SUPPORTING INFORMATION

Alkynyl Benzoxazines and Dihydroquinazolines as Cysteine Targeting Covalent Warheads and Their Application in Identification of Selective Irreversible Kinase Inhibitors

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Contents

Methods

Glutathione Assay Procedure

Half-life of compounds with glutathione was determined by measuring the disappearance of parent over time. Compounds are incubated at 1 µM concentration in the presence of 4.6 mM glutathione (GSH) at 37°C. Each run contains a maximum of 10 compounds including verapamil (used as an internal standard) and two in-house reference compounds. Dimethyl sulfoxide (DMSO) concentration is kept below 0.1%. Data are obtained on Waters UPLC ACQ-TQD with Acquity UPLC system using a Waters XSelect HSS T3 C18, 2.5µm, 2.1 x 50mm column. Gradient analysis was employed using decreasingly polar mixtures using 0.1% formic acid as solvent A and acetonitrile as solvent B (10 - 90% MeCN gradient). The slope value, k, is determined by linear regression of the natural logarithm of the area ratio (parent peak area normalised to verapamil peak area) of the parent drug vs. incubation time curve. The *in vitro* half-life (*in vitro* $t_{1/2}$) is determined from the slope value: *in vitro* $t_{1/2} = -(0.693/k)$.

Kinetics Experiment Procedures

NMR

All NMR spectra were recorded on a Bruker 500 MHz instrument equipped with a 5 mm QNP cryoprobe. Chemical shifts (δ values) are given in parts per million (ppm) and referenced to the DMSO signal (2.50 ppm). NMR experiments used standard pulse sequences available in TopSpin 4.0 (Bruker GmbH). 1H qNMR experiments were carried out using the standard 1D 1H 30° pulse sequence at a constant temperature of 27 °C, and acquired as the summation of 4 transients and 1 equilibrating transient (signal unrecorded) with 30 s of relaxation time between transients. 64k points were used for acquisition covering a spectral width of 20 ppm. Data were collected at regular intervals for a defined number of experiments. Samples were retained within the spectrometer for the duration of the experiment and maintained at a steady 20 Hz rotation throughout. The experiment was controlled using a multi-acquisition automation program, which included Fourier-transform and basic phase-correcting commands. Data were processed using standard Bruker phase-correcting algorithm. Line broadening was maintained at 0.30 Hz throughout and a baseline correction was applied to the whole spectrum. Signals were integrated between defined chemical shift values at appropriate intervals throughout the course of the experiment.

NMR experiments were carried out according to a procedure reported by Golding et al.[1] Heterocycle (690 µL from a stock solution in DMSO-d6 containing 4.2 µmol of compound) was added to a DMSO-d6 solution (10 µL) containing DABCO (0.14 mg, 1.26 µmol) and DMF (0.33 µL, 4.2 µmol) to afford a heterocycle concentration of 6 mM in a total volume of 700 µL. *N*-acetylcysteine methyl ester (7.48 mg, 42 µmol) was added and the NMR tube containing the reagents was quickly inverted several times to aid mixing and dissolution. The thoroughly mixed solution was inserted into the NMR machine cavity and the acquisition of ^{1}H NMR data was immediately initiated. The time between the addition of the *N*-acetylcysteine methyl ester and completion of the first ^{1}H experiment was monitored and subsequent time intervals between experiments were calculated based on the defined parameters.

$$-\frac{d[sm]}{dt} = k'[sm]\frac{K[cysteine]_0[DABCO]_e}{[DABCO^+]_e + K[DABCO]_e}$$

$$-\frac{d[sm]}{dt} = k_{app}[sm]$$

$$ln\frac{[sm]}{[sm]_{t=0}} = -k_{app}[sm]$$

$$\frac{ln2}{k_{app}} = t_{1/2}$$

LCMS

A solution of heterocycle (0.1 mL, 0.70 µmol) in DMSO (7 mM) and (±) Verapamil HCl (0.1 mL, 0.70 µmol) in a 1:1 mixture of MeCN:water (7 mM) was added to pH8 buffer (1.2 mL) in an LCMS vial. 'Capped' amino acid (100 µL, 10.00 µmol) in water (0.1 M) was subsequently added and the reaction monitored by LCMS with sampling every 15 minutes using a 2 µL injection. UPLC was performed on CSH C18 reverse-phase silica, using a Waters XSelect CSH

C18 column with dimensions 2.1 x 50mm and particle size 1.7 micron. Gradient analysis was employed using decreasingly polar mixtures using 0.1% ammonia as solvent A and acetonitrile as solvent B (3 - 97% MeCN gradient). The slope value, k, is determined by linear regression of the natural logarithm of the area ratio (parent peak area normalised to verapamil peak area) of the parent drug vs. incubation time curve. The *in vitro* half-life (*in vitro* $t_{1/2}$) is determined from the slope value: *in vitro* $t_{1/2} = -(0.693/k)$.

X-ray Crystallography

Protein expression and purification was carried out as described previously[2]. All structures were obtained by co-crystallization after 2-hour pre-incubation with a 3-fold molar excess of inhibitor. Crystallization conditions as well as data collection and refinement statistics are available from the PDB database. X-ray diffraction data were collected at Diamond Light source beamlines I03 and I04, and Soleil beamline Proxima I. Images were processed using XDS[3], Aimless[4] and other programmes from the CCP4 suite[5]. Structures were solved by molecular replacement using Phaser and automatic refinement with Buster[6] was interspersed with manual refinement using Coot[7]. Initial ligand topologies and restraints were generated by Grade[8]. PDB depositions are available for compounds **24** (6XV9 and 6XVJ), **25** (6XVA) and **26** (6XVB and 6XVK).

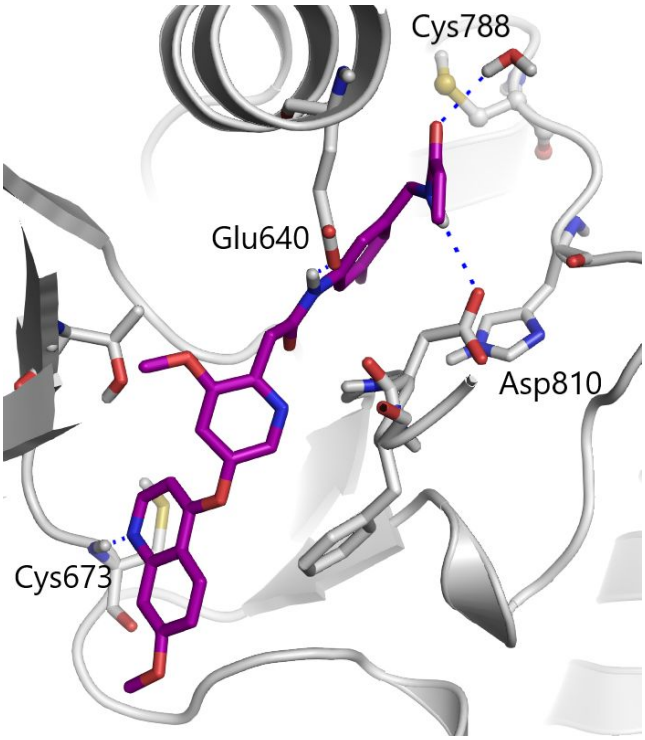


Figure S1. Crystal structure of compound **25** bound in c-KIT (6XVA).

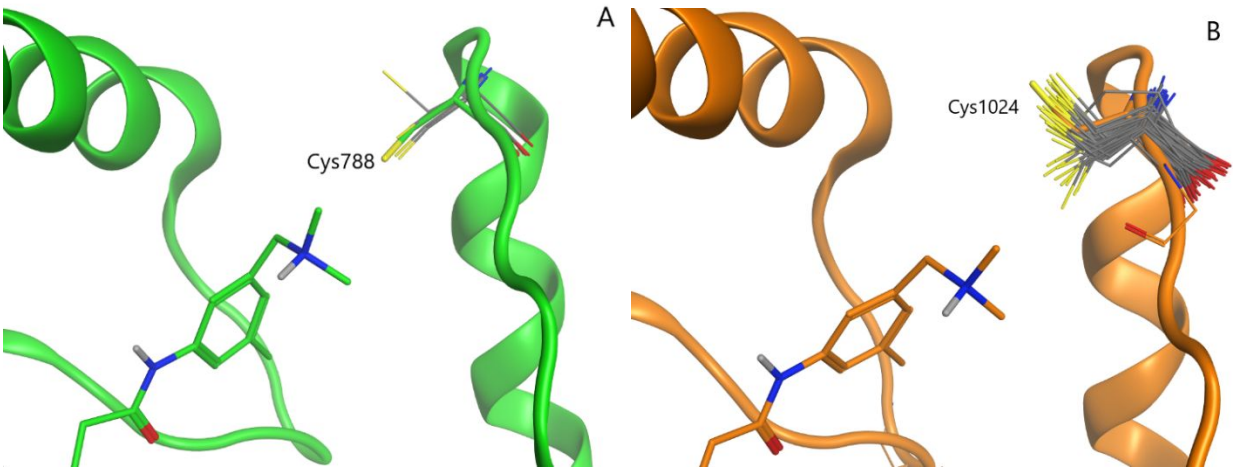


Figure S2. Analysis of cysteine positioning in c-KIT and KDR. A. Overlay of Cys788 position of c-KIT in PDB crystal structures with compound **24** bound (6XV9). B. Overlay of Cys1024 position of KDR in PDB crystal structures with compound **24** bound (6XVJ).

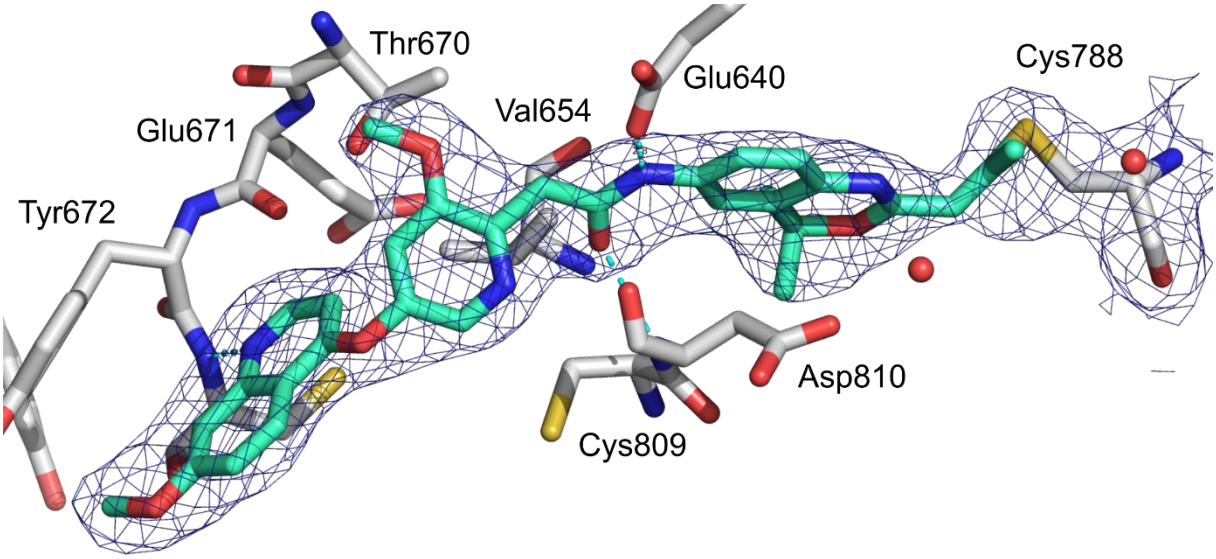


Figure S3. Crystal structure (2.15 Å) of compound **26** covalently bound to c-KIT Cys788. Composite $2|F_o|-|F_c|$ electron density map is shown contoured at 1 σ.

Table S1. Comparison of the *in vitro* pharmacokinetic properties of compounds **25** and **26**

	26	***25***
KIT IC_{50} (μM)*	2.12	0.21

KDR IC_{50} (μM)**	0.98	0.53
GSH $t_{1/2}$ (min)[a]	65	136
PPB (% free)[b]	<0.35	1.4
Rat Heps (μL/min)[c, d]	93	289
Hu Mics (μL/min/mg)[c, e]	36	62
LogD	4.2	4.0
Solubility (μM)[f]	2	0.7

* [ATP] 300 μM. ** [ATP] 75 μM. [a]Determined by rate of disappearance of parent using LCMS. [b]Determined by rapid equilibrium dialysis at 5 μM compound concentration using 100% plasma at 37ºC. [c] Intrinsic clearances are obtained from the first order rate constant of loss of parent compound at 37ºC [d]Compound concentration 1 μM, hepatocyte concentration 10^6 cells/mL. [e]Compound concentration 1 μM, microsomal protein 1 mg/mL, 1 mM NADPH. [f]Solubility from dried DMSO solution in PBS (phosphate buffer solution) pH 7.4.

Quantum Mechanical Modelling of Adduct Formation and Transition State Barrier

Compounds **4a**-**14** and MeS^- (surrogate for GSH) were imported into Maestro in SMILES format and prepared using LigPrep for the addition of hydrogen atoms. All compounds were preliminarily geometry optimized with Jaguar at the B3LYP/6-31G(d,p) level of theory, subsequently compounds were overlaid using SMARTS matching to ensure modelling consistency. Compound adducts were modelled using the compound optimized geometries and the MeS^- was added in the s-cis conformation following observations that this conformation was approximately 1.5 kcal/mol lower in energy than s-trans,[9] which was also in agreement with this study [data not included]. Resulting energies and lowest energy molecular orbital (LUMO) of both compounds and compound adducts were calculated using Guassian16 M06-2X/6-31G(d,p)-IEF-PCM(water) level of theory QM. For a subset of compounds (**4a**-**5a**, **5e**, **8**, **12** and **13**) transition state energy was calculated using quadratic synchronous transit method (qst3) using Guassian16, this method is significantly more computationally expensive hence, a smaller subset was used to confirm the close linear relationship with adduct formation energy. One imaginary frequency was confirmed for all transition states.

