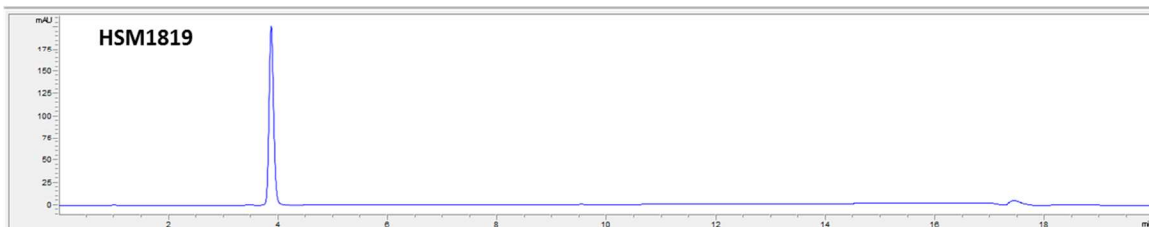
Table S3. % inhibition of TrkC/c-Kit activities by compounds with stable linkers at concentration of 500 nM, in the presence of 100 μ M radiolabeled ATP.

Entry	Compound name	% inhibition of TrkC at 500 nM	% inhibition of c-Kit at 500 nM
1	HSW630-1	99%	68%
2	HSM1651	64%	83%
3	HSM1703	49%	60%
4	HSM1721	52%	80%
5	HSM1743	44%	78%
7	HSM1611	16%	63%
8	HSM1796	7%	3%
9	HSM1798	11%	15%
10	HSM1813	17%	39%
11	HSM1820	74%	86%
12	HSM1819	80%	82%
13	HSM1859	42%	62%

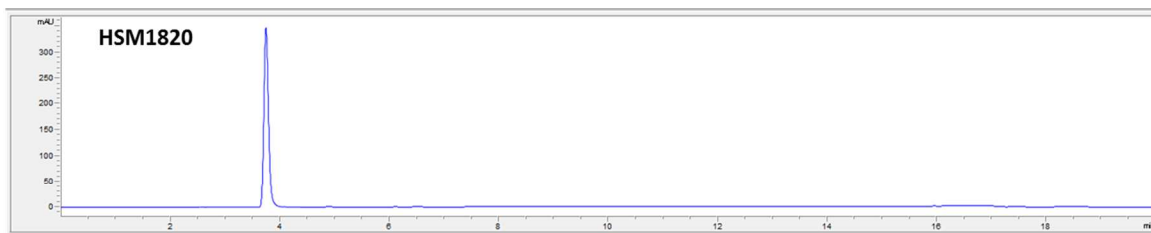
HPLC of key compounds



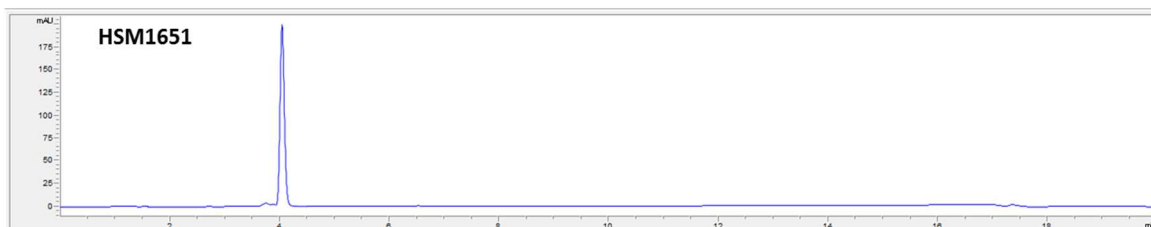
Condition: Agilent Eclipse plus C18 column, 3.5 μ m, 4.6x100 mm, 0 \rightarrow 15 min, 50% B \rightarrow 100% B (A: 0.1% NH_4OH in H_2O , B: MeOH), 55 $^\circ\text{C}$.



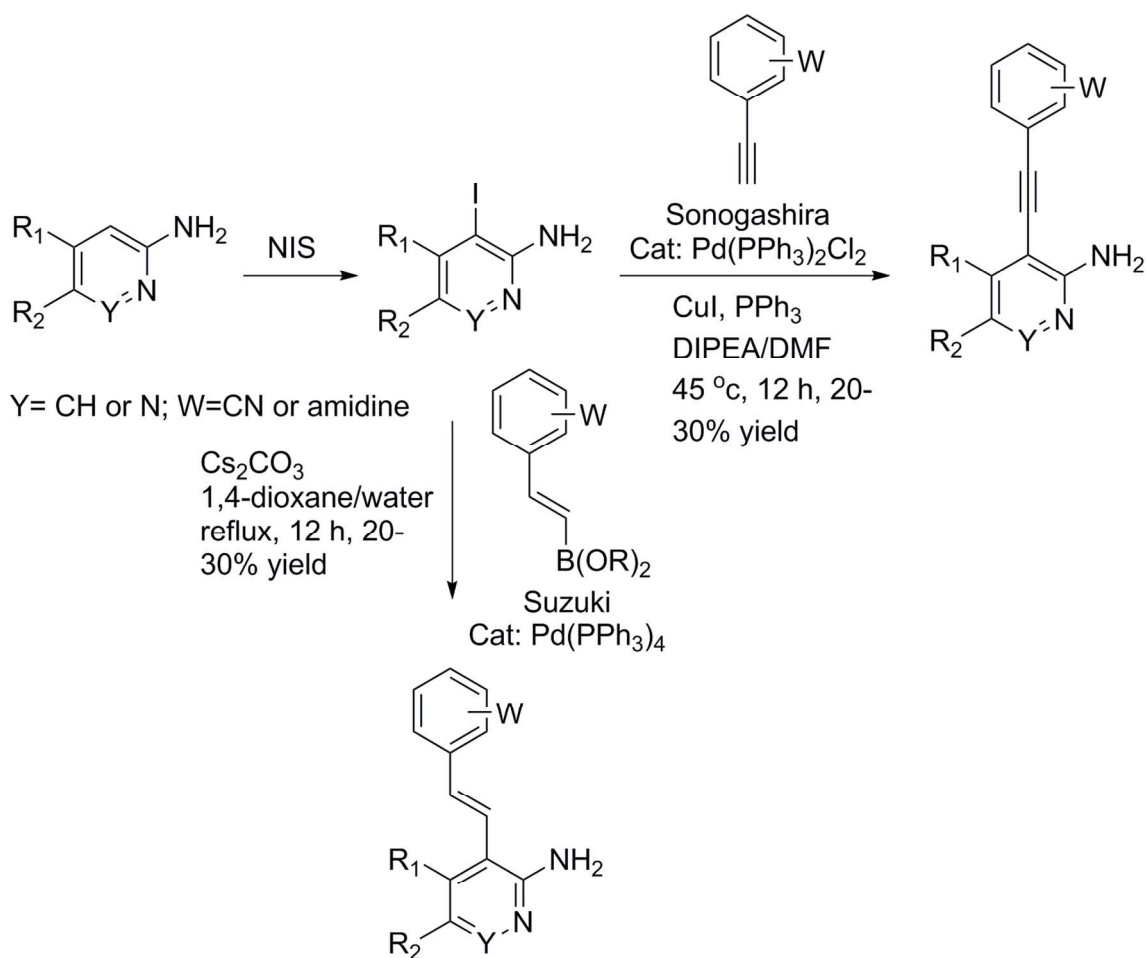
Condition: Agilent Eclipse plus C18 column, 3.5 μ m, 4.6x100 mm, 0 \rightarrow 15 min, 50% B \rightarrow 100% B (A: 0.1% TFA in H_2O , B: MeOH), 55 $^\circ\text{C}$.



Condition: Agilent Eclipse plus C18 column, 3.5 μm , 4.6x100 mm, 0 \rightarrow 15 min, 50% B \rightarrow 100% B (A: 0.1% TFA in H₂O, B: MeOH), 55 $^{\circ}\text{C}$.

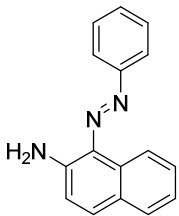


Condition: Agilent Eclipse plus C18 column, 3.5 μm , 4.6x100 mm, 0 \rightarrow 15 min, 50% B \rightarrow 100% B (A: 0.1% TFA in H₂O, B: MeOH), 55 $^{\circ}\text{C}$.



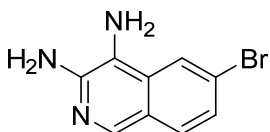
Scheme S1. Synthesis of alkyne and alkene analogs of HSW630-1. NIS (N-iodosuccinimide); DIPEA (diisopropylethylamine); DMF (dimethylformamide).

Characterization



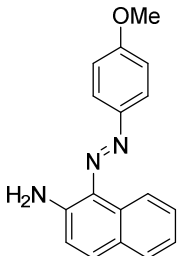
HSW971

(E)-1-(phenyldiazenyl)naphthalen-2-amine. Following the described general procedure I, an orange solid was obtained. ^1H NMR (400 MHz, MeOD) δ 8.80 (d, $J = 8.5$ Hz, 1H), 7.92 – 7.83 (m, 2H), 7.64 (d, $J = 8.8$ Hz, 2H), 7.48 (t, $J = 7.7$ Hz, 3H), 7.35 (t, $J = 7.3$ Hz, 1H), 7.29 – 7.22 (m, 1H), 7.01 (d, $J = 9.0$ Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 155.41, 141.15, 136.41, 134.84, 130.28, 129.86, 129.15, 128.64, 128.56, 127.53, 123.99, 122.76, 122.71, 120.84. HRMS (ESI⁺) [M+H] calcd for C₁₆H₁₄N₃ 248.1188, found 248.1189.



HS001

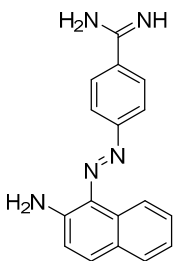
(E)-4-((3-amino-6-bromoisoquinolin-4-yl)diazenyl)benzimidamide. Following the described, procedure III, brownish solid was obtained. ^1H NMR (600 MHz, CDCl₃) δ 8.46 (s, 1H), 7.83 (s, 1H), 7.66 (d, $J = 8.7$ Hz, 1H), 7.35 (d, $J = 8.7$ Hz, 1H), 4.29 (s, 2H), 3.63 (s, 2H). ^{13}C NMR (151 MHz, CDCl₃) δ 143.56, 141.57, 129.93, 128.54, 126.60, 124.12, 123.19, 121.26, 118.86. LRMS (ESI⁺) [M+H] calcd for C₉H₉BrN₃ 237.9980, found 237.9852.



HSW310-4

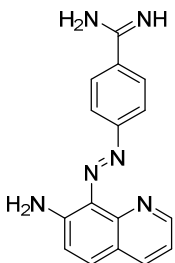
(E)-1-((4-methoxyphenyl)diazenyl)naphthalen-2-amine. Following the described general procedure I, orange solid was obtained. ^1H NMR (400 MHz, MeOD) δ 8.83 – 8.74 (m, 1H), 7.90 – 7.82 (m, 2H), 7.64 (t, $J = 8.7$ Hz, 2H), 7.47 (ddd, $J = 8.4$ Hz, 6.9 Hz, 1.3 Hz, 1H), 7.26 (ddd, $J = 8.1$ Hz, 6.9 Hz, 1.2 Hz, 1H), 7.07 – 6.99 (m, 3H), 3.86 (s, 3H). ^{13}C NMR (100 MHz, MeOD) δ 162.17, 149.60, 140.57, 136.25, 133.98, 129.09,

128.73, 128.33, 127.48, 124.22, 123.84, 122.81, 120.91, 115.49, 56.14. HRMS (ESI⁺) [M+H] calcd for C₁₇H₁₆N₃O 278.1293, found 278.1278.



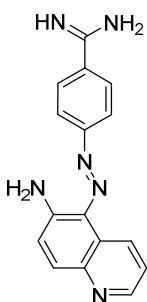
HSW419

(E)-4-((2-aminonaphthalen-1-yl)diazenyl)benzimidamide. Following the described general procedure II, brown solid was obtained. ¹H NMR (400 MHz, MeOD) δ 8.77 (d, *J* = 8.4 Hz, 1H), 7.94 (d, *J* = 8.5 Hz, 2H), 7.86 (d, *J* = 8.5 Hz, 2H), 7.66 (t, *J* = 7.9 Hz, 2H), 7.49 (t, *J* = 7.7 Hz, 1H), 7.28 (t, *J* = 7.4 Hz, 1H), 7.02 (d, *J* = 9.1 Hz, 1H). ¹³C NMR (100 MHz, MeOD) δ 167.57, 157.24, 141.90, 136.38, 135.78, 135.16, 129.28, 129.19, 128.83, 128.67, 127.88, 124.28, 122.74, 122.64, 120.81. HRMS (ESI⁺) [M+H] calcd for C₁₇H₁₆N₅ 290.1406, found 290.1411.