Table S2. Key Experimental and Calculated values for compounds **4-14**

Compound number	**GSH $t_{1/2}$ (min)**	**Buffer $t_{1/2}$ (min)**	**LUMO (Hartree)**	**Adduct Formation Energy (kcal/mol)**	**Transition State Energy Barrier (kcal/mol)**
4a	299	>10000	-0.02663	1.370480748	4.627255053
4b	>10000	>10000	-0.01983	3.847260744	6.885661743

4c	>10000	>10000	-0.01733	4.223138935	6.947157674
4d	19	>10000	-0.02594	-8.089224964	4.453434922
4e	1268	>10000	-0.01887	-2.353160625	7.123487844
5a	NV	2680	-0.03106	-4.025473443	2.025600666
5b	1.6	3464	-0.02666	-3.514680709	
5c	1.4	609	-0.0299	-1.65599757	
5d	7	>10000	-0.03341	-9.70192438	
5e	82	>10000	-0.02739	-4.523715986	5.526476166
***o*-MeO-5e**	74	>10000	-0.03126	-1.505395291	
***m*-MeO-5e**	78	>10000	-0.03041	-3.448792212	
***p*-MeO-5e**	133	>10000	-0.02535	-1.44327185	
***m'*-MeO-5e**	77	>10000	-0.02707	-3.194023355	
6	206	>10000	-0.02638	0.224020891	
7	135	6153	-0.02362	1.456449549	
8	620	6183	-0.02996	-3.933857056	6.744472106
9	37	>10000	-0.02362	-4.700673665	
10	66	5924	-0.01006	0.90298617	
11	1.5	>10000	-0.01737	1.094376568	
12	55	70	-0.0491	-10.3570443	4.149092814
13	79	3390	-0.03235	-3.582451736	6.842991097
14	82	>10000	-0.03885	-4.425824504	

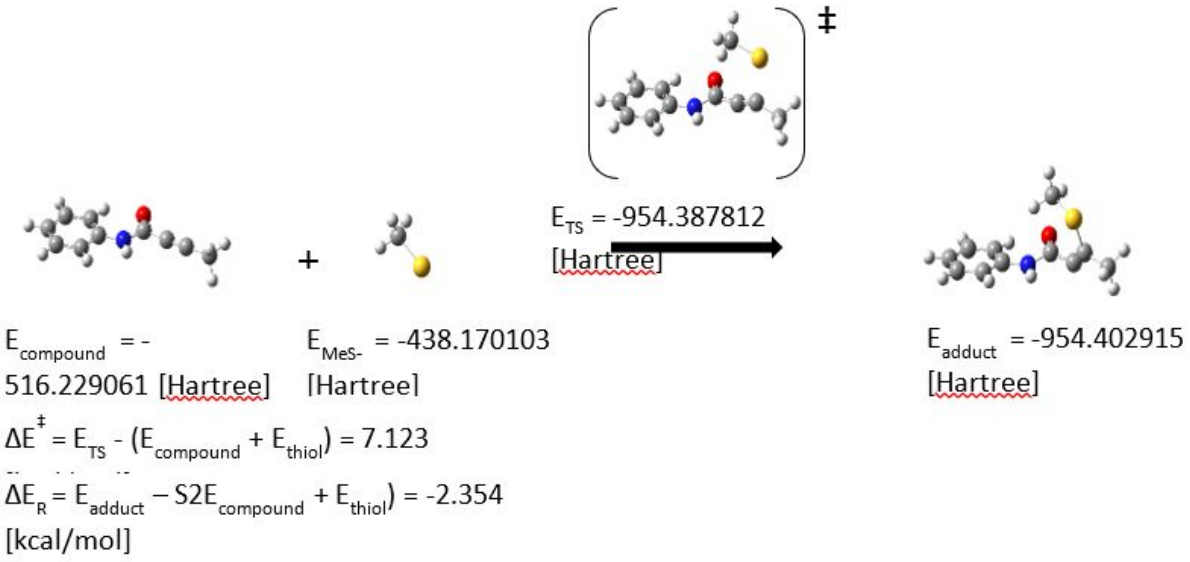


Figure S4. Scheme showing the reaction modelling and resultant energies using M06-2X/6-31G(d,p)-IEF-PCM level of theory for an example un-cyclised β-substituted acetylene warhead.

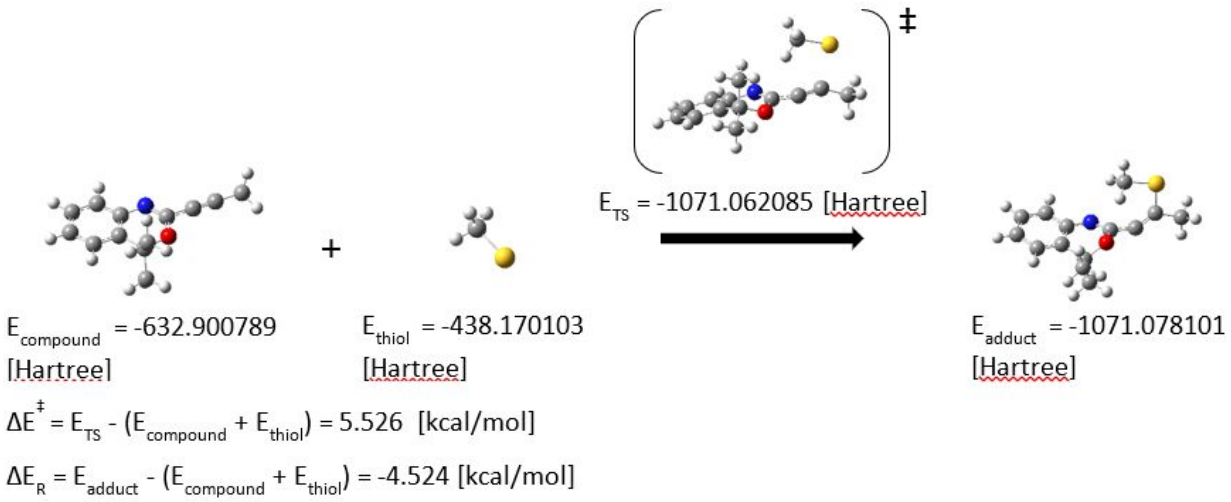


Figure S5. Scheme showing the reaction modelling and resultant energies using M06-2X/6-31G(d,p)-IEF-PCM level of theory for an example cyclised β-substituted acetylene warhead.

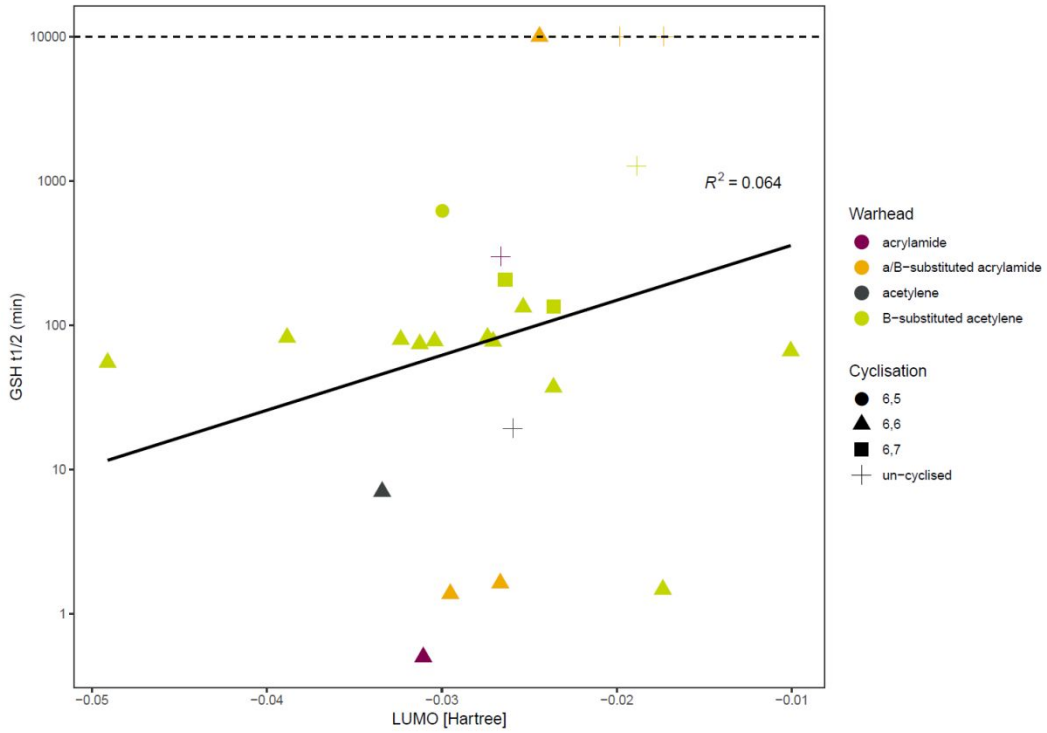


Figure S6. Correlation between glutathione half-life and LUMO energy

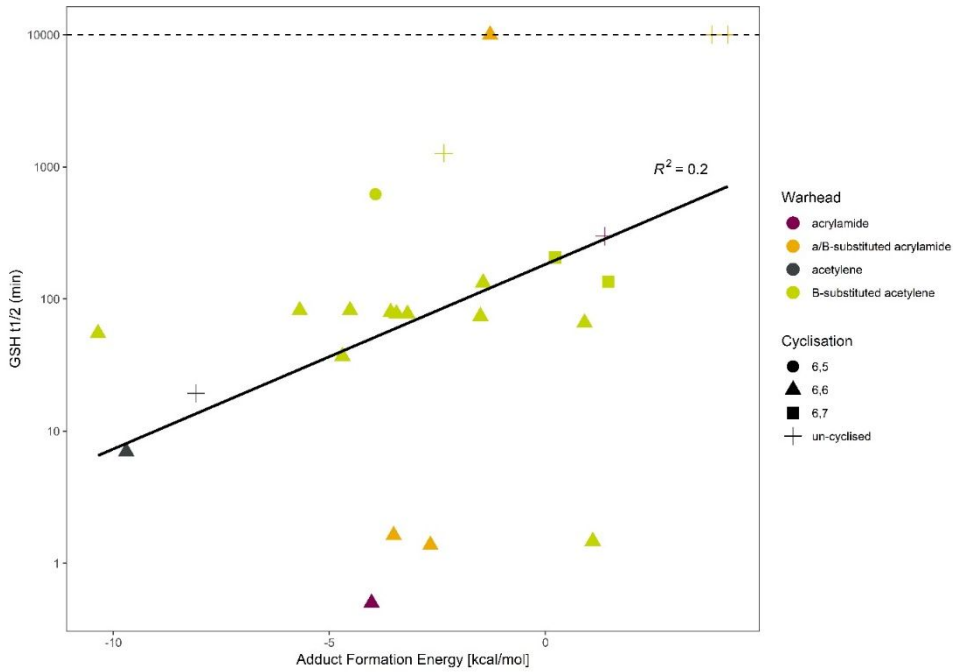


Figure S7. Correlation between glutathione half-life and adduct formation energy

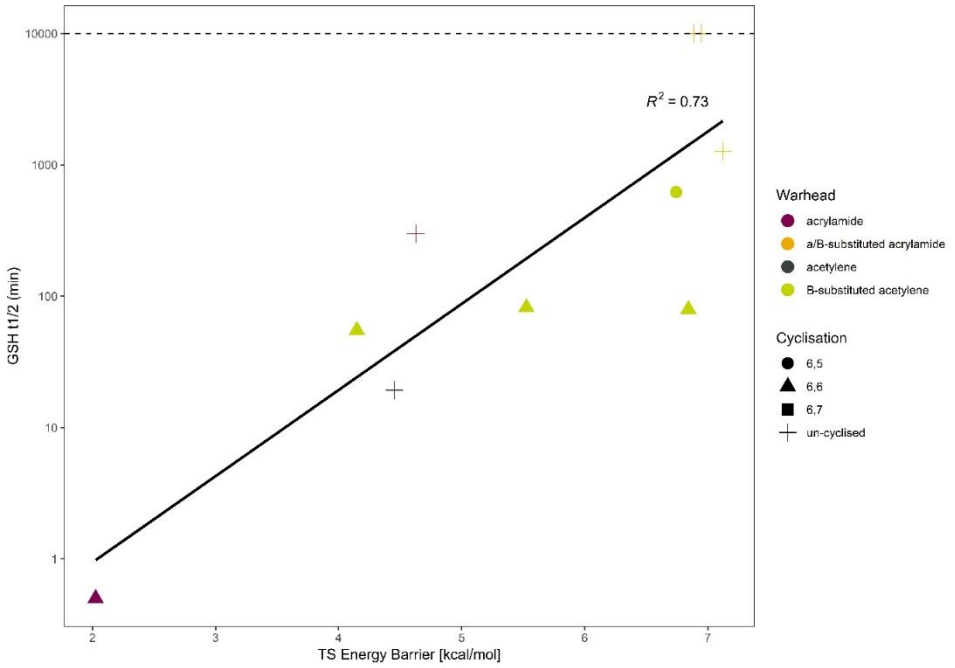


Figure S8. Correlation between glutathione half-life and TS energy barrier

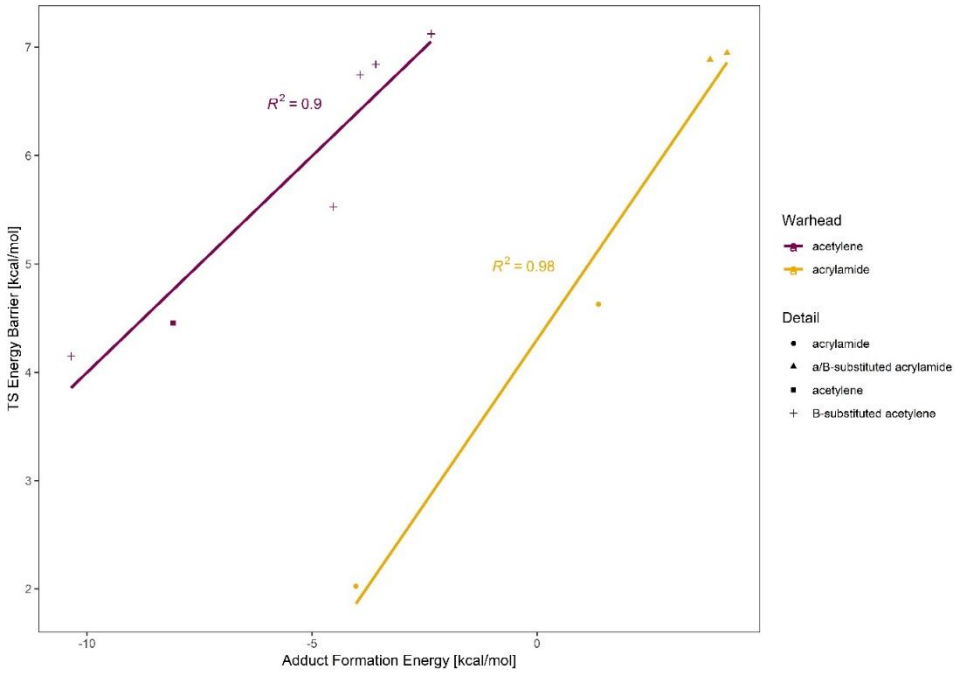


Figure S9. Correlation between adduct formation energy and TS energy barrier for acetylenes and acrylamides

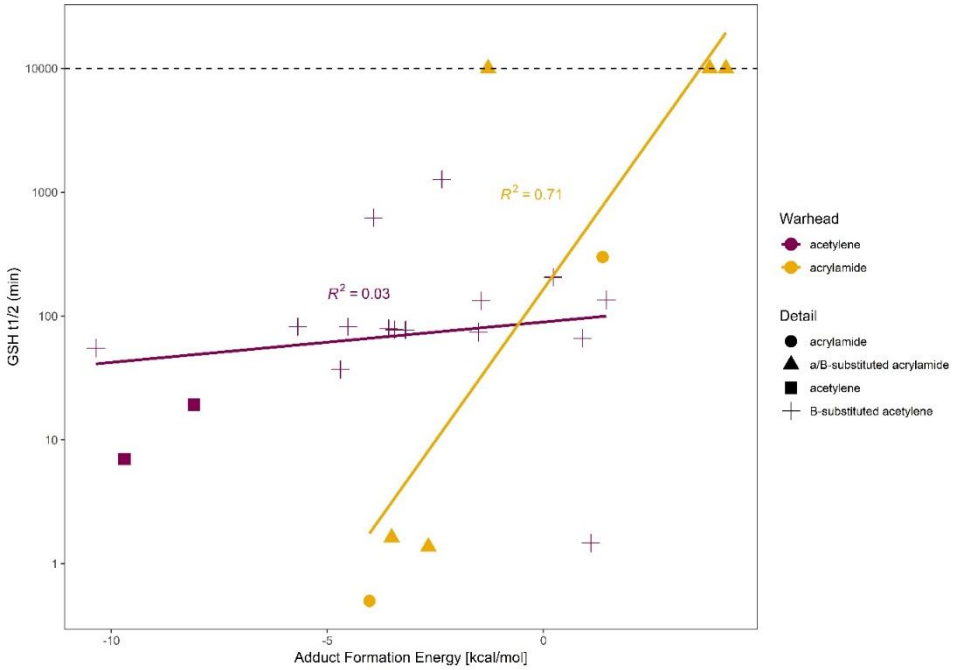


Figure S10. Correlation between glutathione half-life and adduct formation energy by warhead type

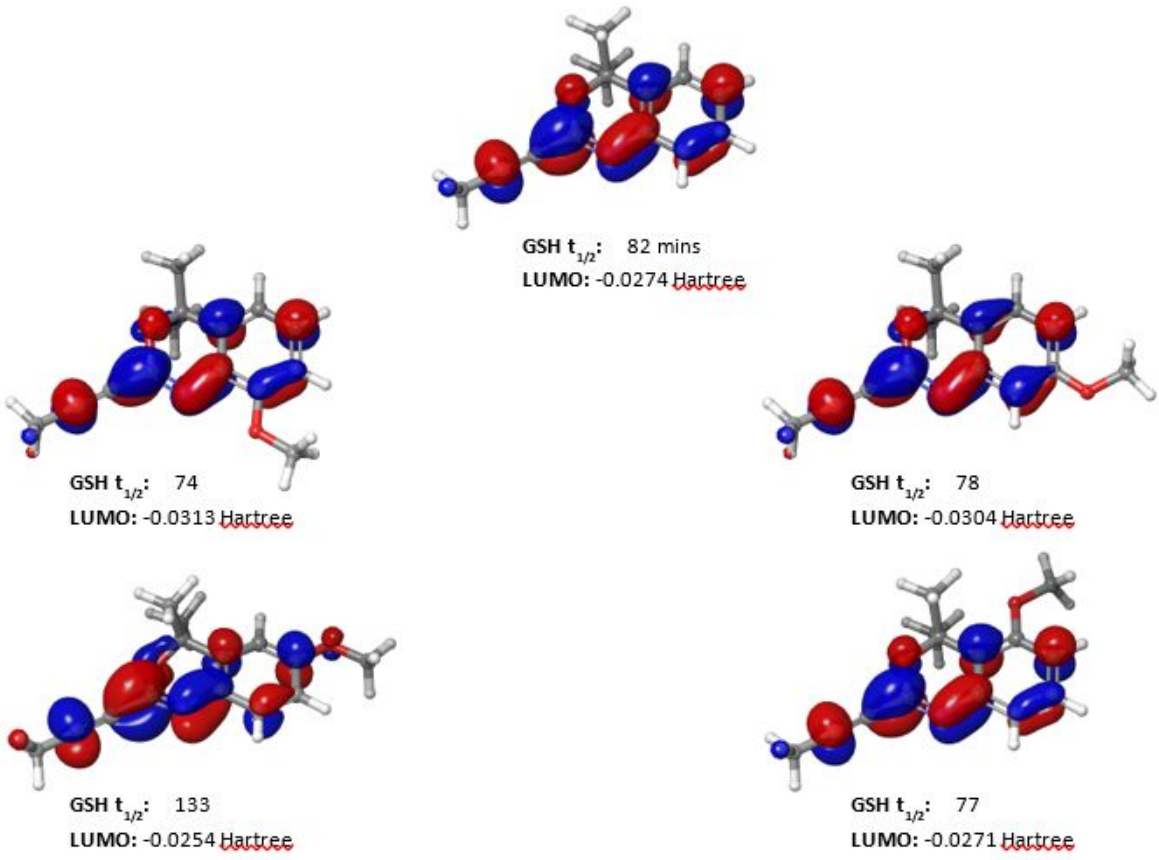


Figure S11. LUMO energy diagrams and glutathione half-lives for compound **5e** and it's corresponding methoxy analogues

JAK3 Protein Incubation and Intact Mass Spec

Incubation of JAK3 protein with covalent ligands

135µl of JAK3 protein (3.6 µM, 0.24 mg/mL) was incubated with 0.675µL of compound **18** (10 mM in DMSO) for 3 hours at room temperature (final concentration 50µM, containing 0.5% DMSO). The sample was then snap frozen and stored at -80 °C. The above procedure was repeated using a literature JAK3 ligand *N*-[3-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl]prop-2-enamide for comparison.[10] JAK3 protein was obtained from ThermoFisher (part no PV3855: 67.4 kDa, amino acids 781-1124, with GST tag) and supplied undiluted in storage buffer; 50mM Tris-HCl, pH 7.5, 150mM NaCl, 2mM DTT, 0.5mM EDTA, 0.02% Triton X-100, 50% glycerol.

Sample Preparation – Desalting

Samples were thawed on ice. To remove glycerol, salts and unreacted compound, protein samples were buffer exchanged and desalted using Thermo Scientific Zeba spin columns (89882). Columns were removed from the fridge 30 minutes before use and brought to room temperature. Columns were pre-equilibrated with 10mM Tris pH 8.0.

Sample Preparation - Intact Mass Analysis

Prior to intact analysis, protein was reduced by the addition of DTT (dithiothreitol) with incubation at 37 ^{o}C for 30 minutes. 100µl of water was added to a tube of Pierce™ DTT, No-Weigh™ Format (Thermo, A39255) to give a 0.5M stock solution. 2µl of this was added to each protein sample to give a final concentration of 10mM reducing agent.

Intact Protein Analysis

Intact protein samples were analysed by reversed phase LC-MS. The reverse-phase separations of reduced samples were performed on a UPLC system (Waters) with an Acquity Protein BEH C4 Column, 300Å, 1.7 µm, 2.1 mm X 50 mm using a gradient of 5-45% B from 3-14 minutes. Mobile phase B was acetonitrile with 0.1% formic acid or 0.01% trifluroacetic acid, whilst solvent A was water with 0.1% formic acid or 0.01% trifluroacetic acid. The flow rate was maintained at 0.150 ml/min, and column temperature was maintained at 65 °C.

Mass spectrometric analysis was performed on an Orbitrap Fusion Tribrid mass spectrometer (Thermo Scientific) operated in intact protein mode. The source voltage was set to 3500V for positive ionisation with a sheath gas (arb) of 35 and aux gas (arb) of 10. The ion transfer tube temperature was set to 300 ^{o}C. Orbitrap scan data was collected at a resolution of 15000 fwhm over an *m/z* range of 1000-3600 with an RF lens value of 80%. The AGC target was set of $4e^5$ with a maximum inject time of 200 msec. All data were acquired using Xcalibur software and visualised using BioPharma Finder (Thermo Scientific).

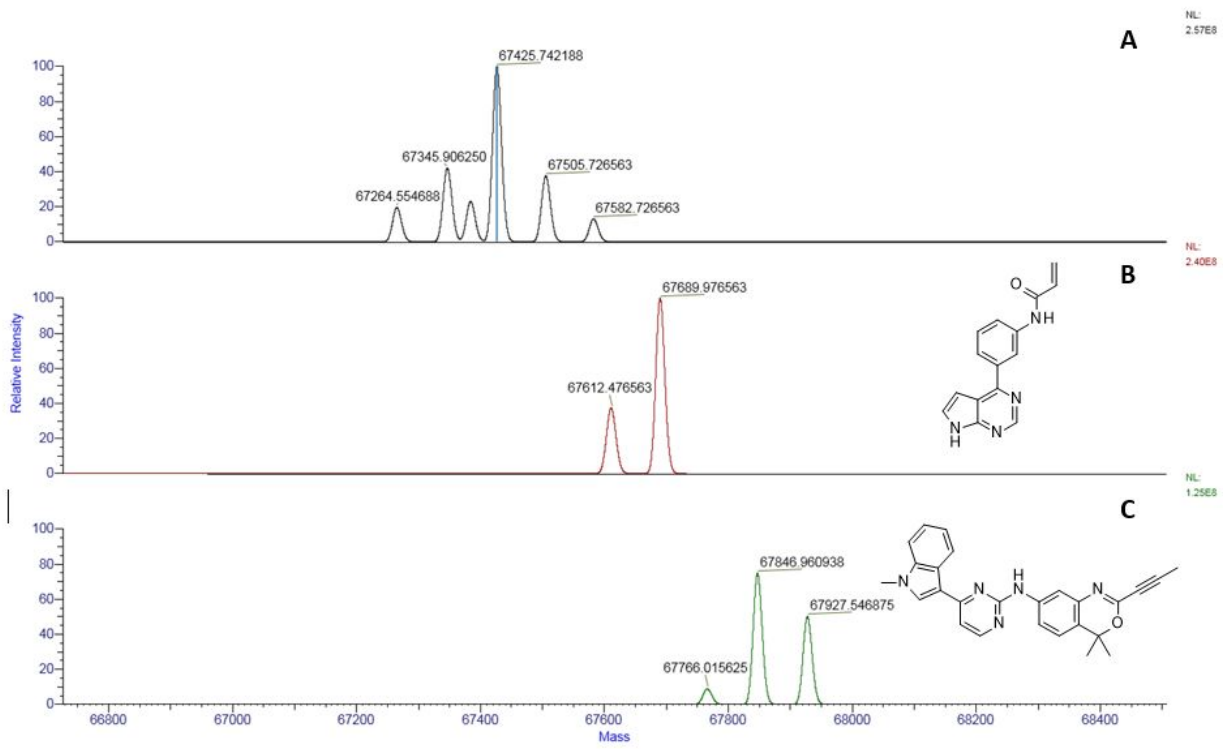
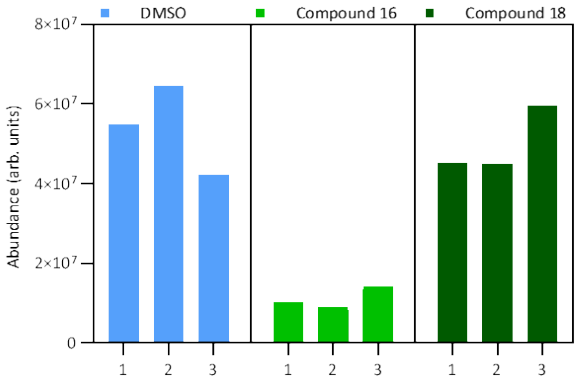


Figure S12. Intact mass anlysis of JAK3 protein. A) MS of native JAK3 protein. B) MS of JAK3 with literature reference compound bound.[10] C) MS of JAK3 with compound **18** bound.

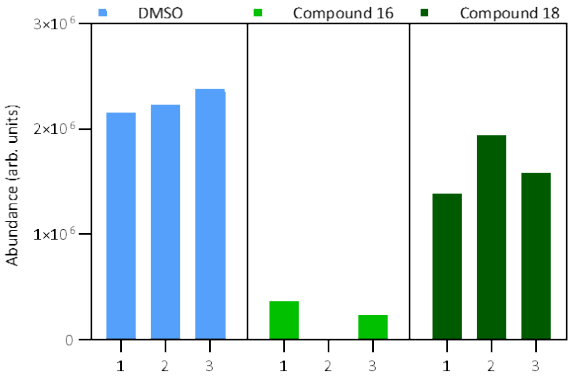
Free cysteine reactivity profiling

NCI-H358 cells were treated in biological triplicate with compound **16**, **18** or vehicle for 2 hours. After washing with phosphate buffer saline, cells were lysed directly in lysis buffer with sonication. Exposed cysteine residues were labelled by incubation with 100µM iodoacetamide desthiobiotin (AstraZeneca) for 1 hour at room temperature. Unreacted iodoacetamide desthiobiotin was removed by acetone precipitation. Proteins were denatured in 8M urea, 50mM ammonium bicarbonate with addition of 10mM dithiothreitol and heating to 65oC for 20 minutes. Newly exposed cysteines were then capped by alkylation with 50mM iodoacetamide. Lysates were de-salted and sequencing grade trypsin added at a ratio of 1:50 (enzyme:protein) with digestion at 37oC overnight. Biotinylated peptides were enriched from the digest by incubation with streptavidin agarose resin (ThermoFisher Scientific) for 2 hours. The resin was sequentially washed with phosphate buffer saline and water before captured peptides were eluted in 50% (v/v) acetonitrile, 0.1% (v/v) trifluoracetic acid. Peptides were dried by centrifugal evaporation and then resuspended in 0.1% (v/v) formic acid for analysis. The LC-MS/MS system consisted of an Acquity M-Class UPLC (Waters) coupled to a Fusion Tribrid Orbitrap mass spectrometer (ThermoFisher Scientific). Peptide separation was achieved across a 25cm HSS T3 column (Waters) using a 60-minute gradient ranging from 5-35% acetonitrile, 0.1%

formic acid at a flow rate of 0.3µl/min. Peptide identification was obtained by searching MS2 spectra against a database of the non-redundant human proteome downloaded from UniProt (ID:00000UP5640). Search parameters allowed variable modification of peptides as follows; cysteine desthiobiotinylation +296.185 Da, cysteine carbamidomethylation +57.021 Da and methionine oxidation +15.995 Da. Peptide identification and label free quantification between compound treated and vehicle samples was completed within Proteome Discoverer version 2.2 (ThermoFisher Scientific). The figure below highlights acrylamide **16** but not benzoxazine **18** binds to RTN4 and PAF1.