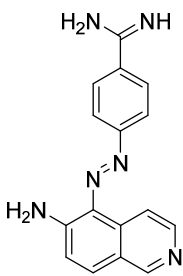
HSW220

(E)-4-((7-aminoquinolin-8-yl)diazenyl)benzimidamide. Following the described general procedure II, red solid was obtained. ¹H NMR (400 MHz, DMSO-d₆) δ 9.26 (d, *J* = 8.5 Hz, 1H), 8.85 (d, *J* = 3.0 Hz, 1H), 8.01 (d, *J* = 8.7 Hz, 1H), 7.94 (q, *J* = 8.5 Hz, 4H), 7.70 (dd, *J* = 8.5 Hz, 4.1, 1H), 7.21 (s, 2H), 6.98 (d, *J* = 8.7 Hz, 1H), 6.83 (s, 2H). ¹³C NMR (100 MHz, DMSO-d₆) δ 162.10, 153.89, 150.74, 147.69, 136.10, 135.87, 135.49, 131.48, 128.13, 127.78, 123.35, 121.56, 116.45, 108.25. HRMS (ESI⁺) [M+H] calcd for C₁₆H₁₅N₆ 291.1358, found 291.1336.



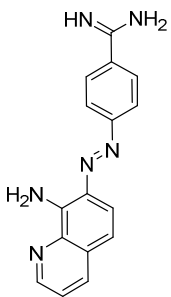
HSW308-3

(E)-4-((6-aminoquinolin-5-yl)diazenyl)benzimidamide. Following the described general procedure II, red solid was obtained. ^1H NMR (400 MHz, MeOD) δ 9.14 (ddd, $J = 8.5$ Hz, 1.5, 0.7, 1H), 8.56 (dd, $J = 4.4$ Hz, 1.6, 1H), 7.98 – 7.91 (m, 2H), 7.91 – 7.84 (m, 2H), 7.78 (dd, $J = 9.4$ Hz, 0.6, 1H), 7.50 (dd, $J = 8.5$ Hz, 4.4 Hz, 1H), 7.31 (d, $J = 9.4$ Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 167.44, 156.75, 147.21, 143.38, 141.86, 136.03, 134.64, 132.09, 131.99, 129.26, 126.19, 125.17, 123.79, 122.93. HRMS (ESI⁺) [M+H] calcd for C₁₆H₁₅N₆ 291.1358, found 291.1355.



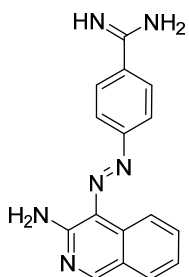
HSW610-2

(E)-4-((6-aminoisoquinolin-5-yl)diazenyl)benzimidamide. Following the described general procedure II, brown solid was obtained. ^1H NMR (400 MHz, MeOD) δ 8.87 (s, 1H), 8.58 (d, $J = 6.0$ Hz, 1H), 8.38 (d, $J = 6.0$ Hz, 1H), 8.07 – 8.01 (m, 2H), 7.96 – 7.90 (m, 2H), 7.86 (d, $J = 9.1$ Hz, 1H), 7.19 (d, $J = 9.1$ Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 167.77, 157.58, 151.63, 145.44, 145.25, 141.08, 134.50, 129.85, 125.82, 124.18, 123.23, 122.91, 116.53. HRMS (ESI⁺) [M+H] calcd for C₁₆H₁₅N₆ 291.1358, found 291.1358.



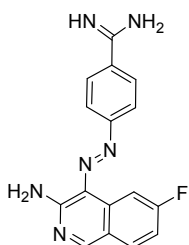
HSW610-1

(E)-4-((8-aminoquinolin-7-yl)diazenyl)benzimidamide. Following the described general procedure II, brown solid was obtained. ^1H NMR (400 MHz, MeOD) δ 9.24 (dd, $J = 8.6$ Hz, 1.6, 1H), 8.78 (dd, $J = 4.1$ Hz, 1.6 Hz, 1H), 8.03 (d, $J = 8.7$ Hz, 1H), 8.00 – 7.94 (m, 2H), 7.92 – 7.86 (m, 2H), 7.59 (dd, $J = 8.6$ Hz, 4.1 Hz, 1H), 6.96 (d, $J = 8.7$ Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 167.75, 157.55, 152.33, 149.02, 138.31, 137.89, 133.06, 132.85, 130.14, 129.67, 124.26, 123.61, 123.41, 118.11, 109.81. HRMS (ESI⁺) [M+H] calcd for C₁₆H₁₅N₆ 291.1358, found 291.1347.



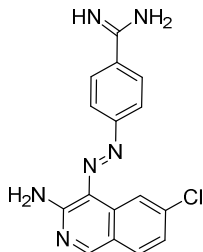
HSW308-4

(E)-4-((3-aminoisoquinolin-4-yl)diazenyl)benzimidamide. Following the described general procedure II, brown solid was obtained. ^1H NMR (400 MHz, MeOD) δ 8.86 (s, 1H), 8.68 (dd, $J = 8.5$ Hz, 0.6, 1H), 7.97 – 7.90 (m, 2H), 7.89 – 7.82 (m, 3H), 7.69 (ddd, $J = 8.4$ Hz, 6.9 Hz, 1.3 Hz, 1H), 7.36 (ddd, $J = 8.0$ Hz, 6.9 Hz, 1.0 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 167.34, 158.63, 156.28, 147.86, 139.27, 137.27, 133.48, 131.96, 129.72, 129.08, 125.11, 125.04, 123.05, 121.89, 121.85. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{16}\text{H}_{15}\text{N}_6$ 291.1358, found 291.1339.



HSW1107-1

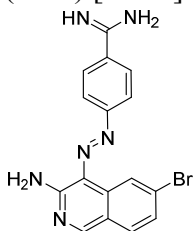
(E)-4-((3-amino-6-fluoroisoquinolin-4-yl)diazenyl)benzimidamide. Following the described general procedure II, brown solid was obtained. ^1H NMR (500 MHz, MeOD) δ 8.90 (s, 1H), 8.30 (dd, $J = 11.4$ Hz, 2.1 Hz, 1H), 8.05 – 7.96 (m, 3H), 7.95 – 7.88 (m, 2H), 7.17 (td, $J = 8.7$ Hz, 2.4 Hz, 1H). ^{13}C NMR (125 MHz, MeOD) δ 167.57, 165.56, 158.50, 156.84, 148.77, 141.73, 141.64, 135.39, 133.49, 133.40, 129.53, 123.25, 122.40, 114.99, 114.78, 105.95, 105.76. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{16}\text{H}_{14}\text{FN}_6$ 309.1264, found 309.1260.



HSW1107-2

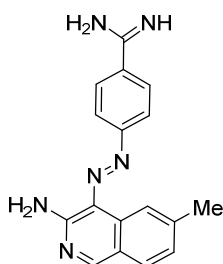
(E)-4-((3-amino-6-chloroisoquinolin-4-yl)diazenyl)benzimidamide. Following the described general procedure II, brownish red solid was obtained. ^1H NMR (600 MHz, MeOD) δ 8.92 (s, 1H), 8.65 (d, $J = 2.0$ Hz, 1H), 8.05 – 8.00 (m, 2H), 7.95 – 7.92 (m, 2H),

7.91 (d, $J = 8.6$ Hz, 1H), 7.35 (dd, $J = 8.6$ Hz, 2.0 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 167.60, 158.95, 156.91, 140.30, 139.92, 131.96, 129.64, 125.85, 123.25, 120.81. HRMS (ESI⁺) [M+H] calcd for C₁₆H₁₄ClN₆ 325.0968, found 325.0961.



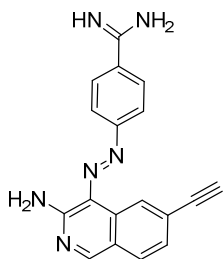
HSW630-1

(E)-4-((3-amino-6-bromoisoquinolin-4-yl)diazenyl)benzimidamide. Following the described general procedure II, brownish red solid was obtained. ^1H NMR (400 MHz, DMSO-d₆) δ 9.87 (s, 1H), 9.06 (s, 1H), 8.78 (d, $J = 1.8$, 1H), 8.01 – 7.91 (m, 5H), 7.53 (dd, $J = 8.5$, 1.9, 1H). ^{13}C NMR (100 MHz, DMSO-d₆) δ 162.27, 157.85, 153.71, 147.41, 137.99, 136.37, 131.07, 127.92, 126.82, 126.63, 122.26, 121.50, 121.44, 118.22. HRMS (ESI⁺) [M+H] calcd for C₁₆H₁₄N₆Br 369.0463 found 369.0477.



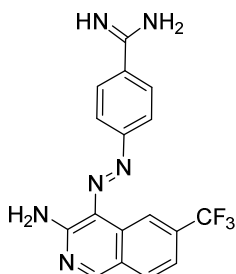
HSW1009-1

(E)-4-((3-amino-6-methylisoquinolin-4-yl)diazenyl)benzimidamide. Following the described general procedure II, brown solid was obtained. ^1H NMR (400 MHz, MeOD) δ 8.84 (d, $J = 0.6$ Hz, 1H), 8.53 (d, $J = 0.7$ Hz, 1H), 8.05 – 7.97 (m, 2H), 7.94 – 7.88 (m, 2H), 7.81 (d, $J = 8.3$ Hz, 1H), 7.26 (dd, $J = 8.3$ Hz, 1.4 Hz, 1H), 2.57 (s, 3H). ^{13}C NMR (100 MHz, MeOD) δ 166.35, 157.33, 155.65, 146.99, 143.54, 138.43, 134.62, 128.68, 128.27, 126.17, 122.45, 121.99, 120.82, 119.76, 21.41. HRMS (ESI⁺) [M+H] calcd for C₁₇H₁₇N₆ 305.1515, found 305.1501.



HSW1017-1

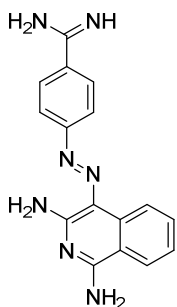
(E)-4-((3-amino-6-ethynylisoquinolin-4-yl)diazenyl)benzimidamide. Following the described general procedure II, brown solid was obtained. ^1H NMR (500 MHz, MeOD) δ 8.91 (s, 1H), 8.78 (s, 1H), 8.01 (d, $J = 8.6$ Hz, 2H), 7.94 – 7.90 (m, 2H), 7.87 (d, $J = 8.3$ Hz, 1H), 7.39 (dd, $J = 8.3$ Hz, 1.4 Hz, 1H). ^{13}C NMR (125 MHz, MeOD) δ 167.63, 158.80, 157.04, 148.55, 138.77, 134.73, 130.07, 129.64, 127.81, 127.62, 125.76, 124.17, 123.30, 121.14, 84.35, 80.94. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{18}\text{H}_{15}\text{N}_6$ 315.1358, found 315.1353.



HST2-138

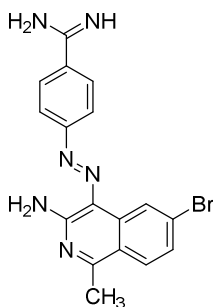
(E)-4-((3-amino-6-(trifluoromethyl)isoquinolin-4-yl)diazenyl)benzimidamide.

Following the described general procedure II, brown solid was obtained. ^1H NMR (500 MHz, DMSO- d_6) δ 10.00 (1H, br. s), 9.41 (3H, br), 9.26 (1H, s), 8.93 (1H, s), 8.24 (1H, d, $J = 8.4$ Hz), 8.14 (2H, d, $J = 8.5$ Hz), 8.03 (2H, d, $J = 8.5$ Hz), 7.67 (1H, d, $J = 8.4$ Hz). ^{13}C NMR (125 MHz, DMSO- d_6) δ 165.0, 159.1, 155.9, 147.8, 136.1, 132.1, 131.6, 131.4, 130.7, 129.6, 128.1, 125.1, 123.8, 123.0, 121.9, 119.2, 117.5. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{17}\text{H}_{14}\text{F}_3\text{N}_6$ 359.1232, found 359.1264.



HSZ5-164

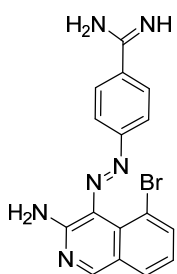
(E)-4-((1,3-diaminoisoquinolin-4-yl)diazenyl)benzimidamide. Following the described general procedure II, brown solid was obtained. ^1H NMR (500 MHz, MeOD, with D_2O exchange) δ 8.52 (d, $J = 8.3$ Hz, 1H), 8.16 (d, $J = 8.1$ Hz, 1H), 7.93 – 7.84 (m, 4H), 7.72 (s, 1H), 7.40 (s, 1H). ^{13}C NMR (126 MHz, MeOD) δ 165.55, 161.15, 158.77, 158.20, 134.18, 132.15, 129.82, 129.45, 128.50, 124.98, 123.88, 120.59, 118.83, 117.85, 116.45, 114.87. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{16}\text{H}_{16}\text{N}_7$ 306.1467, found 306.1448.



HST2-181

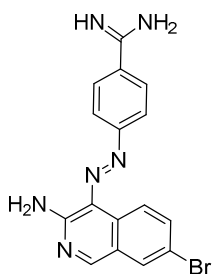
(E)-4-((3-amino-6-bromo-1-methylisoquinolin-4-yl)diazenyl)benzimidamide.