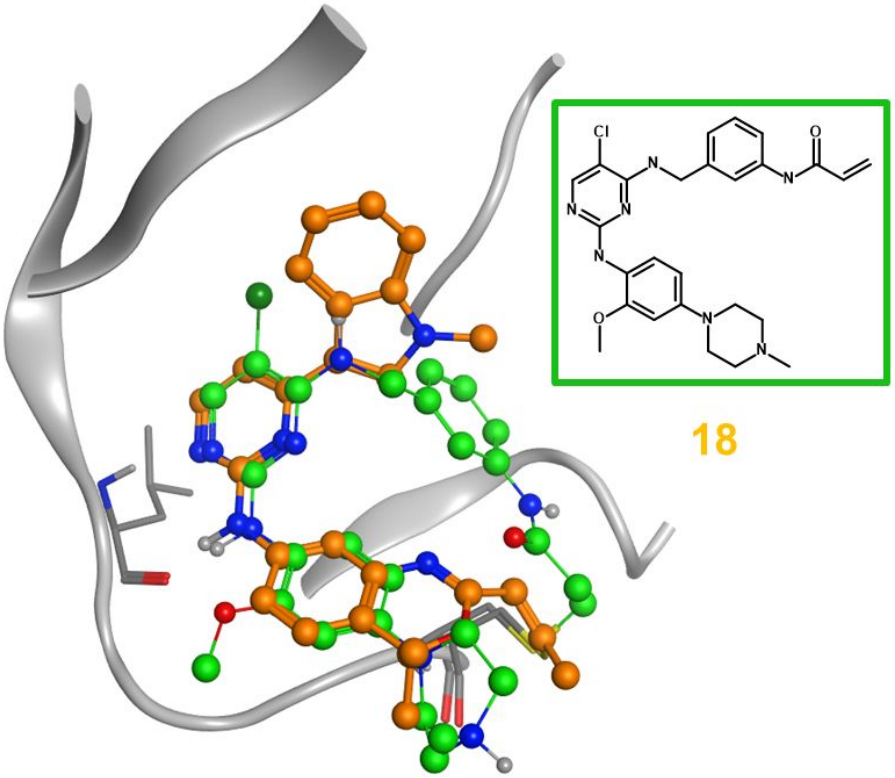
RTN4, Reticulon-4
Cysteine 1101: YSNSALGHVNC[+296.185]TIK



PAF1, RNA polymerase II-associated factor 1 homolog
Cysteine 36: YC [+296.185] NSLPDIPFDPK

JAK3 Ligand Docking

Molecular modelling was utilized to identify the likely binding mode of **18** in JAK3. **18** was covalently docked into the JAK3 crystal structure 4Z16. 4Z16 was prepared using the protein preparation modules in Maestro (version 2019-2) and overlaid, specifically adding and optimizing hydrogen positions. All water molecules were removed from the X-ray structures for modelling purposes. The covalent bond to Cys909 was manually formed using the builder module, followed by optimization of ligand and protein sidechains to identify a low energy binding mode. The predicted binding mode is shown to be in excellent agreement with the 4Z16 structure, shown in the green box.



c-KIT ligand docking

Molecular modelling was utilized to rationally design **26** to c-KIT. 6XVA was prepared using the protein preparation modules in Maestro (version 2019-2), specifically adding and optimizing hydrogen positions. All water molecules were removed from the X-ray structures for modelling purposes. The predicted distance of the alkenyl warhead of **26** to Cys788 was 3.3 Å, consistent with the potential to form a covalent bond.

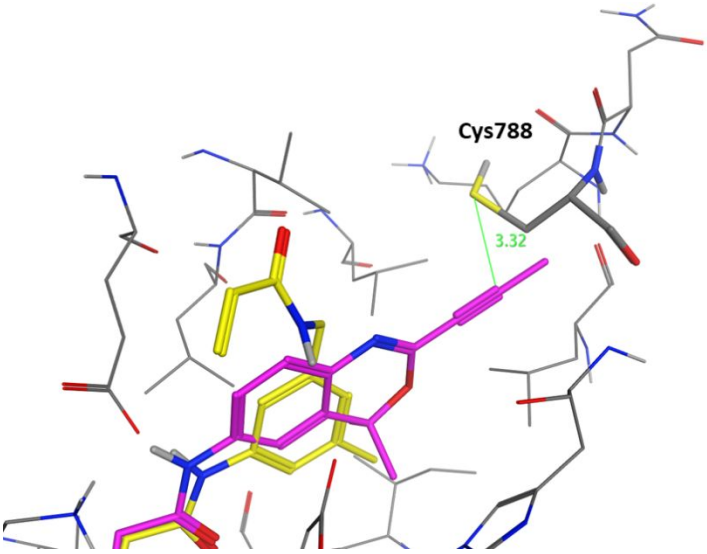


Figure S13. Modelling of the **26** predicts potential to access Cys788 in KIT.

PDGFR ligand docking

Molecular modelling was utilized to identify the likely binding mode of **26** in an isoform of PDGFR. A binding site similarity analysis was carried out using PSILO (version 2019.01) from Chemical Computing Group which identified 5GRN[*] as the PDGF isoform with the most similar ATP pocket (Score 108.015 and DPI 0.098Å). The crystal structure is of the PDGFRA isoform with WQ-C-159 bound, at 1.77 Å resolution. 0AKIT and 5GRN X-ray structures were then prepared using the protein preparation modules in Maestro (version 2019-2) and overlaid, specifically adding and optimizing hydrogen positions. All water molecules were removed from the X-ray structures for modelling purposes. WQ-C-159 was deleted form the 5GRN binding site and replaced with **26** from 0AKIT, with the covalent bond to Cys814 manually formed using the builder module. Ligand and protein side-chains were then optimized using macromodel to identify a suitable low energy binding mode.

[*]X.E.Yan, C.H.Yun. Crystal structure of PDGFRA in complex with WQ-C-15. *To be published*

Protein Labelling Reactions

Reaction Evaluation by LC-MS

All protein conjugation reactions were assessed using the LC-MS methods described below. As an example, the total ion chromatogram (TIC), combined ion series, and deconvoluted spectrum are shown for a C2Am-Cys95 control sample.

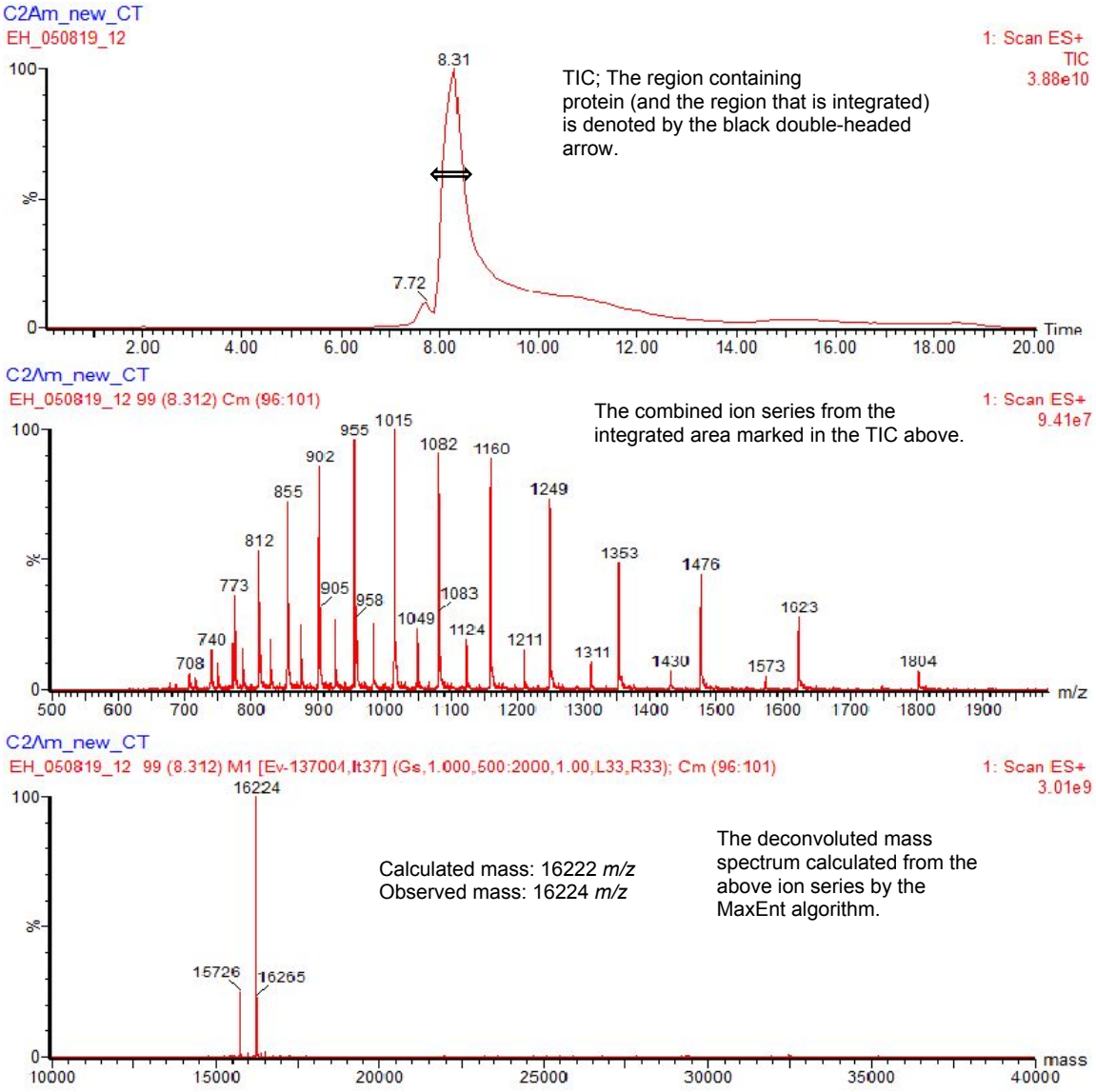


Figure S14. ESI-MS of C2Am-Cys95 control stored in the presence of DTT. Peak at 15726 m/z represents an impurity.

C2Am Studies

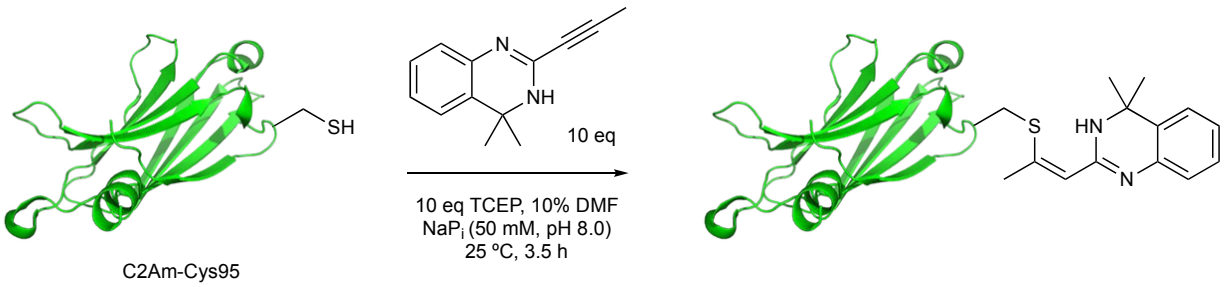
C2Am-Cys95 (obtained from collaborator)[11]

Sequence (inserted Cys residue in bold and underlined):

GSPGISGGGGGILDSMVEKLGKLQYSLDYDFQNNQLLVGIIQAAELPALDMGGTSDPYVKVFLLPDKKKKFETKVHRKTLNPVFNEQ FTFKVPY**C**ELGGKTLVMAVYDFDRFSKHDIIGEFKVPMNTVDFGHVTEEWRDLQSAEK

M_w = 16222.58 Da

C2Am-Cys95 reaction with Compound 11



C2Am-Cys95

In a 35 µL total reaction volume, C2Am-Cys95 (4.32 µL at 81 µM in 50 mM NaP_i pH 8 buffer, 350 pmol, 10 µM final concentration), **11** (1.75 µL at 2 mM in DMF, 3.50 nmol, 100 µM final concentration), and tris(2-carboxyethyl)phosphine (TCEP; 2.00 µL at 1.75 mM in water, 3.50 nmol, 100 µM final concentration) were added to DMF/NaP_i (1.75 µL DMF in 25.18 µL 50 mM NaP_i pH 8 buffer) and vortexed for 30 seconds. Samples of 10 µL were taken from the reaction mixture for LC-MS analysis. Approximately 83% conversion was observed after 3.5 h at 25 ºC with full conversion observed upon using higher equivalents of **11** under the same conditions (calculated mass: 16420 *m/z*; observed mass: 16420 *m/z*).*

*$(M+41)^+$ peaks for the products were observed, where the additional 41 Da is contributed from the acetonitrile in the mobile phase.

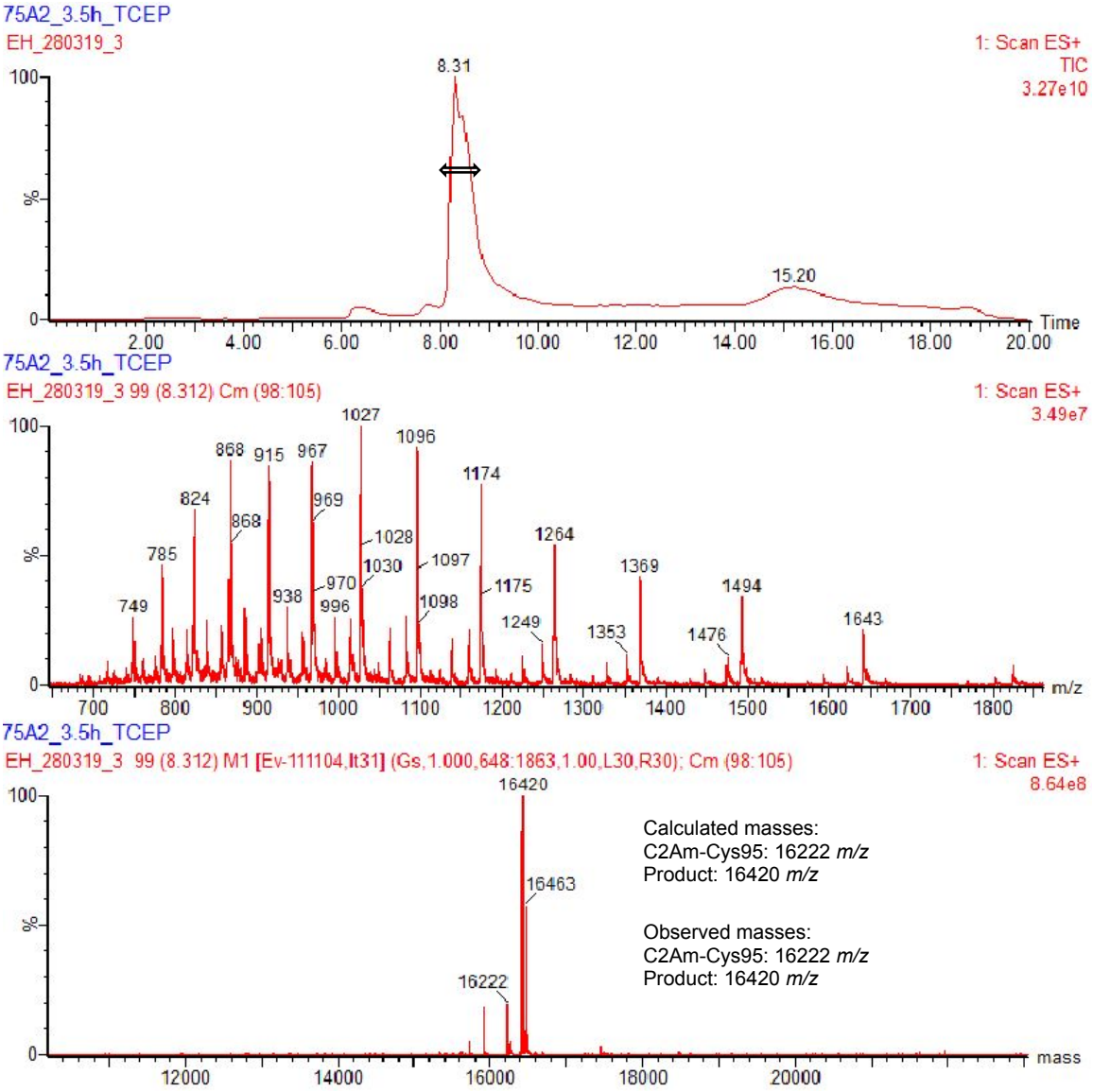
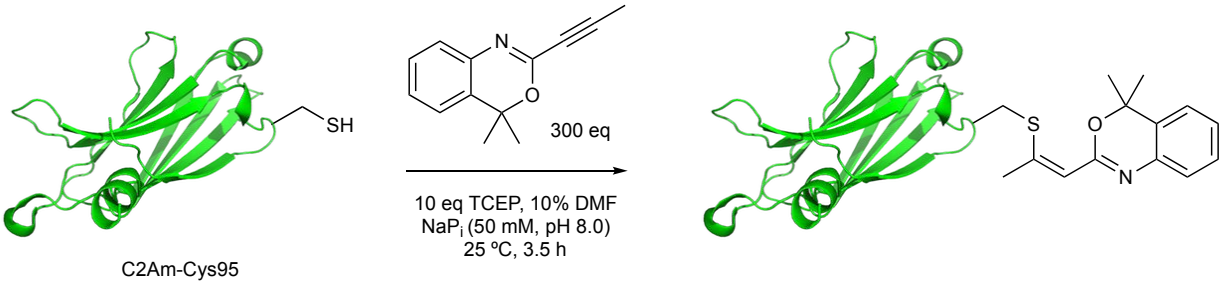