Following the described general procedure II, reddish brown solid was obtained. ^1H NMR (500 MHz, DMSO- d_6) δ = 9.99 (1H, brs), 8.82 (1H, s), 8.04 (1H, d, J = 8.7 Hz), 8.01 (2H, d, J = 8.4 Hz), 7.54 (2H, d, J = 8.4 Hz), 2.83 (3H, s). ^{13}C NMR (101 MHz, MeOD- d_6) δ : 166.3, 165.3, 163.6, 158.1, 155.9, 146.6, 138.3, 129.1, 128.7, 128.3, 126.4, 122.6, 121.7, 118.3, 23.9. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{17}\text{H}_{16}\text{BrN}_6$ 383.0620, found 383.0612.



HSW919-1

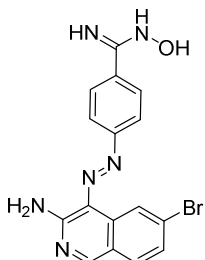
(E)-4-((3-amino-5-bromoisoquinolin-4-yl)diazenyl)benzimidamide. Following the described general procedure II, brown solid was obtained. ^1H NMR (400 MHz, MeOD) δ 8.94 (s, 1H), 8.15 – 8.10 (m, 3H), 7.95 (dd, J = 7.9 Hz, 1.2, 1H), 7.91 – 7.86 (m, 2H), 7.23 (t, J = 7.7 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 167.60, 160.19, 156.59, 148.33, 141.31, 135.33, 134.42, 131.05, 129.41, 127.12, 125.44, 123.77, 121.23, 115.57. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{16}\text{H}_{14}\text{BrN}_6$ 369.0463, found 369.0485.



HSW1107-3

(E)-4-((3-amino-7-bromoisoquinolin-4-yl)diazenyl)benzimidamide. Following the described general procedure II, brown solid was obtained. ^1H NMR (500 MHz, DMSO-

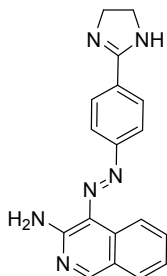
d6) δ 9.90 (s, 1H), 9.07 (s, 1H), 8.64 (d, $J = 9.0$ Hz, 1H), 8.27 (d, $J = 2.1$ Hz, 1H), 8.08 (d, $J = 8.7$ Hz, 2H), 7.98 (d, $J = 8.7$ Hz, 2H), 7.85 (dd, $J = 9.0$ Hz, 2.1 Hz, 1H). ^{13}C NMR (125 MHz, DMSO- d_6) δ 163.38, 157.47, 154.71, 147.00, 135.44, 134.83, 132.26, 130.41, 128.54, 124.02, 122.84, 121.56, 119.26, 115.93. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{16}\text{H}_{14}\text{BrN}_6$ 369.0463, found 369.0441.



HSW818-3

(E)-4-((E)-(3-amino-6-bromoisoquinolin-4-yl)diazenyl)-N'-hydroxybenzimidamide.

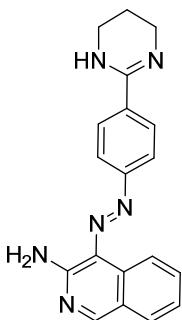
Following the described general procedure II, brownish red solid was obtained. ^1H NMR (500 MHz, DMSO- d_6) δ 9.82 (d, $J = 15.8$ Hz, 2H), 9.05 (d, $J = 16.2$ Hz, 1H), 8.79 (d, $J = 15.5$ Hz, 1H), 8.43 (s, 1H), 8.02 – 7.90 (m, 3H), 7.90 – 7.80 (m, 2H), 7.54 (d, $J = 8.6$ Hz, 1H), 5.91 (d, $J = 14.2$ Hz, 2H). ^{13}C NMR (125 MHz, DMSO- d_6) δ 157.47, 152.91, 150.35, 147.36, 137.90, 134.11, 130.97, 126.67, 126.51, 126.34, 122.24, 121.58, 121.40, 118.13. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{16}\text{H}_{14}\text{BrN}_6\text{O}$ 385.0412, found 385.0439.



HSW925-1

(E)-4-((4-(4,5-dihydro-1H-imidazol-2-yl)phenyl)diazenyl)isoquinolin-3-amine.

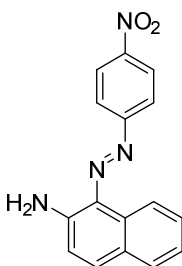
Following the described general procedure II, brown solid was obtained. ^1H NMR (600 MHz, MeOD) δ 8.94 (s, 1H), 8.77 (d, $J = 8.4$ Hz, 1H), 8.01 (d, $J = 8.6$ Hz, 2H), 7.94 (dd, $J = 18.9$ Hz, 8.3 Hz, 3H), 7.75 (t, $J = 7.7$ Hz, 1H), 7.42 (t, $J = 7.4$ Hz, 1H), 3.83 (s, 4H). ^{13}C NMR (151 MHz, MeOD) δ 167.19, 158.91, 156.67, 148.01, 139.35, 133.62, 131.23, 129.81, 129.73, 125.23, 125.16, 123.10, 122.02, 121.94, 49.72. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{18}\text{H}_{17}\text{N}_6$ 317.1515, found 317.1537.



HSW925-2

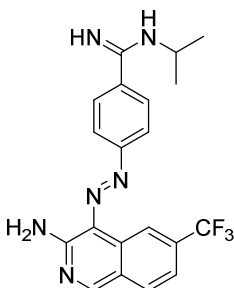
(*E*)-4-((4-(1,4,5,6-tetrahydropyrimidin-2-yl)phenyl)diazenyl)isoquinolin-3-amine.

Following the described general procedure II, brown solid was obtained. ^1H NMR (500 MHz, MeOD) δ 8.94 (s, 1H), 8.77 (d, $J = 8.5$ Hz, 1H), 8.01 (d, $J = 7.2$ Hz, 2H), 7.93 (d, $J = 8.0$ Hz, 1H), 7.84 – 7.79 (m, 2H), 7.75 (t, $J = 7.7$ Hz, 1H), 7.42 (t, $J = 7.5$ Hz, 1H), 3.52 (t, $J = 5.3$ Hz, 4H), 1.99 – 1.91 (m, 2H). ^{13}C NMR (125 MHz, MeOD) δ 159.00, 158.80, 156.43, 147.99, 139.35, 133.58, 129.80, 129.12, 125.21, 125.17, 123.12, 121.98, 121.92, 42.52, 21.22. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{19}\text{H}_{19}\text{N}_6$ 331.1671, found 331.1661.



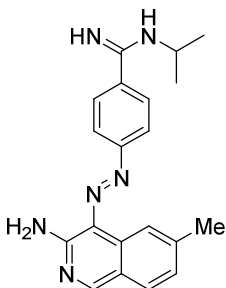
HSW972

(*E*)-1-((4-nitrophenyl)diazenyl)naphthalen-2-amine. Following the described general procedure I, a brown solid was obtained. ^1H NMR (400 MHz, DMSO- d_6) δ 10.32 (s, 1H), 8.70 (d, $J = 8.1$ Hz, 1H), 8.35 (d, $J = 7.9$ Hz, 2H), 8.10 (d, $J = 7.8$ Hz, 2H), 7.82 (d, $J = 8.9$ Hz, 1H), 7.74 (d, $J = 7.7$ Hz, 1H), 7.55 (t, $J = 7.4$ Hz, 1H), 7.34 (t, $J = 7.1$ Hz, 1H), 7.15 (d, $J = 8.9$ Hz, 1H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 157.25, 146.00, 141.75, 135.87, 134.08, 128.35, 128.15, 126.60, 126.41, 124.97, 123.47, 121.85, 120.96, 119.90. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{16}\text{H}_{13}\text{N}_4\text{O}_2$ 293.1039, found 293.1050.



HST2-142

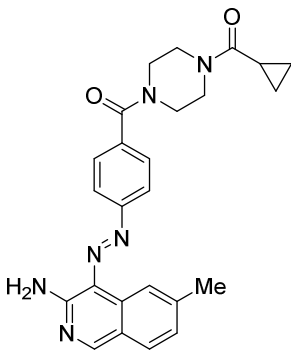
(E)-4-((3-amino-6-(trifluoromethyl)isoquinolin-4-yl)diazenyl)-N-isopropylbenzimidamide. Following the described general procedure II, a brownish solid was obtained. ^1H NMR (400 MHz, MeOD) δ 8.95 (s, 1H), 8.62 (s, 1H), 8.00 (d, $J = 8.4$ Hz, 1H), 7.92 (d, $J = 8.4$ Hz, 2H), 7.79 (d, $J = 8.4$ Hz, 2H), 7.50 (dd, $J = 8.4, 1.2$ Hz, 1H), 3.97 (q, $J = 6.4$ Hz, 1H), 1.32 (d, $J = 6.4$ Hz, 6H). ^{13}C NMR (100 MHz, MeOD) δ 163.99, 158.85, 156.28, 148.76, 138.41, 136.92, 131.28, 130.06, 129.62, 128.48, 125.54, 123.24, 121.27, 120.31, 119.30. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{20}\text{H}_{20}\text{F}_3\text{N}_6$ 401.1702, found 401.1693.



HST2-143

(E)-4-((3-amino-6-methylisoquinolin-4-yl)diazenyl)-N-isopropylbenzimidamide.

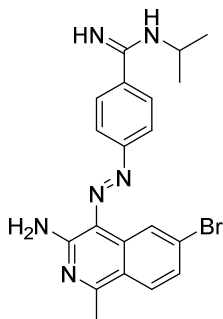
Following the described general procedure II, a brownish solid was obtained. ^1H NMR (400 MHz, MeOD) δ 9.89 (s, 1H), 8.97 (s, 1H), 8.47 (s, 1H), 8.30 (s, 1H), 7.98 (d, $J = 8.4$ Hz, 2H), 7.92-7.88 (m, 3H), 7.25 (d, $J = 8.2$ Hz, 1H), 3.8 (q, $J = 6.2$ Hz, 1H), 2.54 (s, 3H), 1.16 (d, $J = 6.2$ Hz, 6H). ^{13}C NMR (100 MHz, MeOD) δ 157.46, 153.59, 146.97, 142.49, 137.07, 128.63, 127.82, 125.63, 121.51, 121.27, 119.31, 119.26, 48.61, 44.22, 22.98, 22.31. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{20}\text{H}_{23}\text{N}_6$ 347.1984, found 347.1966.



HST2-144

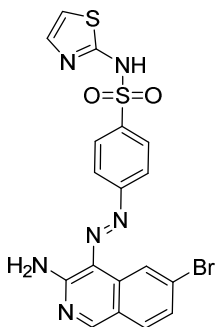
(E)-4-(4-((3-amino-6-methylisoquinolin-4-yl)diazenyl)benzoyl)piperazin-1-yl(cyclopropyl)methanone.

Following the described general procedure II, a brownish solid was obtained. ^1H NMR (400 MHz, MeOD) δ 9.87 (s, 1H), 8.98 (s, 1H), 8.47 (s, 1H), 8.31 (s, 1H), 8.02 (d, $J = 8.0$ Hz, 2H), 7.88 (d, $J = 8.0$ Hz, 1H), 7.61 (d, $J = 8.0$ Hz, 2H), 7.25 (d, $J = 8.0$ Hz, 1H), 3.76-3.55 (b, 8H), 1.98 (s, 1H), 0.75-0.72 (m, 4H). ^{13}C NMR (100 MHz, MeOD) δ 172.58, 170.42, 157.08, 154.37, 146.49, 143.04, 138.14, 135.45, 128.50, 128.40, 126.36, 122.50, 122.31, 121.15, 120.18, 45.58, 42.52, 22.92, 11.22, 7.93. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{25}\text{H}_{27}\text{N}_6\text{O}_2$ 443.2195, found 443.2162.



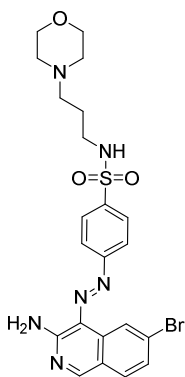
HST2-172

(E)-4-((3-amino-6-bromo-1-methylisoquinolin-4-yl)diazenyl)-N-isopropylbenzimidamide. Following the described general procedure II, a brownish solid was obtained. ^1H NMR (400 MHz, MeOD) δ 8.77 (d, J = 1.8 Hz, 1H), 7.94 (d, J = 8.4 Hz, 2H), 7.90 (d, J = 8.8 Hz, 1H), 7.80 (d, J = 8.4 Hz, 2H), 7.43 (dd, J = 8.8, 1.8 Hz, 1H), 3.98 (q, J = 6.4 Hz, 1H), 2.80 (s, 1H), 1.34 (d, J = 6.4 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 165.03, 153.59, 146.35, 138.36, 129.12, 128.12, 127.90, 126.79, 126.20, 126.11, 122.53, 121.18, 120.17, 117.72, 44.21, 22.91, 22.69. HRMS (ESI⁺) [M+H] calcd for $\text{C}_{20}\text{H}_{22}\text{BrN}_6$ 425.1089, found 425.1099.



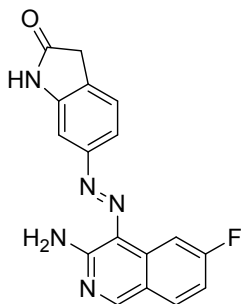
HSY135

(E)-4-((3-amino-6-bromoisoquinolin-4-yl)diazenyl)-N-(thiazol-2-yl)benzenesulfonamide. Following the described general procedure II, red solid was obtained. ^1H NMR (400 MHz, DMSO- d_6) δ 12.84 (br, 1H), 9.94 (br, 1H), 9.06 (s, 1H), 8.74 (s, 1H), 8.65 (br, 1H), 8.07 (d, J = 8.6 Hz, 2H), 7.96 (d, J = 8.6 Hz, 2H), 7.92 (d, J = 8.6 Hz, 1H), 7.53 (dd, J = 8.5, 1.8 Hz, 1H), 7.29 (d, J = 4.6 Hz, 1H), 6.87 (d, J = 4.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 168.95, 158.51, 154.54, 147.50, 141.98, 137.88, 131.04, 127.15, 126.96, 126.75, 124.55, 122.16, 122.08, 121.40, 118.44, 108.41. HRMS (ESI⁺) [M+H] calcd for $\text{C}_{18}\text{H}_{14}\text{BrN}_6\text{O}_2\text{S}_2$ 448.9798, found 448.9776.



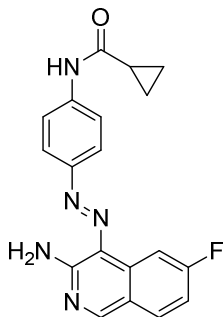
HSY136

(E)-4-((3-amino-6-bromoisoquinolin-4-yl)diazenyl)-N-(3-morpholinopropyl)benzenesulfonamide. Following the described general procedure II, red solid was obtained. ^1H NMR (400 MHz, DMSO- d_6) δ 9.96 (br, 1H), 9.07 (s, 1H), 8.76 (d, $J=1.4$ Hz, 1H), 8.75-8.60 (br, 1H), 8.12 (d, $J=8.5$ Hz, 2H), 8.01-7.86 (m, 3H), 7.72 (t, $J=5.1$ Hz, 1H), 7.54 (dd, $J=8.4$ Hz, 1.4 Hz, 1H), 3.49 (s, 4H), 2.84 (q, $J=6.3$ Hz, 2H), 2.32-2.14 (m, 6H), 1.53 (p, $J=6.8$ Hz, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 158.66, 154.77, 147.55, 140.15, 137.91, 131.08, 127.92, 127.01, 126.80, 122.29, 122.19, 121.44, 118.51, 66.10, 55.22, 53.17, 40.81, 25.82. HRMS (ESI $^+$) $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{22}\text{H}_{27}\text{BrN}_6\text{O}_3\text{S}^+$ 533.0965, found 533.0978.



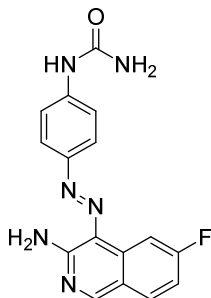
HST3-1

(E)-6-((3-amino-6-fluoroisoquinolin-4-yl)diazenyl)indolin-2-one. ^1H NMR (500 MHz, DMSO- d_6) δ 8.80 (s, 1H), 7.87 (dd, $J=8.8, 6.1$ Hz, 1H), 7.27 (dd, $J=10.9, 2.0$ Hz, 2H), 7.07 (dd, $J=12.7, 7.8$ Hz, 1H), 7.04 – 6.96 (m, 1H), 6.95 – 6.88 (m, 1H), 6.58 (s, 1H), 6.06 (s, 1H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 164.81, 164.40, 163.71, 162.44, 157.46, 151.99, 140.23, 131.79, 131.70, 120.27, 112.42, 112.21, 107.36, 107.19, 97.09. LRMS (ESI $^+$) $[\text{M}+\text{H}]$ calcd for $\text{C}_{17}\text{H}_{13}\text{FN}_5\text{O}$ 322.1104, found 322.0946.



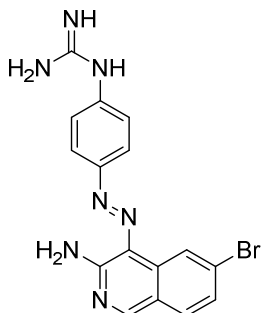
HST3-5

(*E*)-N-(4-((3-amino-6-fluoroisoquinolin-4-yl)diazenyl)phenyl)cyclopropanecarboxamide. ^1H NMR (500 MHz, DMSO- d_6) δ 10.49 (s, 1H), 9.00 (s, 1H), 8.28 (dd, $J = 11.6, 2.3$ Hz, 1H), 8.06 (dt, $J = 17.2, 8.7$ Hz, 1H), 7.94 (d, $J = 8.9$ Hz, 2H), 7.80 (dd, $J = 20.7, 8.9$ Hz, 2H), 7.23 (td, $J = 8.7, 2.5$ Hz, 1H), 1.81 (ddd, $J = 12.4, 7.5, 5.0$ Hz, 1H), 0.84 – 0.80 (m, 4H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 172.37, 165.50, 163.52, 156.63, 148.71, 147.78, 141.25, 139.40, 139.31, 132.80, 132.72, 123.21, 120.82, 119.77, 119.29, 113.59, 113.39, 104.82, 104.63, 15.18, 7.92. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{19}\text{H}_{17}\text{FN}_5\text{O}$ 350.1417, found 350.1422.



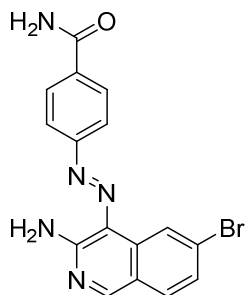
HST3-8

(*E*)-1-(4-((3-amino-6-fluoroisoquinolin-4-yl)diazenyl)phenyl)urea. ^1H NMR (500 MHz, DMSO- d_6) δ 8.96 (s, 1H), 8.92 (s, 1H), 8.26 (dd, $J = 11.6, 2.2$ Hz, 1H), 8.06 (dd, $J = 8.8, 6.0$ Hz, 1H), 7.88 (d, $J = 8.8$ Hz, 2H), 7.58 (d, $J = 8.8$ Hz, 2H), 7.22 (td, $J = 8.7, 2.4$ Hz, 1H), 5.99 (s, 2H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 165.42, 163.44, 156.21, 156.11, 147.67, 147.54, 142.89, 139.31, 132.76, 127.43, 123.31, 120.80, 119.21, 118.31, 113.53, 113.33, 104.75, 104.56. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{16}\text{H}_{14}\text{FN}_6\text{O}$ 325.1213, found 325.1218.



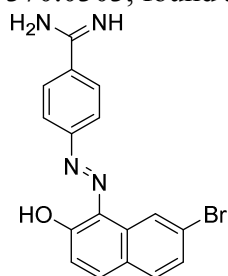
HSW821-3

(E)-1-(4-((3-amino-6-bromoisoquinolin-4-yl)diazenyl)phenyl)guanidine. ^1H NMR (500 MHz, MeOD) δ = 8.93 (s, 1H), 8.88 (s, 1H), 8.06 – 8.02 (m, 2H), 7.85 (d, J =8.6, 1H), 7.50 (dd, J =8.5, 1.8, 1H), 7.47 (d, J =8.6, 2H). ^{13}C NMR (125 MHz, MeOD) δ 158.33, 153.52, 140.15, 137.63, 131.68, 128.58, 128.39, 127.06, 124.60, 124.26, 123.43, 120.51, 111.10, 105.79. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{16}\text{H}_{15}\text{N}_7\text{Br}$ 384.0572, found 384.0588.



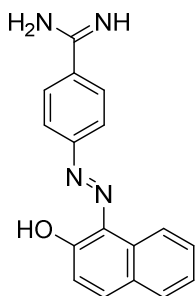
HSW819-1

(E)-4-((3-amino-6-bromoisoquinolin-4-yl)diazenyl)benzamide. Following the described general procedure II, red solid was obtained. ^1H NMR (400 MHz, DMSO- d_6) δ = 9.91 (s, 1H), 9.08 (s, 1H), 8.80 (d, J =1.8, 1H), 8.56 (s, 1H), 8.09 (s, 1H), 8.07 – 8.00 (m, 4H), 7.96 (d, J =8.5, 1H), 7.55 (dd, J =8.5, 1.9, 1H), 7.47 (s, 1H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 167.33, 158.07, 154.34, 147.44, 137.93, 134.49, 131.04, 128.76, 126.83, 126.64, 122.22, 121.50, 121.41, 118.28. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{16}\text{H}_{13}\text{BrN}_5\text{O}$ 370.0303, found 370.0272.



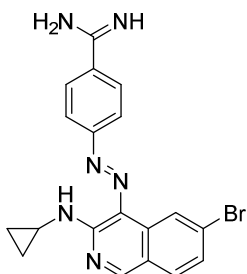
HST3-12

(E)-4-((7-bromo-2-hydroxynaphthalen-1-yl)diazenyl)benzimidamide. Following the described general procedure II, red solid was obtained. ^1H NMR (500 MHz, DMSO- d_6) δ 8.77 (s, 1H), 7.91 (q, J = 8.8 Hz, 4H), 7.82 (d, J = 9.6 Hz, 1H), 7.63 (d, J = 8.3 Hz, 1H), 7.52 (dd, J = 8.3, 1.7 Hz, 1H), 6.77 (d, J = 9.5 Hz, 1H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 175.00, 165.26, 134.61, 131.37, 130.31, 129.84, 129.20, 127.65, 126.72, 124.57, 123.61, 119.07. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{17}\text{H}_{14}\text{BrN}_4\text{O}$ 369.0463, found 369.0493.



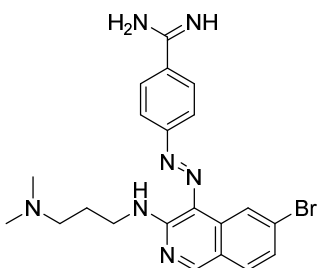
HSW0113-2

(E)-4-((2-hydroxynaphthalen-1-yl)diazenyl)benzimidamide. Following the described general procedure II, red solid was obtained. ^1H NMR (500 MHz, DMSO- d_6) δ 9.42 (s, 2H), 9.20 (s, 2H), 8.44 (d, $J = 8.0$ Hz, 1H), 8.04 – 7.87 (m, 5H), 7.70 (d, $J = 7.6$ Hz, 1H), 7.58 (t, $J = 7.6$ Hz, 1H), 7.46 (t, $J = 7.4$ Hz, 1H), 6.73 (d, $J = 9.6$ Hz, 1H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 178.07, 165.12, 147.68, 143.42, 133.11, 131.02, 130.47, 130.20, 129.80, 128.71, 127.70, 126.33, 124.79, 122.55, 117.70. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{17}\text{H}_{15}\text{N}_4\text{O}$ 291.1246, found 291.1260.



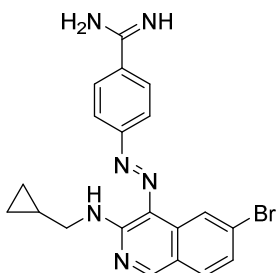
HSY430-1

(E)-4-((6-bromo-3-(cyclopropylamino)isoquinolin-4-yl)diazenyl)benzimidamide. Following the described general procedure II, red solid was obtained. ^1H NMR (400 MHz, MeOD) δ 8.60 (d, $J = 0.8$ Hz, 1H), 8.32 (dd, $J = 1.8, 0.8$ Hz, 1H), 7.86-7.75 (m, 2H), 7.63-7.57 (m, 2H), 7.50 (d, $J = 8.5$ Hz, 1H), 7.25 (dd, $J = 8.5, 1.9$ Hz, 1H), 3.05 (tt, $J = 7.4, 3.9$ Hz, 1H), 0.96-0.85 (m, 2H), 0.67-0.55 (m, 2H). ^{13}C NMR (125 MHz, MeOD) δ 167.07, 158.12, 155.57, 148.71, 139.64, 135.93, 131.30, 129.05, 128.33, 128.13, 123.91, 122.82, 122.59, 120.33, 24.94, 8.12. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{19}\text{H}_{18}\text{BrN}_6$ 409.0771, found 409.0757.