Figure S15. ESI-MS of the reaction of C2Am-Cys95 with **11** (10 eq) after being shaken for 3.5 h at 25 ºC.

C2Am-Cys95 reaction with compound 5e



C2Am-Cys95

In a 35 µL total reaction volume, C2Am-Cys95 (4.32 µL at 81 µM in 50 mM NaP_i pH 8 buffer, 350 pmol, 10 µM final concentration), **5e** (2.58 µL at 40.7 mM in DMF, 105 nmol, 3 mM final concentration), and TCEP (2.00 µL at 1.75 mM in water, 3.50 nmol, 100 µM final concentration) were added to DMF/NaP_i (0.92 µL DMF in 25.18 µL 50 mM NaP_i pH 8 buffer) and vortexed for 30 seconds. Samples of 10 µL were taken from the reaction mixture for LC-MS analysis. Full conversion was observed after 3.5 h at 25 ºC (calculated mass: 16421 *m/z*; observed mass: 16421 *m/z*).*

*$(M+41)^+$ peaks for the products were observed, where the additional 41 Da is contributed from the acetonitrile in the mobile phase.

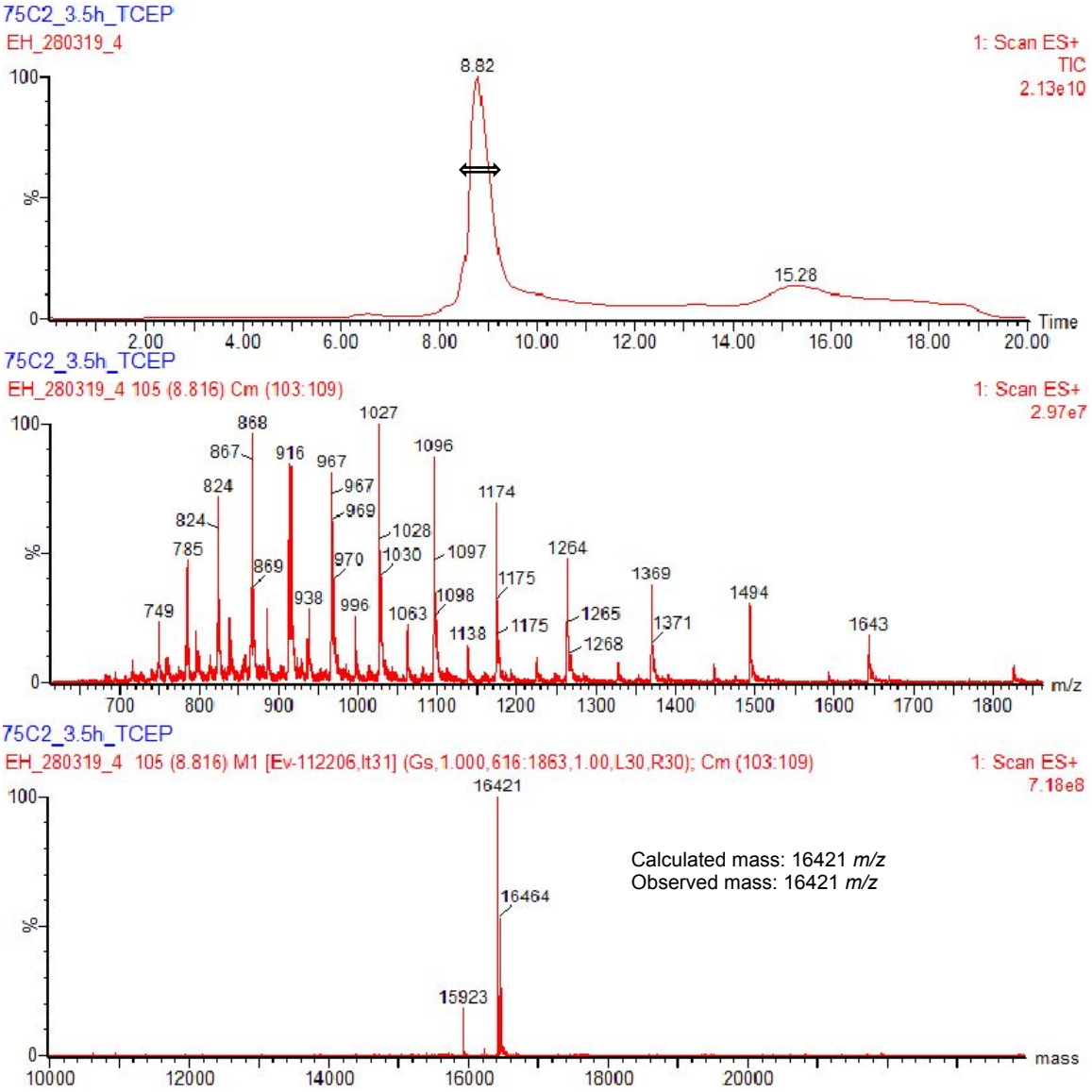


Figure S16. ESI-MS of the reaction of C2Am-Cys95 with **5e** (300 eq) after being shaken for 3.5 h at 25 ºC.

Stability of C2Am-5e/11 conjugates in the presence of GSH (1 mM)

A 10 mM stock solution of glutathione (GSH) was prepared in water. To 18 µL of each C2Am-**5e**/**11** conjugate (20 µM, 360 pmol), 2 µL of the GSH stock solution was added. Each mixture was vortexed and left to shake at 37 ºC. Time points were taken at 24 h and 1 week. Evaluation of the stability of the conjugates was accomplished by LC-MS analysis of 10 µL aliquots from each time point. While noticeable GSH replacement occurs for both conjugates, both conjugates are still present even after a 1 week incubation period.

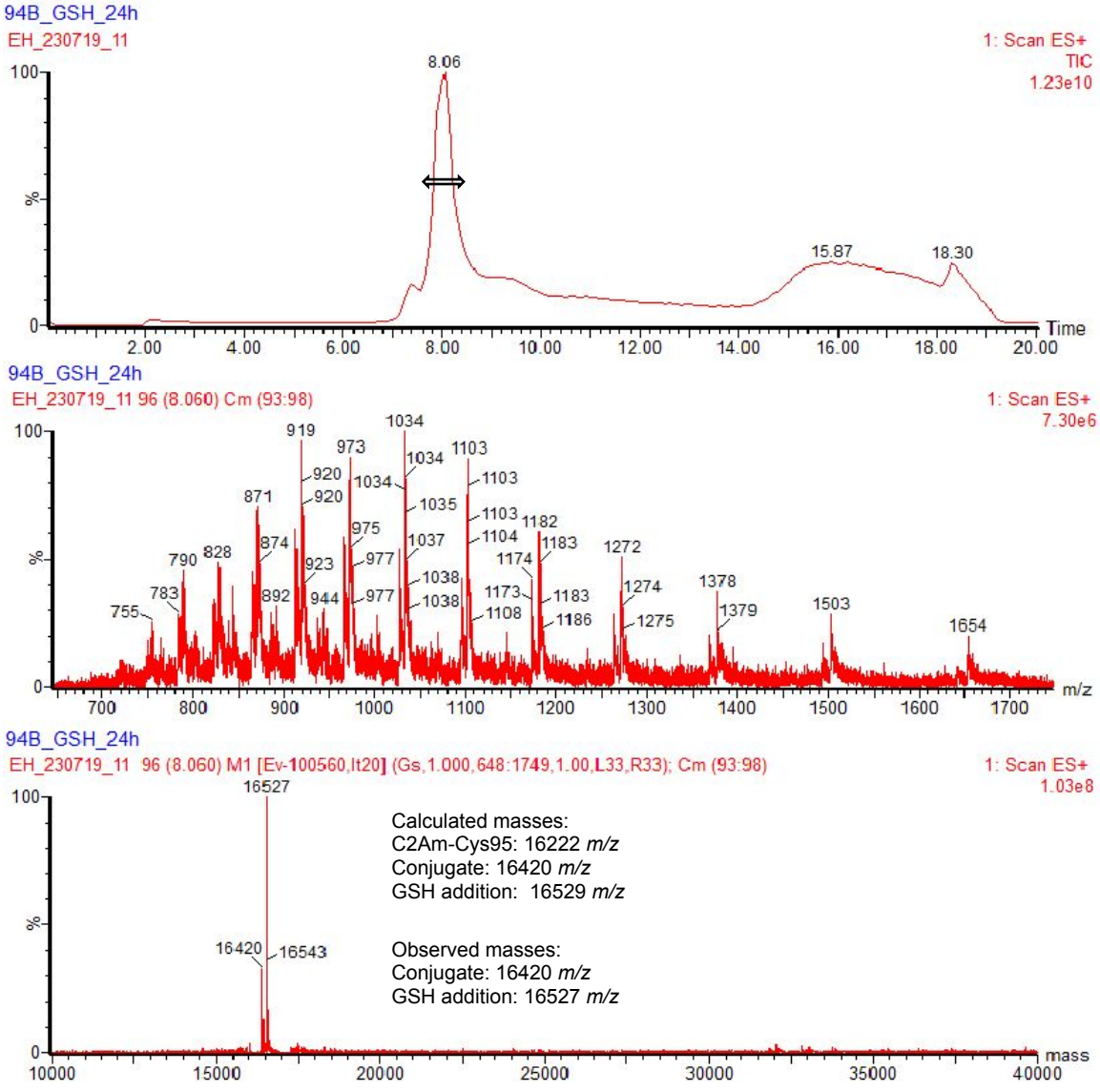


Figure S17. ESI-MS of C2Am-**11** conjugate (N-Cys) after 24 h at 37 ºC in the presence of 1 mM GSH.

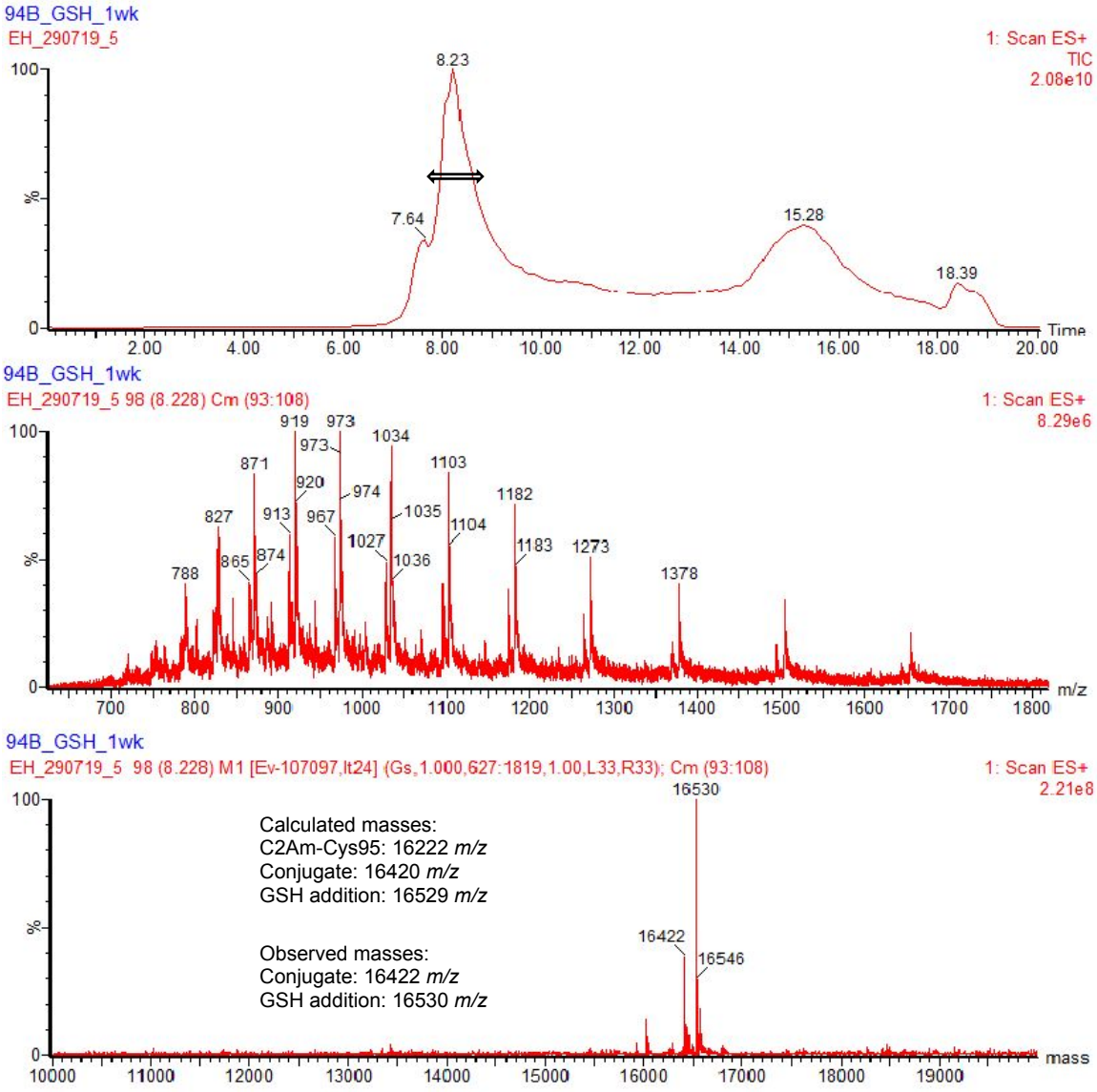


Figure S18. ESI-MS of C2Am-**11** conjugate (N-Cys) after 1 week at 37 ºC in the presence of 1 mM GSH.