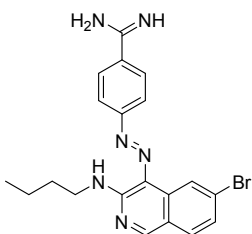
HSY430-2

(E)-4-((6-bromo-3-((3-(dimethylamino)propyl)amino)isoquinolin-4-yl)diazenyl)benzimidamide. Following the described general procedure II, red solid was obtained. ¹H NMR (400 MHz, DMSO-d₆) δ 11.17 (t, *J* = 5.7 Hz, 1H), 9.13 (s, 1H), 8.76 (d, *J* = 1.7 Hz, 1H), 8.00-7.92 (m, 5H), 7.54 (dd, *J* = 8.5, 1.9 Hz, 1H), 3.79 (q, *J* = 6.7 Hz, 2H), 2.33 (t, *J* = 6.9 Hz, 2H), 2.14 (s, 6H), 1.81 (p, *J* = 6.9 Hz, 2H). ¹³C NMR (101 MHz, DMSO-d₆) δ 162.75, 157.93, 153.85, 146.63, 137.94, 134.30, 130.97, 128.18, 127.01, 126.82, 126.58, 122.08, 121.25, 120.78, 118.54, 56.84, 48.59, 45.30, 39.52, 27.07. HRMS (ESI⁺) [M+H] calcd for C₂₁H₂₅BrN₇ 454.1349, found 454.1378.



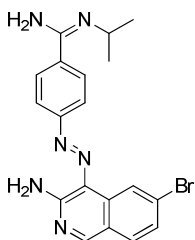
HSY430-3

(E)-4-((6-bromo-3-((cyclopropylmethyl)amino)isoquinolin-4-yl)diazenyl)benzimidamide. Following the described general procedure II, red solid was obtained. ¹H NMR (400 MHz, DMSO-d₆) δ 11.41 (s, 1H), 9.11 (br, 1H), 8.71 (s, 1H), 8.04 (q, *J* = 8.4 Hz, 4H), 7.92 (d, *J* = 8.5 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 1.22 (m, 1H), 0.55 (d, *J* = 7.3 Hz, 2H), 0.37 (d, *J* = 4.2 Hz, 2H). ¹³C NMR (101 MHz, DMSO-d₆) δ 164.95, 159.06, 155.58, 146.64, 138.03, 131.23, 129.57, 127.81, 127.24, 127.00, 122.16, 121.69, 121.02, 118.85, 44.99, 11.04, 3.44. HRMS (ESI⁺) [M+H] calcd for C₂₀H₂₀BrN₆ 423.0927, found 423.0920.



HSY430-4

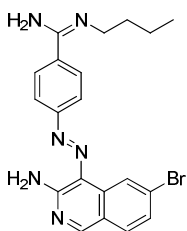
(E)-4-((6-bromo-3-(butylamino)isoquinolin-4-yl)diazenyl)benzimidamide. Following the described general procedure II, red solid was obtained. ¹H NMR (400 MHz, DMSO-d₆) δ 11.09 (t, *J* = 5.3 Hz, 1H), 9.02 (s, 1H), 8.65 (s, 1H), 7.95 (d, *J* = 8.2 Hz, 2H), 7.85 (d, *J* = 8.2 Hz, 3H), 7.46 (d, *J* = 7.9 Hz, 1H), 6.82 (br, 3H), 3.70 (m, 2H), 1.64 (m, 2H), 1.41 (m, 2H), 0.94 (t, *J* = 7.3 Hz, 3H); ¹³C NMR (101 MHz, DMSO-d₆) δ 162.04, 157.72, 153.23, 146.64, 138.01, 136.50, 130.97, 127.79, 126.76, 126.54, 122.08, 121.16, 120.78, 118.39, 31.18, 19.83, 13.67. HRMS (ESI⁺) [M + H] calcd for C₂₀H₂₂BrN₆ 425.1084, found 425.1079.



HSY430-5

(E)-4-((3-amino-6-bromoisoquinolin-4-yl)diazenyl)-N-isopropylbenzimidamide.

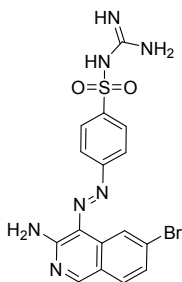
Following the described general procedure II, red solid was obtained. ^1H NMR (400 MHz, DMSO- d_6) δ 9.84 (br, 1H), 9.03 (s, 1H), 8.74 (s, 1H), 8.45 (br, 1H), 7.91 (m, 5H), 7.50 (d, $J = 8.5$ Hz, 1H), 3.81 (m, 6.2 Hz, 1H), 1.15 (d, $J = 6.3$ Hz, 6H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 157.53, 153.23, 147.31, 138.18, 137.91, 130.92, 127.67, 126.64, 126.46, 122.20, 121.35, 121.31, 118.09, 44.08, 23.08. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{19}\text{H}_{20}\text{BrN}_6$ 411.0927, found 411.0911.



HSY430-6

(E)-4-((3-amino-6-bromoisoquinolin-4-yl)diazenyl)-N-butylbenzimidamide.

Following the described general procedure II, red solid was obtained. ^1H NMR (400 MHz, DMSO- d_6) δ 9.85 (br, 1H), 9.06 (s, 1H), 8.79 (s, 1H), 8.47 (br, 1H), 7.95 (m, 5H), 7.50 (dd, $J = 8.4, 0.8$ Hz, 2H), 6.61 (br, 2H), 3.14 (t, $J = 6.8$ Hz, 3H), 1.60 (m, 2H), 1.43 (m, 2H), 0.94 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 157.57, 153.22, 147.33, 137.83, 130.97, 127.58, 126.68, 126.50, 122.22, 121.34, 118.10, 44.79, 32.29, 20.27, 14.00. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{19}\text{H}_{20}\text{BrN}_6$ 425.1084, found 425.1127.

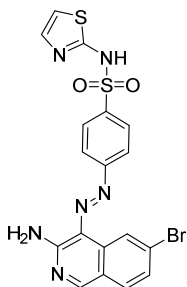


HSY127

(E)-4-((3-amino-6-bromoisoquinolin-4-yl)diazenyl)-N-

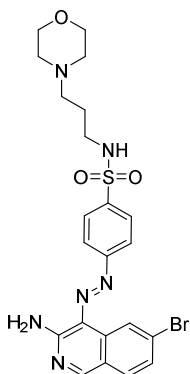
carbamimidoylbenzenesulfonamide. Following the described general procedure II, red solid was obtained. ^1H NMR (400 MHz, DMSO- d_6) δ 9.91 (br, 1H), 9.05 (s, 1H), 8.75 (s,

1H), 8.60 (br, 1H), 8.03 (d, $J = 8.4$ Hz, 2H), 7.92 (d, $J = 8.4$ Hz, 3H), 7.52 (dd, $J = 8.4, 1.0$ Hz, 1H), 6.80 (br, 4H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 158.29, 158.19, 154.03, 147.48, 144.43, 137.91, 131.03, 126.94, 126.91, 126.70, 122.19, 121.88, 121.40, 118.35. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{16}\text{H}_{15}\text{BrN}_7\text{O}_2\text{S}$ 448.0186, found 448.0194.



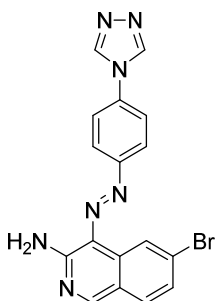
HSY135

(E)-4-((3-amino-6-bromoisoquinolin-4-yl)diazenyl)-N-(thiazol-2-yl)benzenesulfonamide. Following the described general procedure II, red solid was obtained. ^1H NMR (400 MHz, DMSO- d_6) δ 12.84 (br, 1H), 9.94 (br, 1H), 9.06 (s, 1H), 8.74 (s, 1H), 8.65 (br, 1H), 8.07 (d, $J = 8.6$ Hz, 2H), 7.96 (d, $J = 8.6$ Hz, 2H), 7.92 (d, $J = 8.6$ Hz, 1H), 7.53 (dd, $J = 8.5, 1.8$ Hz, 1H), 7.29 (d, $J = 4.6$ Hz, 1H), 6.87 (d, $J = 4.6$ Hz, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 168.95, 158.51, 154.54, 147.50, 141.98, 137.88, 131.04, 127.15, 126.96, 126.75, 124.55, 122.16, 122.08, 121.40, 118.44, 108.41. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{18}\text{H}_{14}\text{BrN}_6\text{O}_2\text{S}_2$ 448.9798, found 448.9776.



HSY136

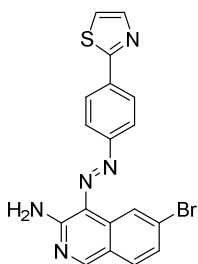
(E)-4-((3-amino-6-bromoisoquinolin-4-yl)diazenyl)-N-(3-morpholinopropyl)benzenesulfonamide. Following the described general procedure II, red solid was obtained. ^1H NMR (400 MHz, DMSO- d_6) δ 9.96 (br, 1H), 9.07 (s, 1H), 8.76 (d, $J = 1.4$ Hz, 1H), 8.75-8.60 (br, 1H), 8.12 (d, $J = 8.5$ Hz, 2H), 8.01-7.86 (m, 3H), 7.72 (t, $J = 5.1$ Hz, 1H), 7.54 (dd, $J = 8.4$ Hz, 1.4 Hz, 1H), 3.49 (s, 4H), 2.84 (q, $J = 6.3$ Hz, 2H), 2.32-2.14 (m, 6H), 1.53 (p, $J = 6.8$ Hz, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 158.66, 154.77, 147.55, 140.15, 137.91, 131.08, 127.92, 127.01, 126.80, 122.29, 122.19, 121.44, 118.51, 66.10, 55.22, 53.17, 40.81, 25.82. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{22}\text{H}_{27}\text{BrN}_6\text{O}_3\text{S}$ 533.0965, found 533.0978.



HSY155

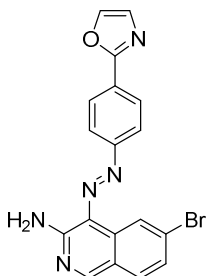
(E)-4-((4-(4H-1,2,4-triazol-4-yl)phenyl)diazenyl)-6-bromoisoquinolin-3-amine.

Following the described general procedure II, red solid was obtained. ¹H NMR (400 MHz, DMSO-d₆) δ 9.84 (br, 1H), 9.24 (s, 2H), 9.08 (s, 1H), 8.82 (s, 1H), 8.55 (br, 1H), 8.16 (d, *J* = 8.6 Hz, 2H), 7.96 (d, *J* = 8.5 Hz, 1H), 7.91 (d, *J* = 8.7 Hz, 2H), 7.55 (d, *J* = 7.1 Hz, 1H); ¹³C NMR (101 MHz, DMSO-d₆) δ 157.89, 151.96, 147.42, 141.28, 137.93, 134.26, 131.02, 126.79, 126.59, 123.25, 122.28, 121.99, 121.40, 118.10. HRMS (ESI⁺) [M+H] calcd for C₁₇H₁₃BrN₇ 394.0410, found 394.0392.



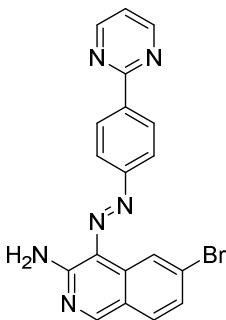
HSY161

(E)-6-bromo-4-((4-(thiazol-2-yl)phenyl)diazenyl)isoquinolin-3-amine. Following the described general procedure II, red solid was obtained. ¹H NMR (400 MHz, DMSO-d₆) δ 9.92 (br, 1H), 9.08 (s, 1H), 8.81 (s, 1H), 8.56 (br, 1H), 8.15 (d, *J* = 8.3 Hz, 2H), 8.09 (d, *J* = 8.4 Hz, 2H), 7.99 (d, *J* = 3.2 Hz, 1H), 7.96 (d, *J* = 8.5 Hz, 1H), 7.86 (d, *J* = 3.3 Hz, 1H), 7.55 (dd, *J* = 8.5, 1.9 Hz, 1H); ¹³C NMR (125 MHz, DMSO-d₆) δ 166.33, 157.85, 153.53, 147.41, 144.14, 137.84, 133.51, 130.97, 127.23, 126.74, 126.58, 122.59, 122.19, 121.40, 120.92, 118.33. HRMS (ESI⁺) [M+H] calcd for C₁₈H₁₃BrN₅S 410.0070, found 410.0052.



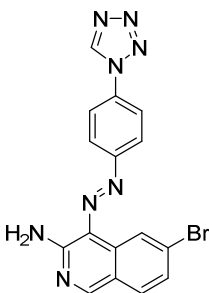
HSY162

(E)-6-bromo-4-((4-(oxazol-2-yl)phenyl)diazenyl)isoquinolin-3-amine. Following the described general procedure II, red solid was obtained. ^1H NMR (400 MHz, DMSO- d_6) δ 9.93 (br, 1H), 9.07 (s, 1H), 8.80 (d, $J = 1.8$ Hz, 1H), 8.57 (br, 1H), 8.28 (s, 1H), 8.15 (d, $J = 8.4$ Hz, 2H), 8.10 (d, $J = 8.4$ Hz, 2H), 7.95 (d, $J = 8.6$ Hz, 1H), 7.54 (dd, $J = 8.4, 1.9$ Hz, 1H), 7.45 (s, 1H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 160.44, 158.06, 153.67, 147.48, 140.48, 137.90, 131.04, 128.90, 127.19, 127.05, 126.84, 126.66, 122.55, 122.21, 121.43, 118.39. HRMS (ESI $^+$) [$M+H$] calcd for $\text{C}_{18}\text{H}_{13}\text{BrN}_5\text{O}$ 394.0298, found 394.0287.