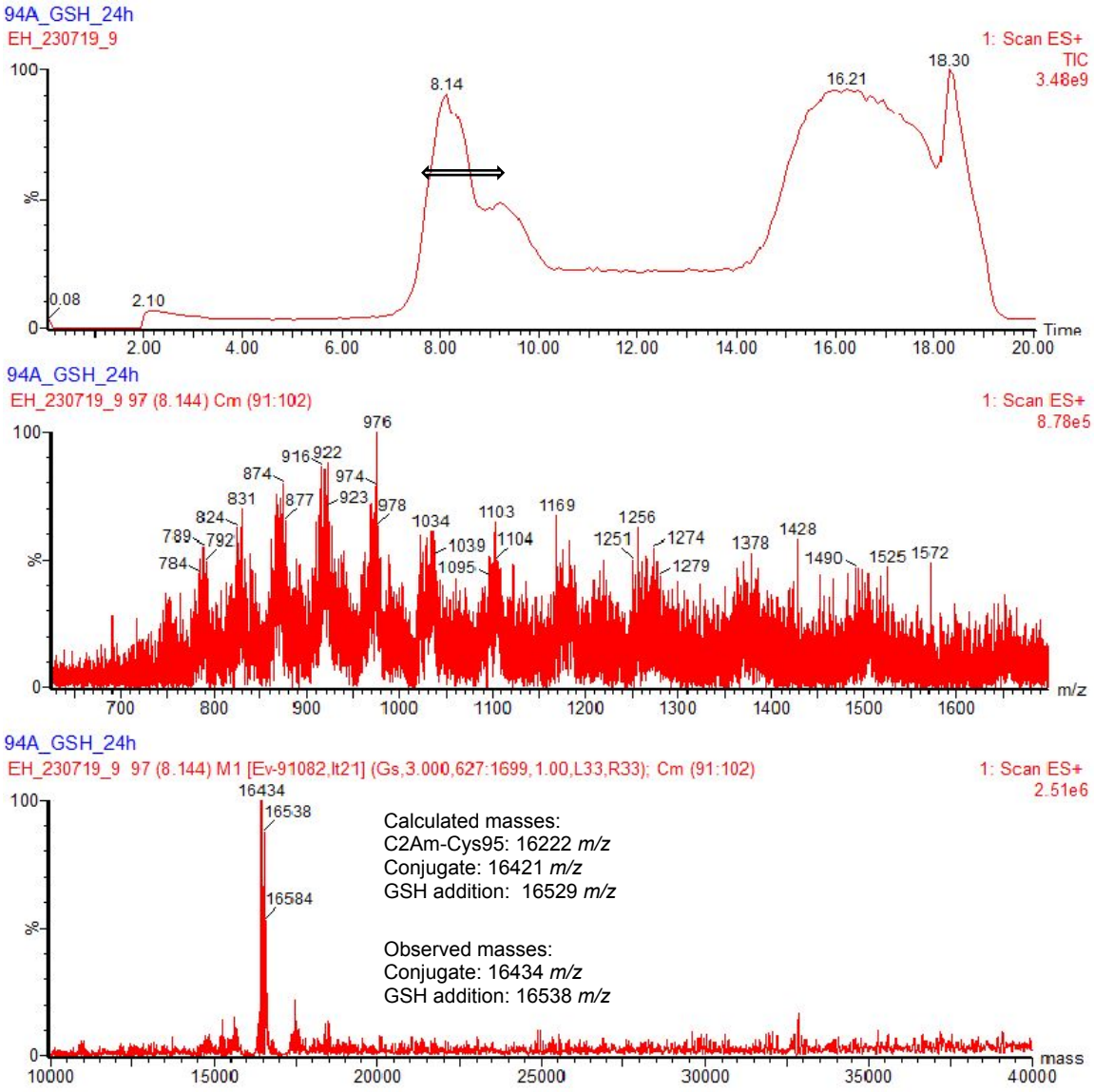


Figure S19. ESI-MS of C2Am-**5e** conjugate (O-Cys) after 24 h at 37 ºC in the presence of 1 mM GSH.

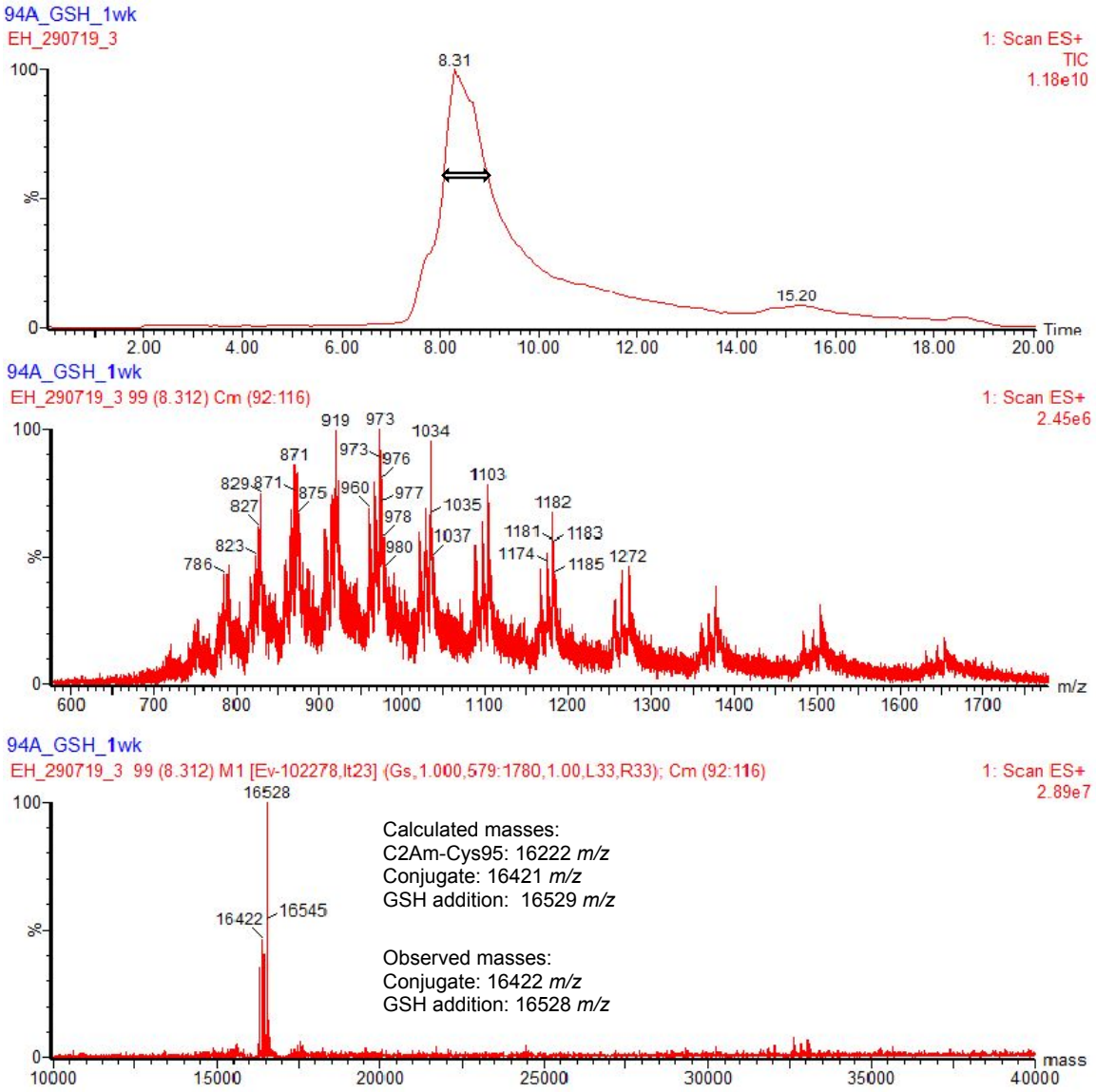


Figure S20. ESI-MS of C2Am-**5e** conjugate (O-Cys) after 1 week at 37 ºC in the presence of 1 mM GSH.

Stability of C2Am-5e/11 conjugates in human plasma

To 18 µL of each C2Am-**5e**/**11** conjugate (20 µM, 360 pmol), 2 µL of reconstituted human plasma (*Sigma Aldrich*) was added. Each mixture was vortexed and left to shake at 37 ºC. Time points were taken at 24 h and 1 week. Evaluation of the stability of the conjugates was accomplished by LC-MS analysis of 10 µL aliquots from each time point. Both conjugates are still present even after a 1 week incubation period with no signs of significant degradation.

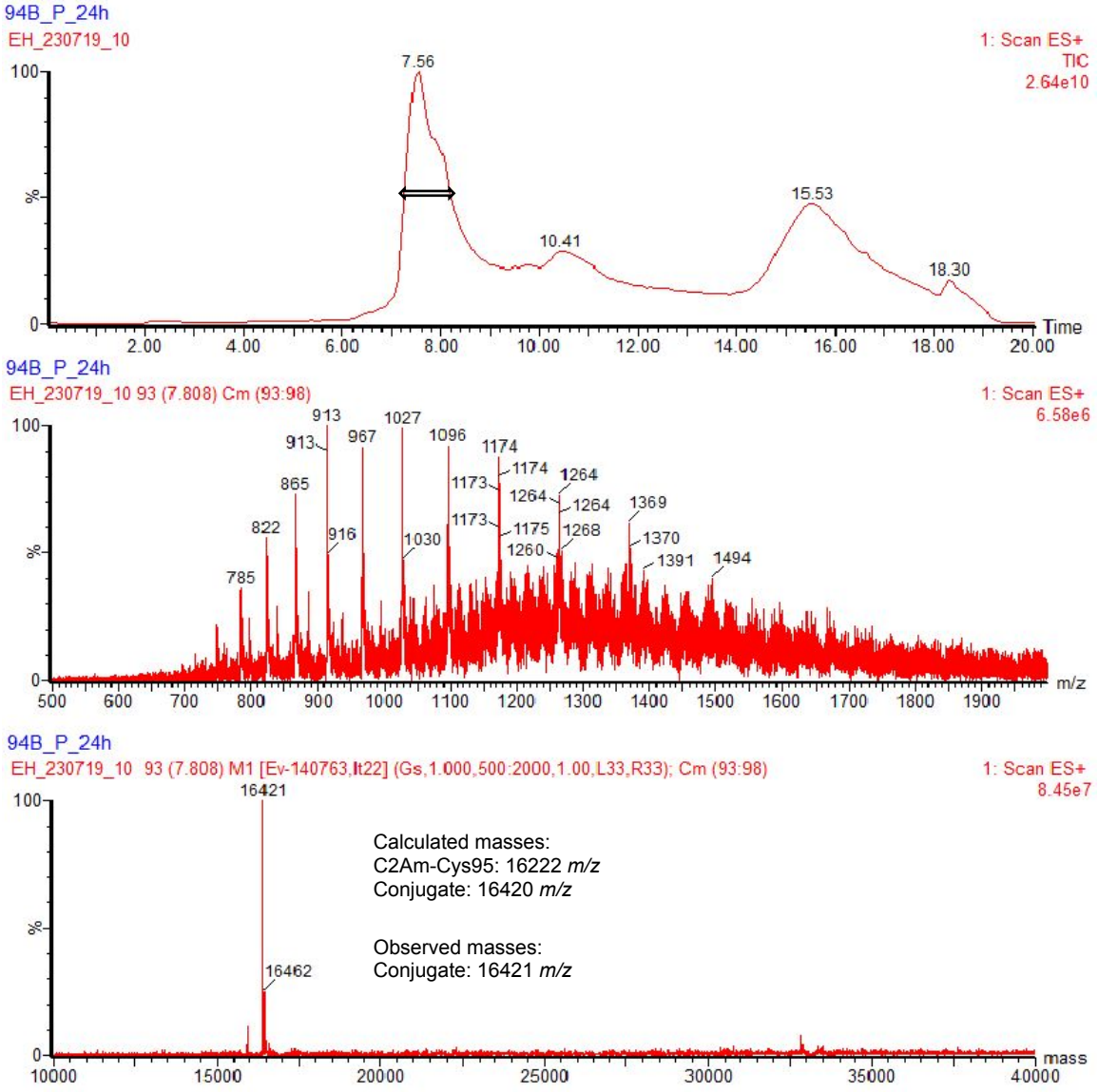


Figure S21. ESI-MS of C2Am-**11** conjugate (N-Cys) after 24 h at 37 ºC in the presence of human plasma.

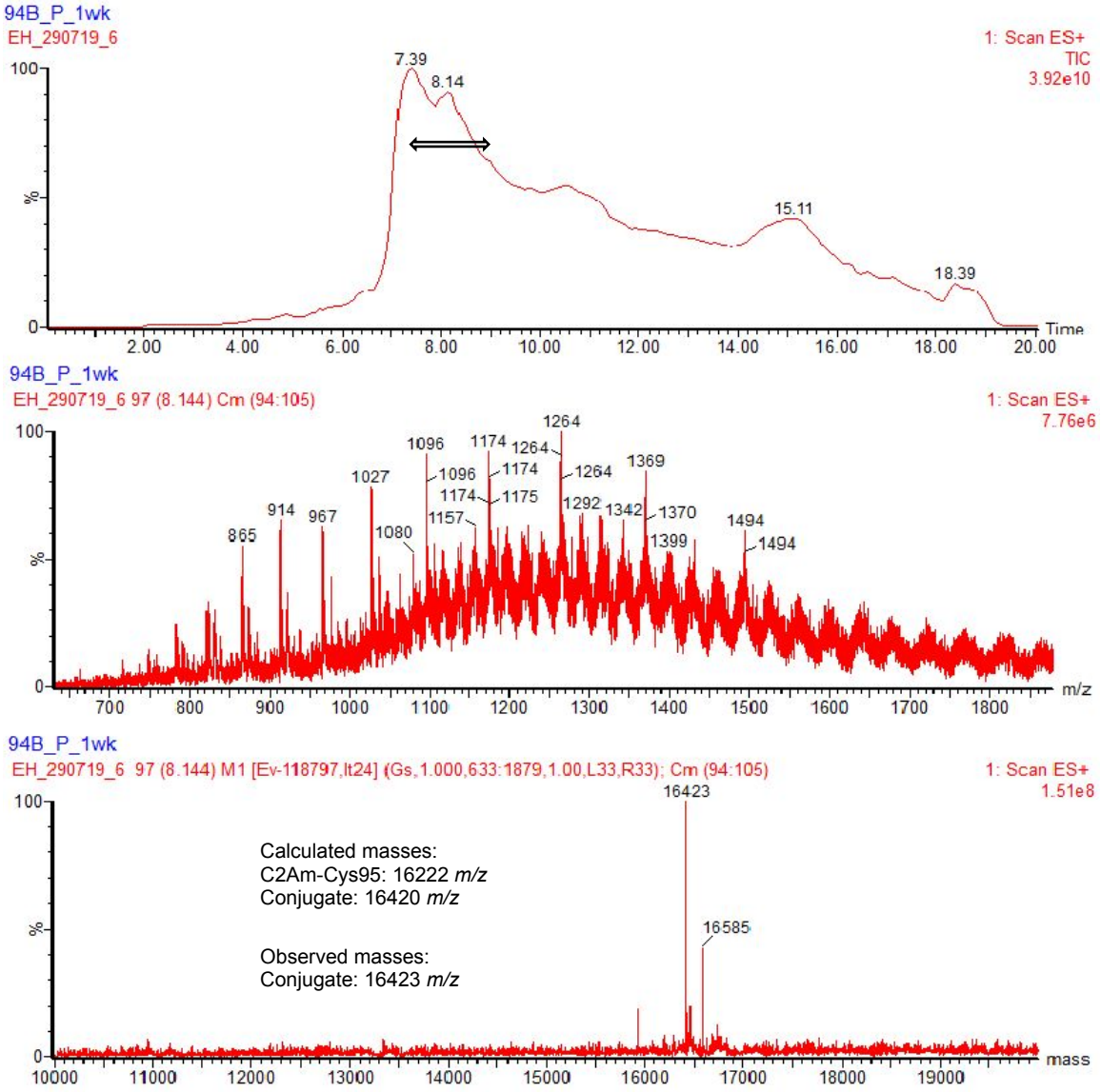


Figure S22. ESI-MS of C2Am-**11** conjugate (N-Cys) after 1 week at 37 ºC in the presence of human plasma.

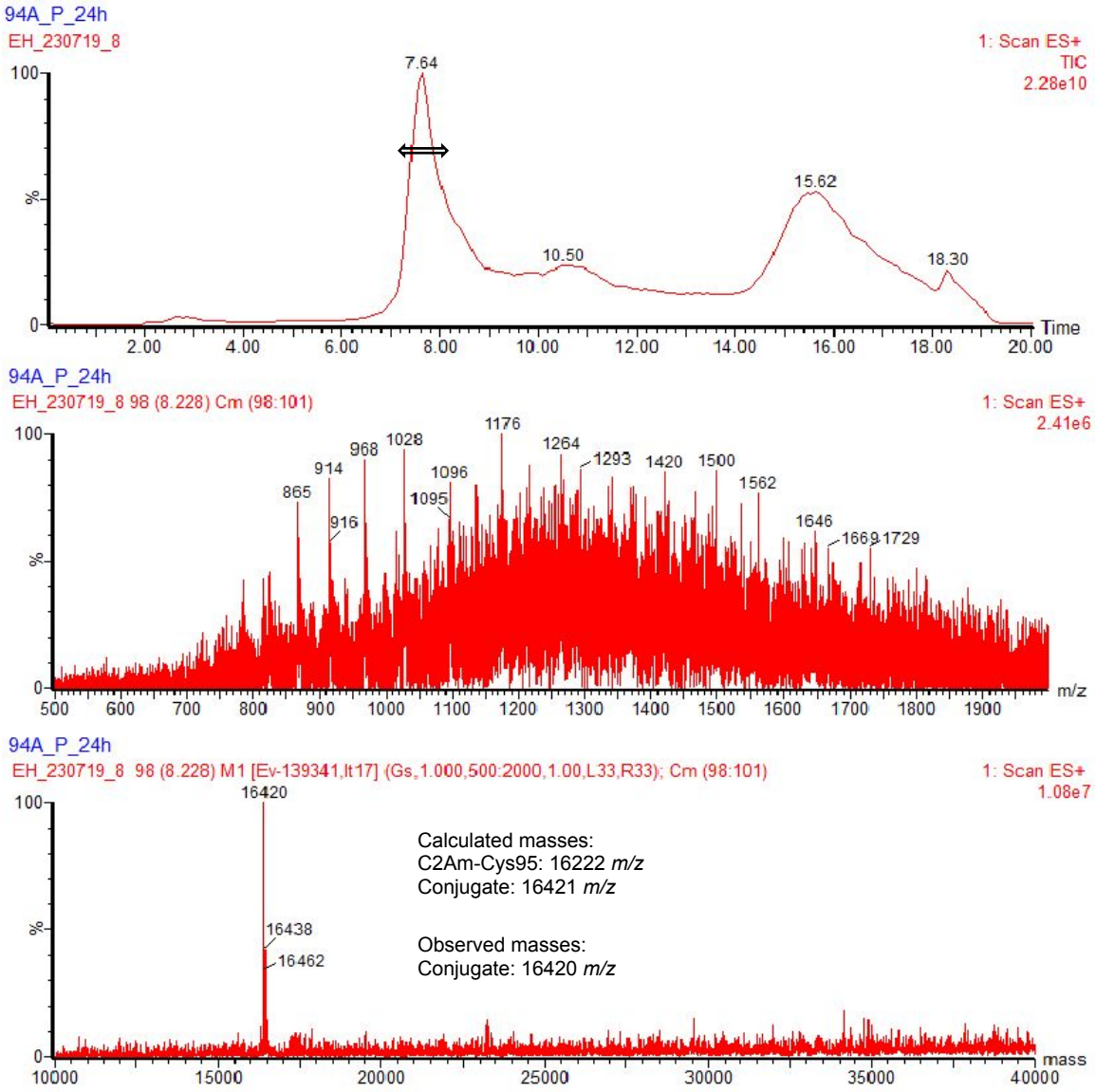


Figure S23. ESI-MS of C2Am-**5e** conjugate (O-Cys) after 24 h at 37 ºC in the presence of human plasma.

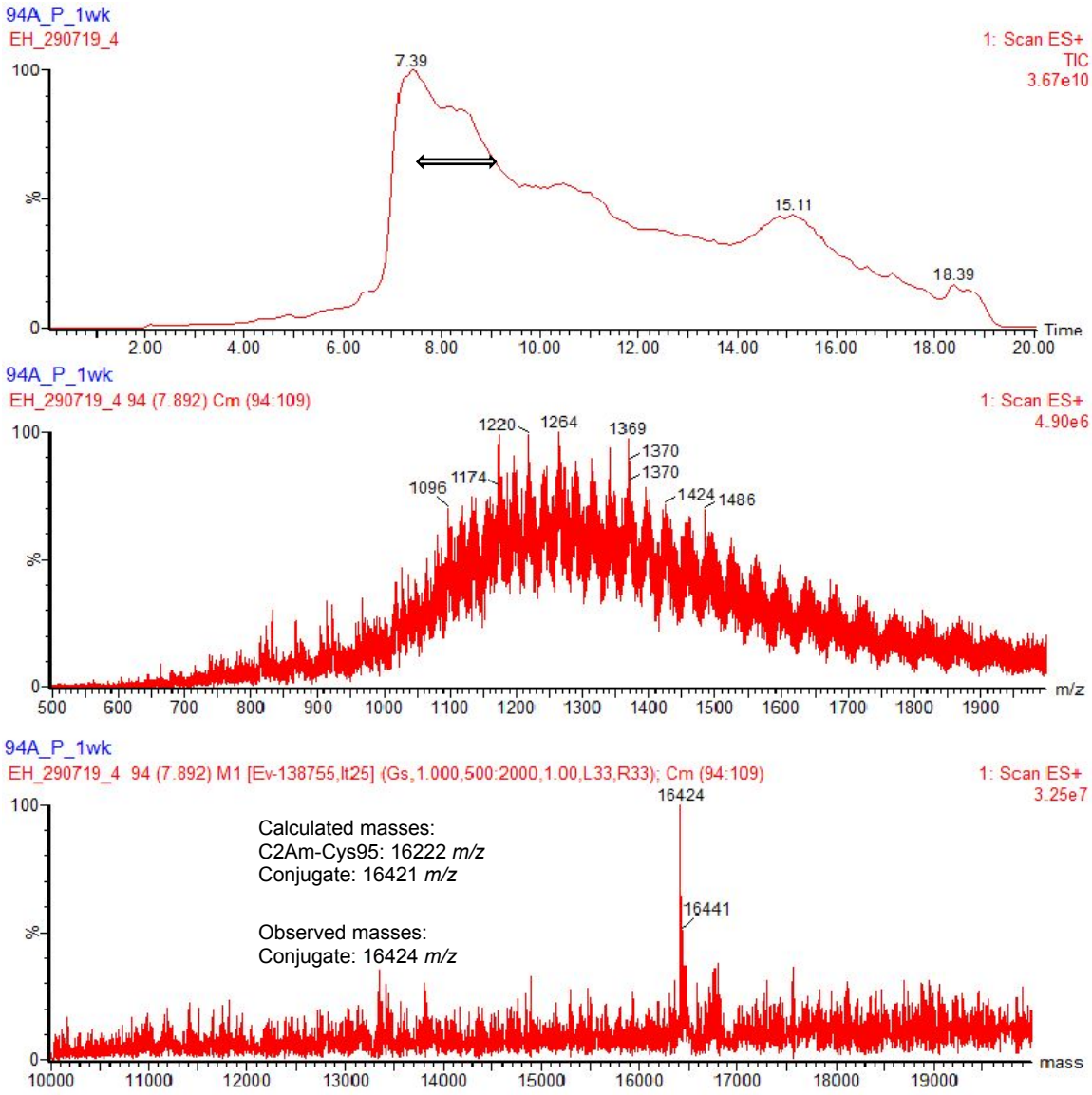
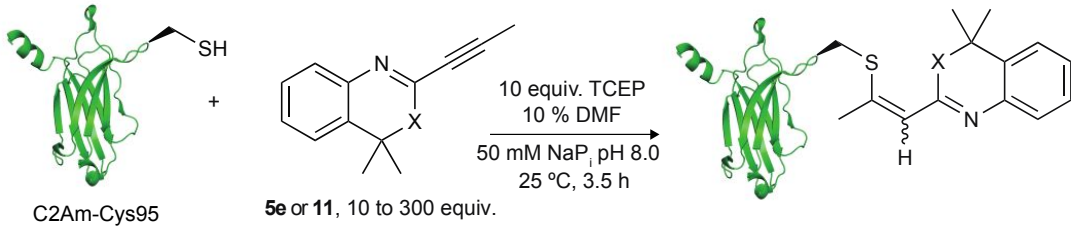


Figure S24. ESI-MS of C2Am-**5e** conjugate (O-Cys) after 1 week at 37 ºC in the presence of human plasma.

LC-MS/MS analysis of C2Am-Cys95 conjugates



Protein solutions were subjected to enzymatic digestion in 50 mM ammonium bicarbonate (pH 8) with trypsin overnight at 37°C. After digestion, the supernatant was pipetted into a sample vial and loaded onto an autosampler for automated LC-MS/MS analysis.

All LC-MS/MS experiments were performed using a Dionex Ultimate 3000 RSLC nanoUPLC (Thermo Fisher Scientific Inc, Waltham, MA, USA) system and a QExactive Orbitrap mass spectrometer (Thermo Fisher Scientific Inc, Waltham, MA, USA). Separation of peptides was performed by reverse-phase chromatography at a flow rate of 300 nL/min and a Thermo Scientific reverse-phase nano Easy-spray column (Thermo Scientific PepMap C18, 2µm particle size, 100Å pore size, 75 µm i.d. x 50cm length). Peptides were loaded onto a pre-column (Thermo Scientific PepMap 100 C18, 5µm particle size, 100A pore size, 300 µm i.d. x 5mm length) from the Ultimate 3000 autosampler with 0.1% formic acid for 3 minutes at a flow rate of 10 µL/min. After this period, the column valve was switched to allow elution of peptides from the pre-column onto the analytical column. Solvent A was water + 0.1% formic acid and solvent B was 80% acetonitrile, 20% water + 0.1% formic acid. The linear gradient employed was 2-40% B in 30 minutes.

The LC eluant was sprayed into the mass spectrometer by means of an Easy-Spray source (Thermo Fisher Scientific Inc.). All *m/z* values of eluting ions were measured in an Orbitrap mass analyzer, set at a resolution of 70000 and was scanned between *m/z* 380-1500. Data dependent scans (Top 20) were employed to automatically isolate and generate fragment ions by higher energy collisional dissociation (HCD, NCE:25%) in the HCD collision cell and measurement of the resulting fragment ions was performed in the Orbitrap analyser, set at a resolution of 17500. Singly charged ions and ions with unassigned charge states were excluded from being selected for MS/MS and a dynamic exclusion window of 20 seconds was employed.

Post-run, the data was processed using Protein Discoverer (version 2.1., Thermo Scientific). Briefly, all MS/MS data were converted to mgf files and the files were then submitted to the Mascot search algorithm (Matrix Science, London UK) and searched against a custom database containing the Synaptotagmin sequence and a common contaminant sequences (123 sequences; 40594 residues). Variable modifications of oxidation (M), deamidation (NQ), OCCL (C) and NCCL (C) were applied. The peptide and fragment mass tolerances were set to 100 ppm and 0.1 Da, respectively. A significance threshold value of $p<0.05$ and a peptide cut-off score of 20 were also applied.