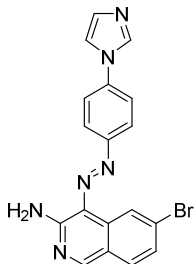
HSY164

(E)-6-bromo-4-((4-(pyrimidin-2-yl)phenyl)diazenyl)isoquinolin-3-amine. Following the described general procedure II, red solid was obtained. ^1H NMR (400 MHz, DMSO- d_6) δ 9.95 (br, 1H), 9.08 (s, 1H), 8.95 (d, $J = 4.8$ Hz, 2H), 8.83 (s, 1H), 8.58 (d, $J = 8.4$ Hz, 3H), 8.11 (d, $J = 8.3$ Hz, 2H), 7.96 (d, $J = 8.5$ Hz, 1H), 7.55 (d, $J = 8.5$ Hz, 1H), 7.49 (d, $J = 5.6$ Hz, 1H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 162.74, 157.96, 157.82, 154.19, 147.49, 137.92, 137.71, 131.06, 128.83, 126.83, 126.66, 122.24, 122.15, 121.44, 120.01, 118.40. HRMS (ESI $^+$) [$M+H$] calcd for $\text{C}_{19}\text{H}_{14}\text{BrN}_6$ 405.0458, found 405.0457.



HSY168

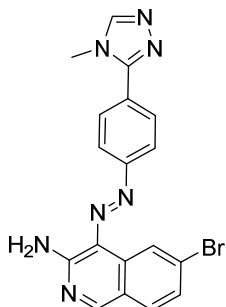
(E)-4-((4-(1H-tetrazol-1-yl)phenyl)diazenyl)-6-bromoisoquinolin-3-amine. Following the described general procedure II, red solid was obtained. ^1H NMR (400 MHz, DMSO- d_6) δ 10.19 (s, 1H), 9.89 (br, 1H), 9.05 (s, 1H), 8.77 (d, $J = 1.9$ Hz, 1H), 8.60 (br, 1H), 8.20 (d, $J = 8.4$ Hz, 2H), 8.08 (d, $J = 8.4$ Hz, 2H), 7.92 (d, $J = 8.5$ Hz, 1H), 7.52 (dd, $J = 8.5, 1.9$ Hz, 1H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 158.21, 152.92, 147.51, 142.24, 137.91, 133.70, 131.01, 126.89, 126.67, 123.24, 122.25, 121.97, 121.41, 118.26. HRMS (ESI $^+$) [$M+H$] calcd for $\text{C}_{16}\text{H}_{12}\text{BrN}_8$ 395.0363, found 395.0375.



HSY169

(E)-4-((4-(1H-imidazol-1-yl)phenyl)diazenyl)-6-bromoisoquinolin-3-amine.

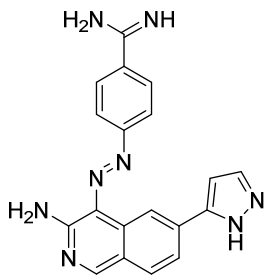
Following the described general procedure II, red solid was obtained. ^1H NMR (400 MHz, DMSO- d_6) δ 9.80 (br, 1H), 9.06 (s, 1H), 8.80 (d, $J = 1.8$ Hz, 1H), 8.46 (br, 1H), 8.39 (s, 1H), 8.16-8.06 (m, 2H), 7.95 (d, $J = 8.6$ Hz, 1H), 7.91-7.81 (m, 3H), 7.53 (dd, $J = 8.5, 1.9$ Hz, 1H), 7.16 (s, 1H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 157.46, 151.12, 147.29, 137.86, 137.29, 135.55, 130.90, 130.12, 126.61, 126.43, 123.17, 122.22, 121.34, 120.89, 117.99, 117.85. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{16}\text{H}_{12}\text{BrN}_8$ 393.0458, found 393.0458.



HSW909-1

(E)-6-bromo-4-((4-(4-methyl-4H-1,2,4-triazol-3-yl)phenyl)diazenyl)isoquinolin-3-amine.

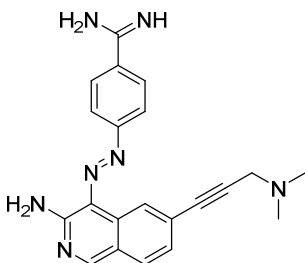
Following the described general procedure II, red solid was obtained. ^1H NMR (400 MHz, CDCl_3) δ = 8.93 (d, $J = 1.5$, 1H), 8.88 (s, 1H), 8.26 (s, 1H), 8.04 (d, $J = 8.6$, 2H), 7.89 (d, $J = 8.6$, 2H), 7.71 (d, $J = 8.5$, 1H), 7.49 (dd, $J = 8.6, 1.8$, 1H), 3.87 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 157.11, 154.06, 146.83, 145.78, 139.05, 130.07, 129.78, 127.81, 127.76, 127.56, 123.85, 122.81, 122.42, 120.18, 32.73. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{18}\text{H}_{15}\text{N}_7\text{Br}$ 408.0572, found 408.0565.



HSS504-1

(E)-4-((3-amino-6-(1H-pyrazol-5-yl)isoquinolin-4-yl)diazenyl)benzimidamide.

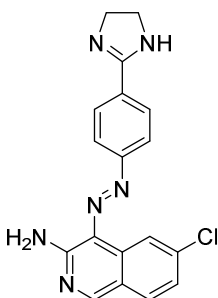
Following the described general procedure II, yellowish brown solid was obtained. ¹H NMR (600 MHz, DMSO-d₆) δ 9.61 (s, 2H), 9.43 (s, 2H), 9.06 (d, *J* = 6.5 Hz, 2H), 8.19 (d, *J* = 8.3 Hz, 2H), 8.08 – 8.00 (m, 3H), 7.90 (d, *J* = 8.3 Hz, 1H), 7.85 (s, 1H), 6.97 (d, *J* = 2.0 Hz, 1H). ¹³C NMR (126 MHz, MeOD) δ 165.31, 158.78, 158.54, 158.29, 158.03, 157.78, 156.25, 147.58, 137.22, 129.57, 127.62, 122.39, 121.81, 120.16, 118.16, 115.80, 115.45, 103.28. HRMS (ESI⁺) [M+H] calcd for C₁₉H₁₇N₈ 372.1576 found 372.1552.



HSS504-2

(E)-4-((3-amino-6-(3-(dimethylamino)prop-1-yn-1-yl)isoquinolin-4-yl)diazenyl)benzimidamide.

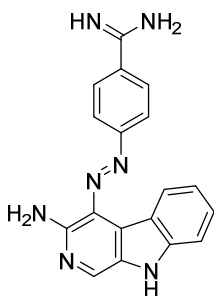
Following the described general procedure II, yellowish brown solid was obtained. ¹H NMR (400 MHz, MeOD) δ 8.72 (s, 1H), 8.47 (s, 1H), 7.83 (s, 4H), 7.68 (s, 1H), 7.20 (s, 1H), 3.49 (s, 2H), 2.36 (s, 6H). ¹³C NMR (101 MHz, MeOD) δ 166.39, 160.61, 157.65, 156.01, 147.29, 137.50, 132.05, 128.82, 128.60, 126.83, 126.48, 123.84, 122.71, 122.15, 119.95, 86.96, 85.92, 43.48. HRMS (ESI⁺) [M+H] calcd for C₂₁H₂₂N₇ 372.1931 found 372.1930.



HST3-14

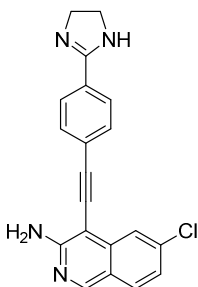
(E)-6-chloro-4-((4-(4,5-dihydro-1H-imidazol-2-yl)phenyl)diazenyl)isoquinolin-3-amine.

Following the described general procedure II, yellow solid was obtained. ¹H NMR (400 MHz, MeOD) δ 8.94 (s, 1H), 8.6 (s, 1H), 8.09 (d, *J* = 8.8 Hz, 2H), 8.01 (d, *J* = 8.4 Hz, 2H), 7.91 (d, *J* = 8.4 Hz, 1H), 7.37 (dd, *J* = 8.8, 2 Hz, 1H), 4.13 (s, 4H). ¹³C NMR (100 MHz, MeOD) δ 164.48, 163.90, 158.92, 155.87, 147.78, 137.74, 134.24, 130.12, 128.72, 124.29, 122.09, 121.35, 119.17, 118.94, 44.56. HRMS (ESI⁺) [M+H] calcd for C₁₈H₁₆ClN₆ 351.1125, found 351.1146.



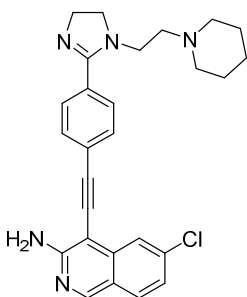
HST3-28

(E)-4-((3-amino-9H-pyrido[3,4-b]indol-4-yl)diazenyl)benzimidamide. Following the described general procedure II, yellow solid was obtained. ^1H NMR (400 MHz, MeOD) δ 8.69 (s, 1H), 8.28 (s, 1H), 8.23 (d, $J = 7.6$ Hz, 1H), 7.84 (d, $J = 8.4$ Hz, 2H), 7.64 (d, $J = 8.4$ Hz, 2H), 7.56 (s, 2H), 7.28 (d, $J = 3.6$ Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 179.73, 167.74, 143.86, 137.36, 132.62, 130.82, 130.29, 123.71, 123.12, 122.83, 121.16, 116.38, 116.31, 113.22, 106.85. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{21}\text{H}_{22}\text{N}_7$ 330.1467, found 330.1482.



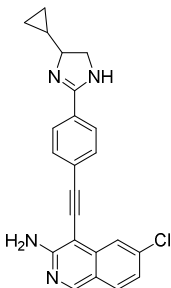
HSM1651

6-chloro-4-((4-(4,5-dihydro-1H-imidazol-2-yl)phenyl)ethynyl)isoquinolin-3-amine. Following the described general procedure IV, yellow solid was obtained. ^1H NMR (500 MHz, DMSO- d_6) δ 8.92 (s, 1H), 7.96 (d, $J = 8.6$ Hz, 1H), 7.93-7.81 (m, 5H), 7.28 (dd, $J = 8.6, 1.8$ Hz, 1H), 6.85 (s, 2H), 3.68 (s, 4H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 163.73, 158.70, 153.30, 138.73, 137.42, 131.64, 131.56, 129.45, 127.78, 125.60, 123.52, 121.33, 120.61, 99.69, 88.45, 85.91, 49.51; HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{20}\text{H}_{16}\text{ClN}_4$ 347.1063, found 347.1057.



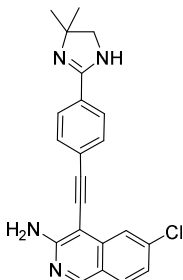
HSM1721

6-chloro-4-((4-(1-(2-(piperidin-1-yl)ethyl)-4,5-dihydro-1H-imidazol-2-yl)phenyl)ethynyl)isoquinolin-3-amine. Following the described general procedure IV, yellow solid was obtained. ¹H NMR (500 MHz, DMSO-d₆) δ 8.93 (s, 1H), 7.99-7.78 (m, 6H), 7.29 (dd, *J* = 8.6, 2.0 Hz, 1H), 6.86 (s, 2H), 3.53-3.46 (m, 2H), 3.42 (dd, *J* = 7.9, 3.1 Hz, 2H), 3.25-3.16 (m, 2H), 2.38 (dd, *J* = 24.1, 22.3 Hz, 6H), 1.55-1.47 (m, 4H), 1.39 (s, 2H); ¹³C NMR (126 MHz, DMSO-d₆) δ 206.36, 166.05, 158.59, 157.55, 153.58, 138.92, 137.12, 133.90, 131.37, 128.40, 126.15, 123.15, 121.23, 120.71, 99.32, 88.09, 86.12, 72.35, 67.21, 60.93, 54.76, 45.46, 32.08, 26.11, 23.84; HRMS (ESI⁺) [M+H] calcd for C₂₇H₂₉ClN₅ 458.2111, found 458.2110.



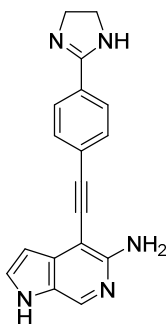
HSM1703

6-chloro-4-((4-(4-cyclopropyl-4,5-dihydro-1H-imidazol-2-yl)phenyl)ethynyl)isoquinolin-3-amine. Following the described general procedure IV, yellow solid was obtained. ¹H NMR (500 MHz, MeOD) δ 8.83 (s, 1H), 7.88 (ddd, *J* = 62.4, 45.2, 5.0 Hz, 6H), 7.29 (dd, *J* = 8.7, 1.9 Hz, 1H), 4.05-3.90 (m, 1H), 3.67 (dd, *J* = 11.9, 8.0 Hz, 1H), 3.53 (dd, *J* = 18.2, 7.9 Hz, 1H), 1.10-0.98 (m, 1H), 0.66-0.29 (m, 4H). HRMS (ESI⁺) [M+H] calcd for C₂₃H₂₀ClN₄ 387.1376, found 387.1.



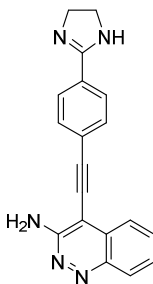
HSM1743

6-chloro-4-((4-(4,4-dimethyl-4,5-dihydro-1H-imidazol-2-yl)phenyl)ethynyl)isoquinolin-3-amine. Following the described general procedure IV, yellow solid was obtained. ¹H NMR (500 MHz, MeOD) δ 8.75 (s, 1H), 7.88 (d, *J* = 1.2 Hz, 1H), 7.81 (d, *J* = 7.4 Hz, 3H), 7.70 (d, *J* = 8.2 Hz, 2H), 7.22 (dd, *J* = 8.7, 1.9 Hz, 1H), 3.53 (s, 2H), 1.35 (s, 6H); ¹³C NMR (126 MHz, MeOD) δ 162.62, 157.51, 151.80, 138.75, 138.02, 131.01, 130.32, 129.49, 127.18, 125.73, 123.67, 121.34, 120.78, 99.38, 90.34, 84.27, 62.77, 61.61, 27.34; HRMS (ESI⁺) [M+H] calcd for C₂₂H₂₀ClN₄ 350.1376, found 375.1368.



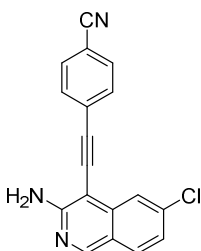
HSM1781

4-((4-(4,5-dihydro-1H-imidazol-2-yl)phenyl)ethynyl)-1H-pyrrolo[2,3-c]pyridin-5-amine. Following the described general procedure IV, yellow solid was obtained. ^1H NMR (500 MHz, MeOD) δ 8.27 (s, 1H), 7.90 (d, $J = 8.5$ Hz, 2H), 7.79 (d, $J = 8.5$ Hz, 2H), 7.53 (d, $J = 2.9$ Hz, 1H), 6.50 (dd, $J = 2.8, 0.7$ Hz, 1H), 3.96 (s, 4H); ^{13}C NMR (126 MHz, MeOD) δ 165.52, 153.08, 143.40, 136.88, 133.96, 132.14, 131.63, 131.27, 128.33, 128.20, 127.85, 99.54, 96.35, 87.85, 41.16; HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{18}\text{H}_{16}\text{N}_5$ 302.1406, found 302.1399.



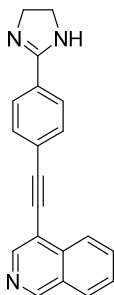
HSM1796

4-((4-(4,5-dihydro-1H-imidazol-2-yl)phenyl)ethynyl)cinnolin-3-amine. Following the described general procedure IV, yellow solid was obtained. ^1H NMR (500 MHz, DMSO- d_6) δ 8.19 (d, $J = 8.5$ Hz, 1H), 8.03 (d, $J = 2.1$ Hz, 1H), 7.91 (d, $J = 11.9$ Hz, 3H), 7.75 – 7.68 (m, 1H), 7.56 – 7.48 (m, 1H), 7.11 (d, $J = 17.1$ Hz, 3H), 3.62 (s, 4H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 163.46, 157.83, 145.17, 132.88, 132.59, 132.25, 131.49, 130.35, 127.69, 127.32, 126.36, 125.17, 124.06, 123.77, 103.13, 94.50, 83.62, 41.23; HRMS(ESI $^+$) [M+H] calcd for $\text{C}_{19}\text{H}_{16}\text{N}_5$ 314.1406, found 314.1396.



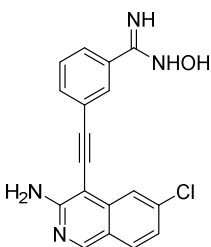
HSM1611

4-((3-amino-6-chloroisoquinolin-4-yl)ethynyl)benzonitrile. Following the described general procedure IV, yellow solid was obtained. ^1H NMR (500 MHz, DMSO- d_6) δ 8.91 (s, 1H), 7.91 (ddd, $J = 12.4, 9.9, 5.0$ Hz, 6H), 7.26 (d, $J = 8.6$ Hz, 1H), 6.95 (s, 2H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 159.04, 153.96, 138.83, 137.63, 132.78, 132.41, 131.61, 128.36, 123.61, 121.33, 120.58, 119.18, 110.60, 98.86, 88.51, 87.73; HRMS (ESI^+) $[\text{M}+\text{H}]$ calcd for $\text{C}_{18}\text{H}_{11}\text{ClN}_3$ 304.0642, found 304.1411.



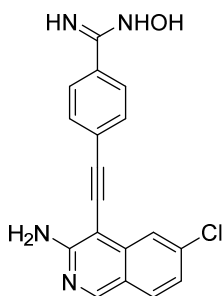
HSM1813

4-((4-(4,5-dihydro-1H-imidazol-2-yl)phenyl)ethynyl)isoquinoline. Following the described general procedure IV, yellow solid was obtained. ^1H NMR (500 MHz, DMSO- d_6) δ 9.37 (s, 1H), 8.79 (s, 1H), 8.35 (d, $J = 8.2$ Hz, 1H), 8.24 (d, $J = 8.0$ Hz, 1H), 7.96 (dd, $J = 19.4, 7.8$ Hz, 3H), 7.81 (dd, $J = 13.7, 7.7$ Hz, 3H), 3.65 (s, 4H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 163.43, 153.15, 146.66, 134.95, 132.48, 131.96, 131.31, 128.96, 128.85, 127.93, 127.85, 124.84, 124.02, 115.01, 96.65, 86.36, 50.06. HRMS (ESI^+) $[\text{M}+\text{H}]$ calcd for $\text{C}_{20}\text{H}_{16}\text{N}_3$ 298.1344, found 298.1341.



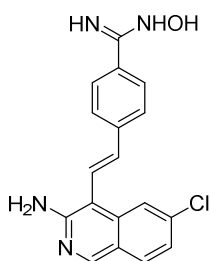
HSM1819

3-((3-amino-6-chloroisoquinolin-4-yl)ethynyl)-N-hydroxybenzimidamide. Following the described general procedure IV, yellow solid was obtained. ^1H NMR (500 MHz, DMSO- d_6) δ 9.75 (s, 1H), 8.92 (s, 1H), 8.04 (s, 1H), 7.96 (d, $J = 8.6$ Hz, 1H), 7.88 (s, 1H), 7.78 (d, $J = 7.5$ Hz, 1H), 7.72 (d, $J = 7.8$ Hz, 1H), 7.46 (t, $J = 7.7$ Hz, 1H), 7.28 (d, $J = 8.5$ Hz, 1H), 6.80 (s, 2H), 5.93 (s, 2H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 158.57, 153.00, 150.66, 138.73, 137.33, 134.15, 132.13, 131.55, 128.89, 128.65, 125.84, 123.48, 123.22, 121.31, 120.63, 99.88, 88.73, 83.91. HRMS (ESI^+) $[\text{M}+\text{H}]$ calcd for $\text{C}_{18}\text{H}_{14}\text{ClN}_4\text{O}$ 337.0856, found 337.0873.



HSM1820

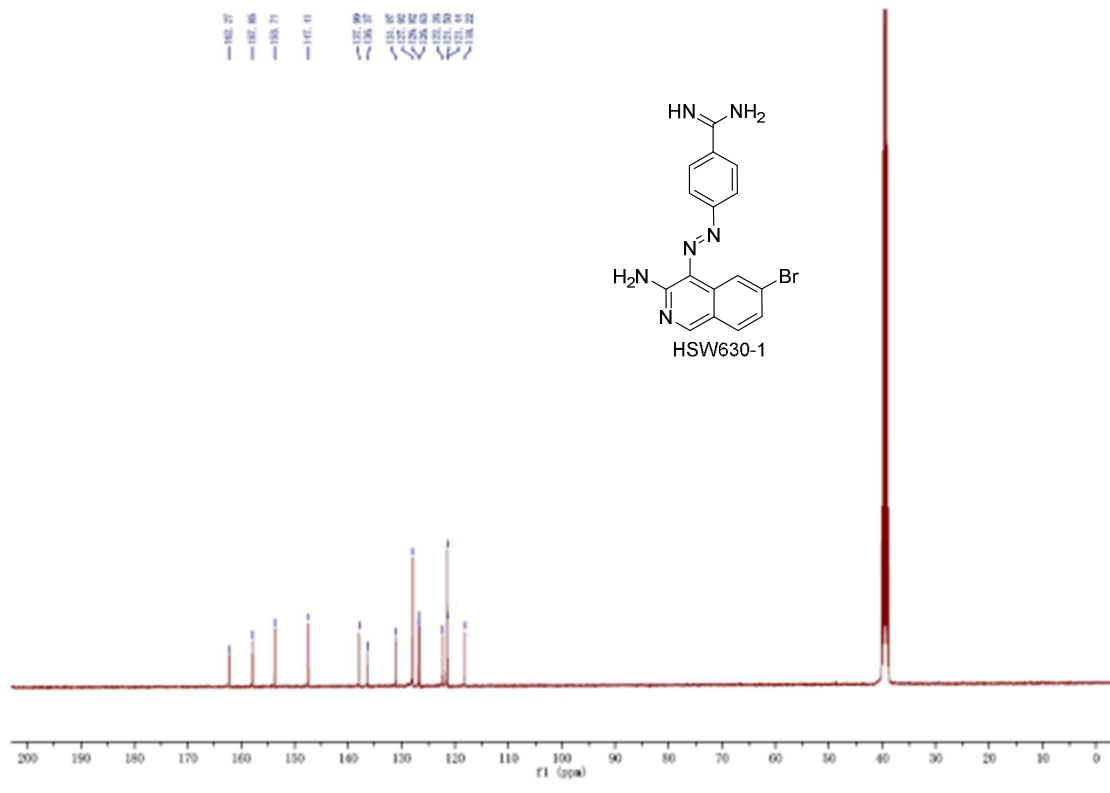
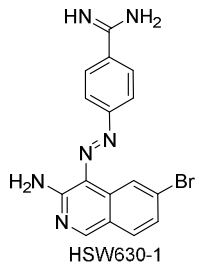
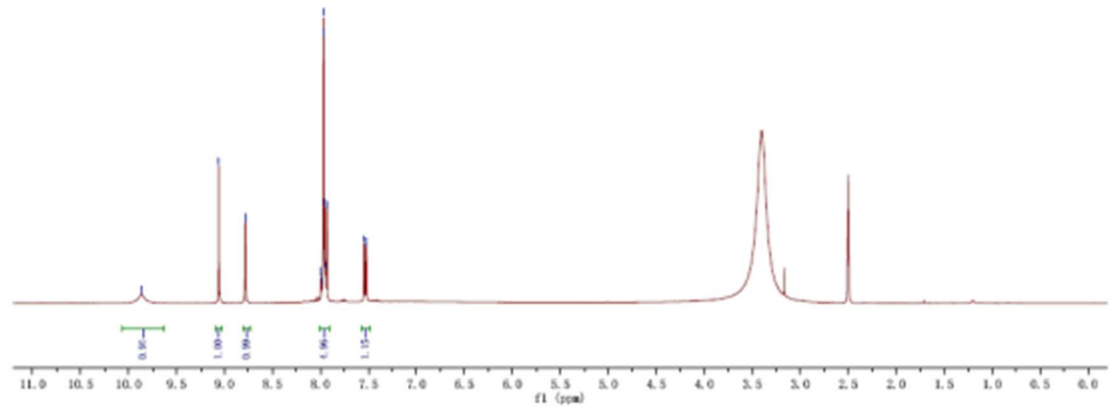
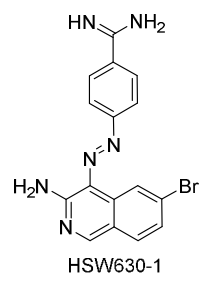
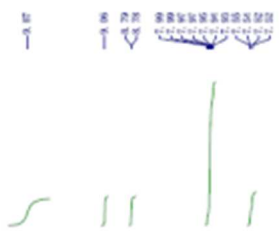
4-((3-amino-6-chloroisoquinolin-4-yl)ethynyl)-N-hydroxybenzimidamide. Following the described general procedure IV, yellow solid was obtained. ^1H NMR (500 MHz, DMSO- d_6) δ 9.79 (s, 1H), 8.91 (s, 1H), 7.95 (d, J = 8.6 Hz, 1H), 7.88 (d, J = 1.4 Hz, 1H), 7.80 – 7.72 (m, 4H), 7.27 (dd, J = 8.6, 1.9 Hz, 1H), 6.80 (s, 2H), 5.90 (s, 2H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 158.53, 153.00, 150.71, 138.69, 137.32, 133.41, 131.53, 125.81, 123.66, 123.47, 121.35, 120.62, 99.94, 88.76, 84.85. HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{18}\text{H}_{14}\text{ClN}_4\text{O}$ 337.0856, found 337.0865.

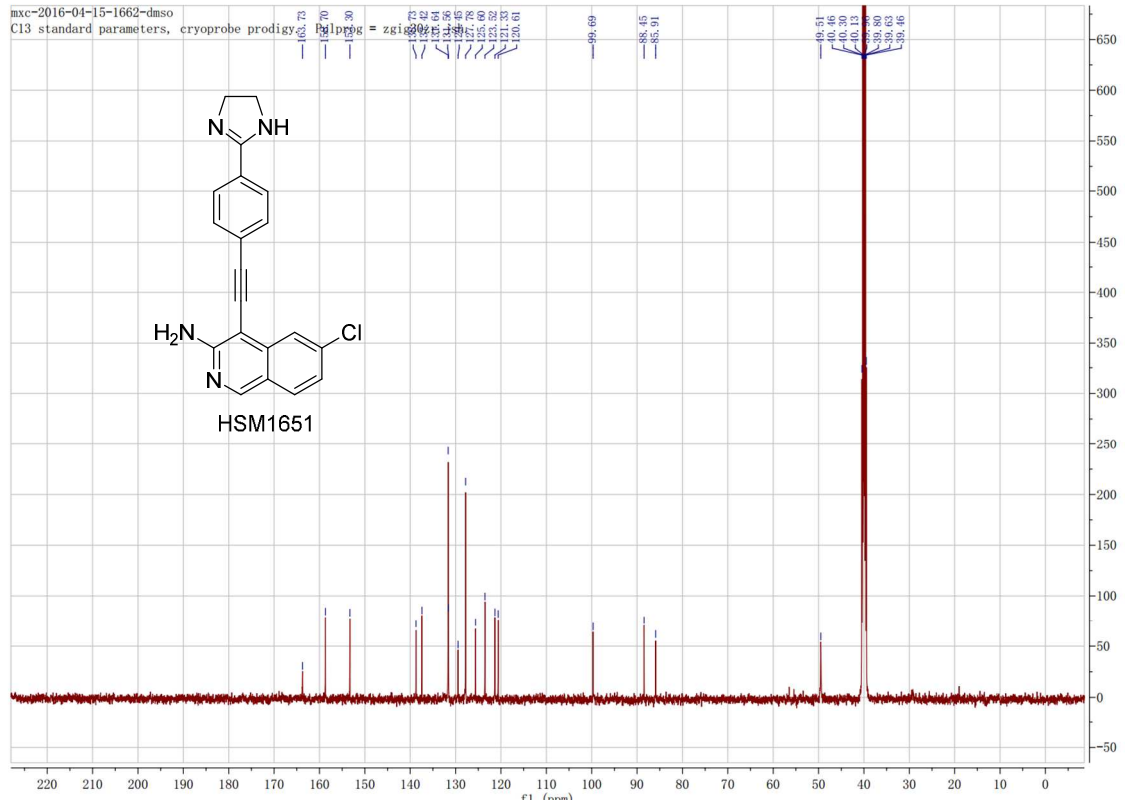
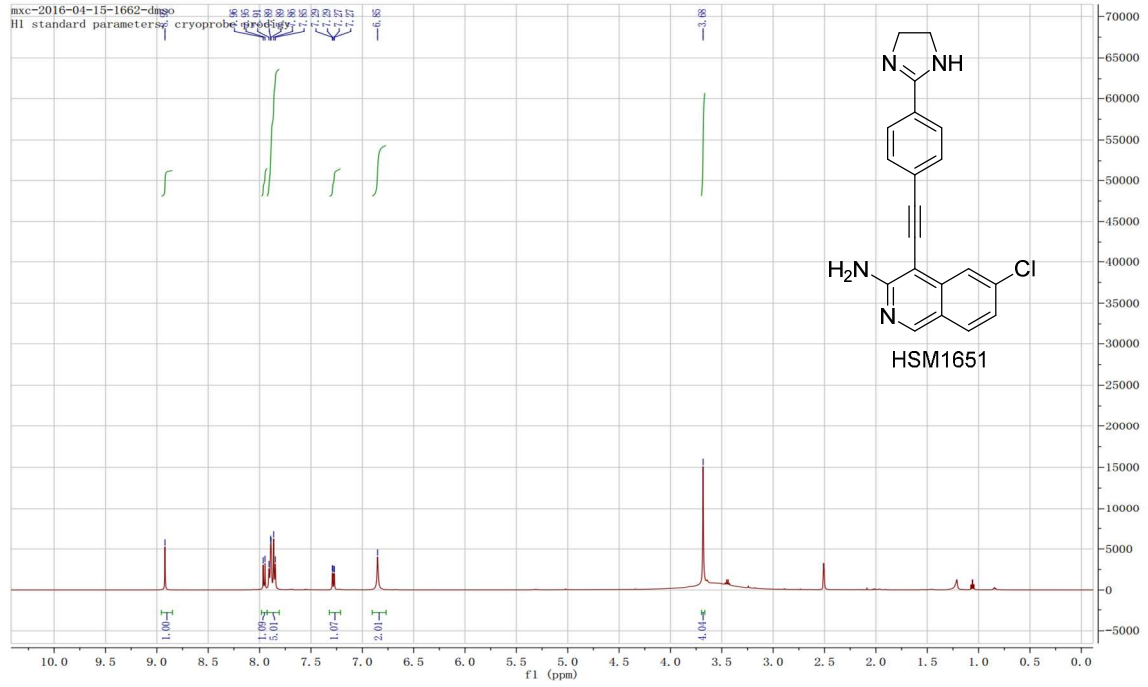


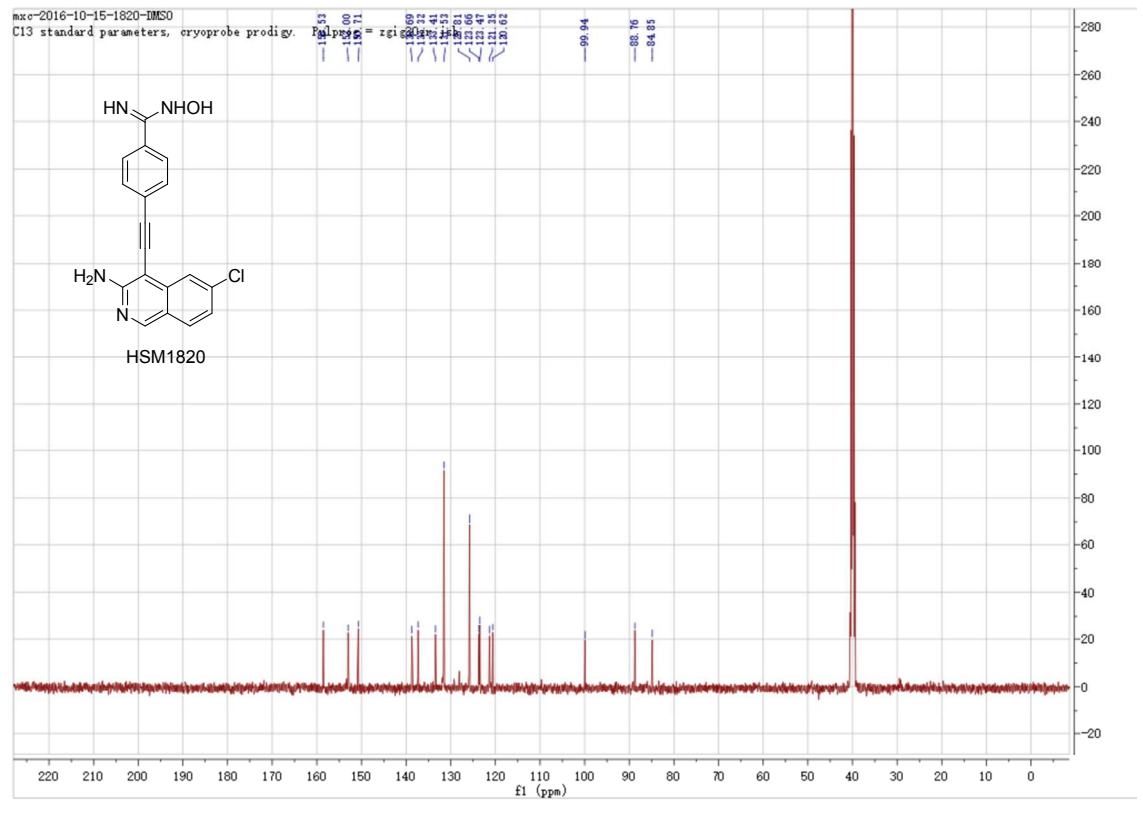
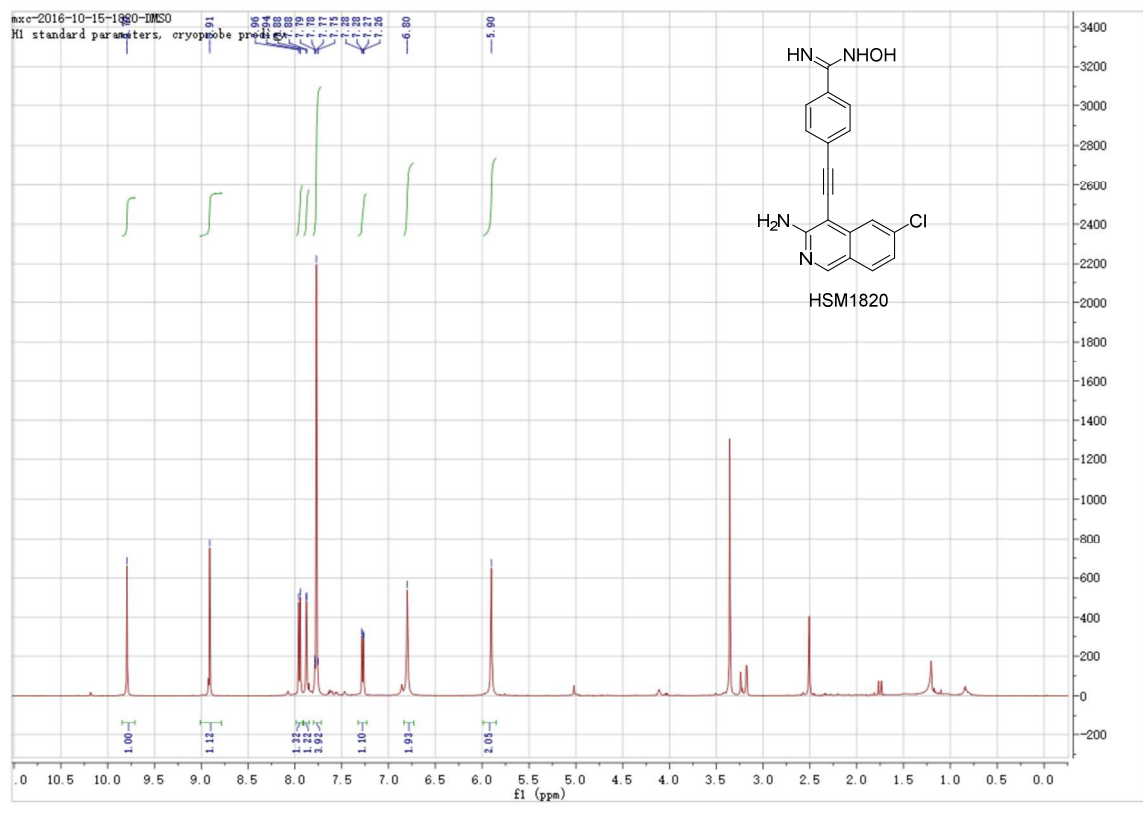
HSM1859

(E)-4-(2-(3-amino-6-chloroisoquinolin-4-yl)vinyl)-N-hydroxybenzimidamide. Following the described synthesis procedure V, yellow solid was obtained. ^1H NMR 500 MHz, DMSO- d_6) δ 9.66 (d, J = 6.2 Hz, 1H), 8.88 – 8.75 (m, 1H), 7.89 (dt, J = 17.8, 5.5 Hz, 2H), 7.77 – 7.63 (m, 4H), 7.33 (t, J = 17.4 Hz, 1H), 7.28 – 7.09 (m, 1H), 7.06 – 6.94 (m, 1H), 6.24 (s, 2H), 5.83 (s, 2H), ^{13}C NMR (126 MHz, DMSO- d_6) δ 154.75, 151.48, 151.00, 138.28, 136.75, 136.24, 134.02, 132.80, 131.32, 126.82, 125.88, 122.80, 122.59, 121.50, 121.20, 104.46, HRMS (ESI $^+$) [M+H] calcd for $\text{C}_{18}\text{H}_{16}\text{ClN}_4\text{O}$ 339.1013, found 339.1010.

Spectra of key compounds







Reference

1. Chopra, G.; Samudrala, R., Exploring Polypharmacology in Drug Discovery and Repurposing Using the CANDO Platform. *Curr Pharm Des* **2016**, *22* (21), 3109-23.