Anti-HER2 2Rb17c nanobody Studies

anti-HER2 2Rb17c nanobody (obtained from collaborator)[12]

Sequence:

EVQLQESGGGLVQPGGSLRLSCAASGFIFSNDAMTWVRQAPGKGLEWVSSINWSGT

HTNYADSVKGRFTISRDNAKRTLYLQMNSLKDEDTALYYCVTGYGVTKTPTGQGT QVTVSSHHHHHHSPSTPPTPSPSTPPC

Calculated M_w = 14861.46 Da

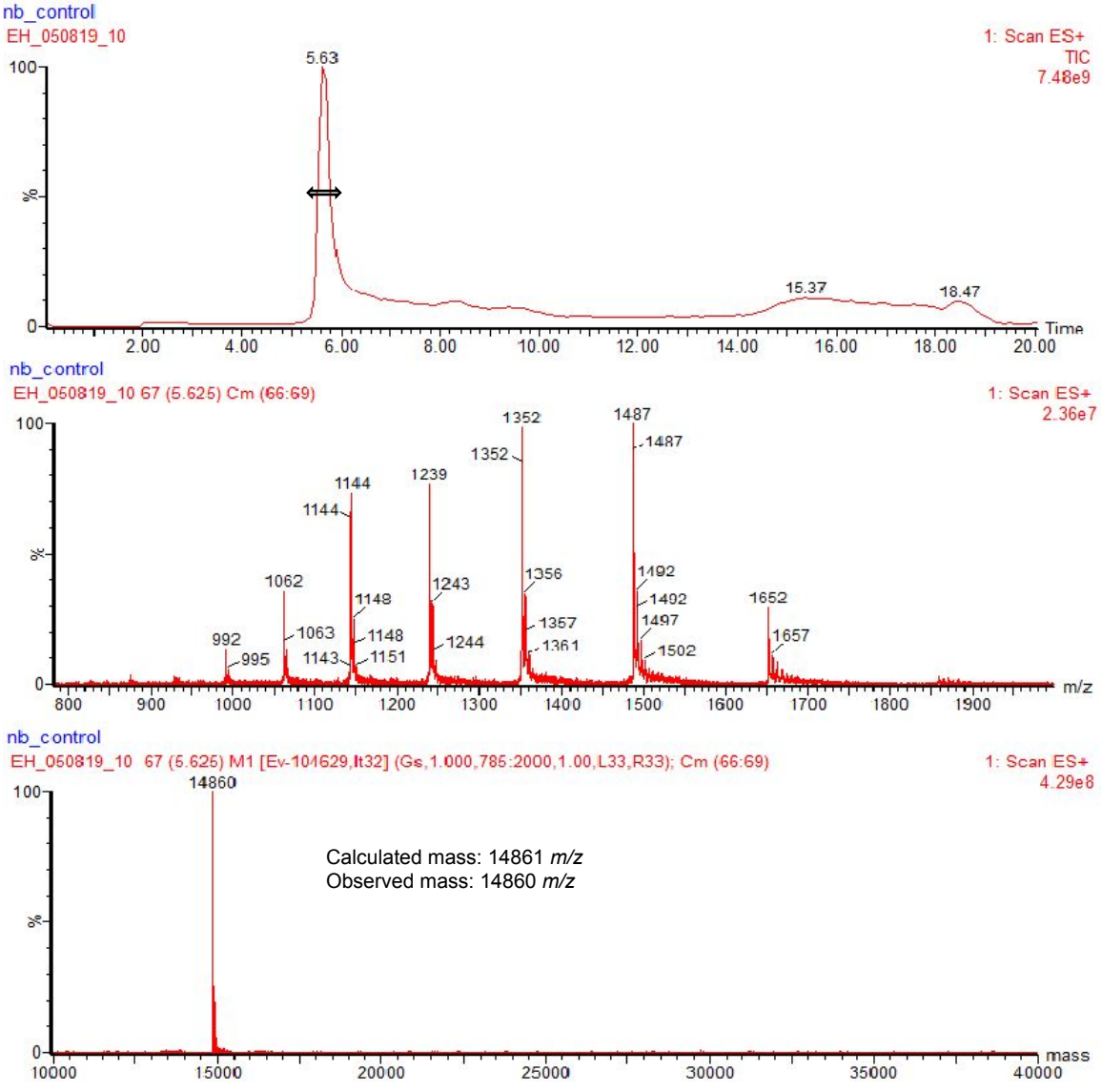
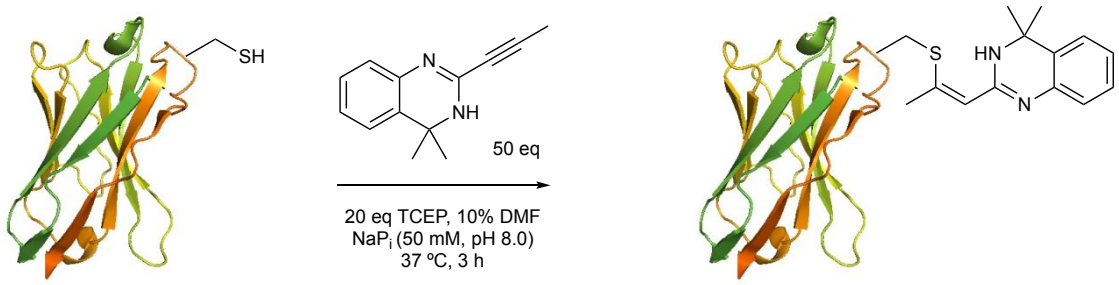


Figure S25. ESI-MS of anti-HER2 2Rb17c control in the presence of TCEP.

anti-HER2 2Rb17c reaction with 11



In a 12.5 µL total reaction volume, anti-HER2 2Rb17c (2.35 µL at 53 µM in 50 mM NaP_i pH 8 buffer, 125 pmol, 10 µM final concentration), **11** (0.5 µL at 12.6 mM in DMF, 6.25 nmol, 500 µM final concentration), and TCEP (1 µL at 2.5 mM in water, 2.50 nmol, 200 µM final concentration) were added to DMF/NaP_i (0.5 µL DMF in 8.15 µL 50 mM NaP_i pH 8 buffer) and vortexed for 30 seconds. Samples of 10 µL were taken from the reaction mixture for LC-MS analysis. Full conversion was observed after 3 h at 37 ºC (calculated mass: 15059 *m/z*; observed mass: 15064 *m/z*).*

*$(M+41)^+$ peaks for the products were observed, where the additional 41 Da is contributed from the acetonitrile in the mobile phase.

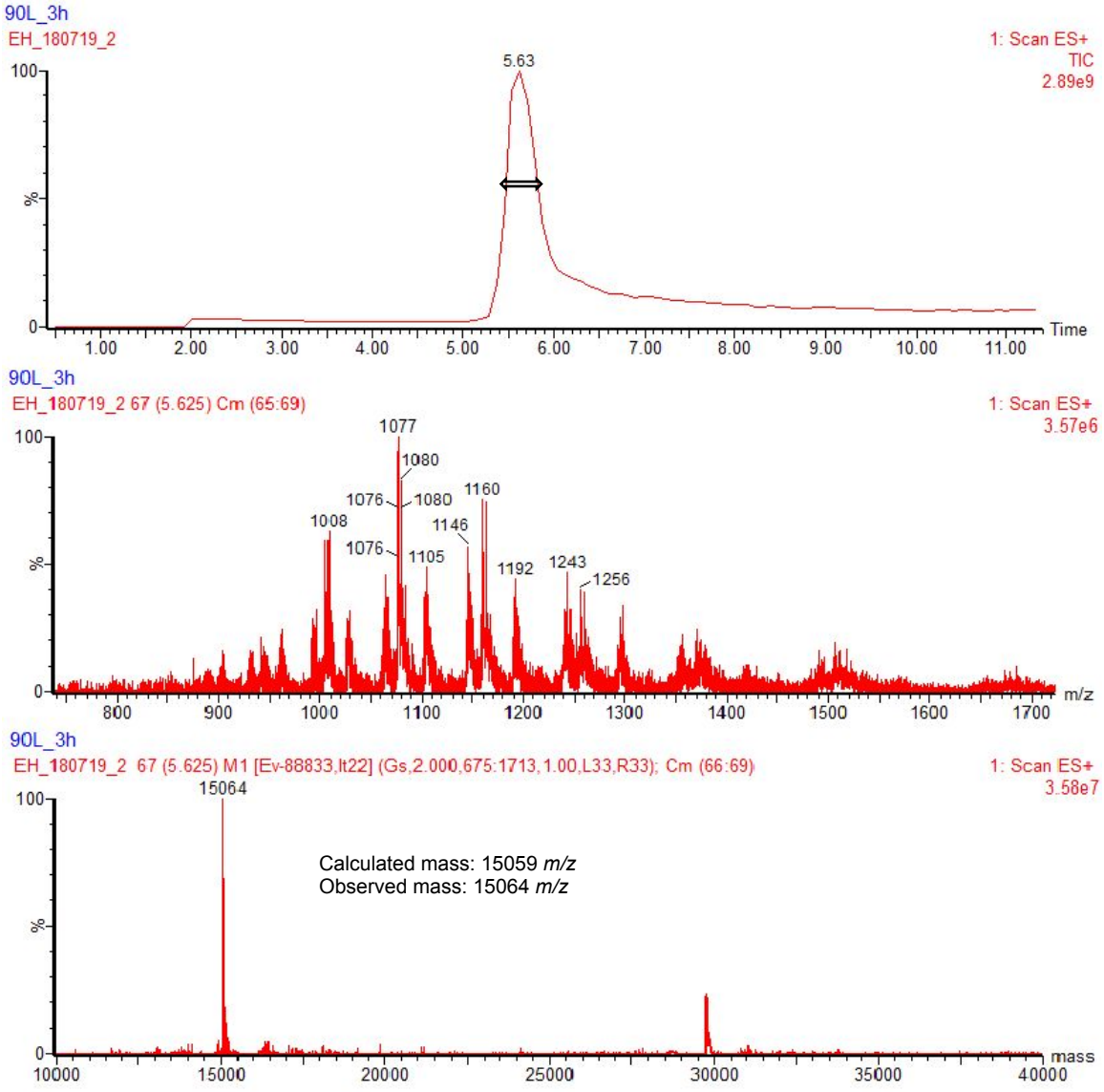
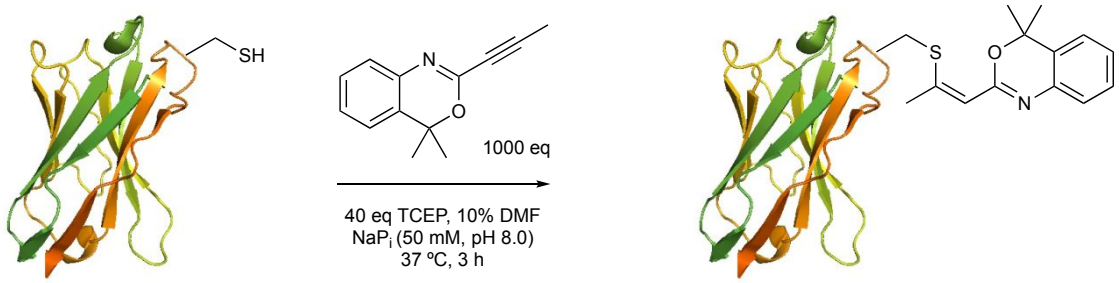


Figure S26. ESI-MS of the reaction of anti-HER2 2Rb17c with **11** (50 eq) after being shaken for 3 h at 37 ºC.

anti-HER2 2b17c reaction with 5e



In a 12.5 µL total reaction volume, anti-HER2 2Rb17c (2.35 µL at 53 µM in 50 mM NaP_i pH 8 buffer, 125 pmol, 10 µM final concentration), **5e** (1 µL at 125 mM in DMF, 125 nmol, 10 mM final concentration), and TCEP (2 µL at 2.5 mM in water, 2.50 nmol, 400 µM final concentration) were added to NaP_i (7.15 µL 50 mM NaP_i pH 8 buffer) and vortexed for 30 seconds. Samples of 10 µL were taken from the reaction mixture for LC-MS analysis. Approximately 40% conversion was observed after 3 h at 37 ºC (calculated mass: 15060 *m/z*; observed mass: 15061 *m/z*).*

*$(M+41)^+$ peaks for the products were observed, where the additional 41 Da is contributed from the acetonitrile in the mobile phase.

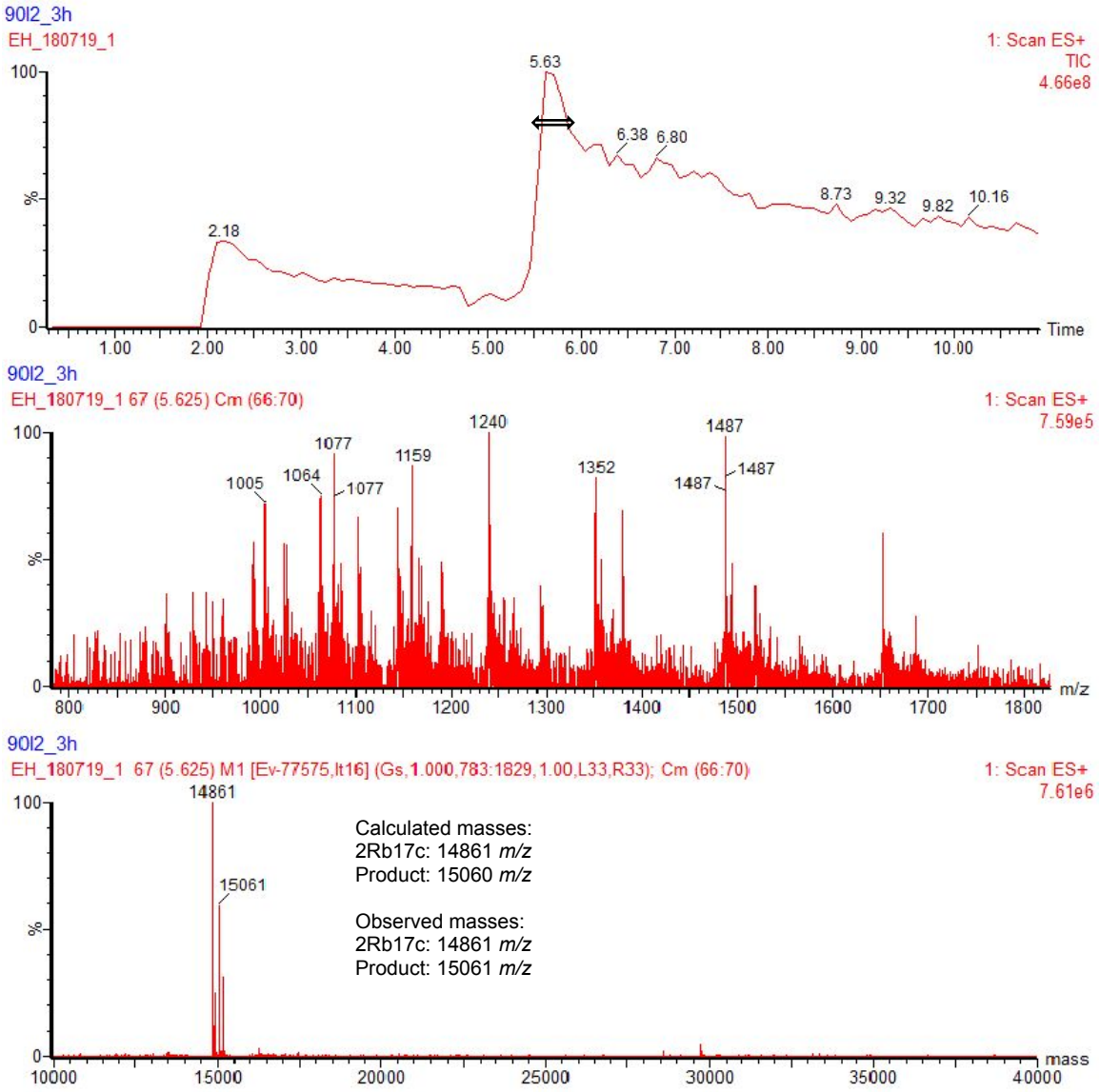


Figure S27. ESI-MS of the reaction of anti-HER2 2Rb17c with **5e** (1000 eq) after being shaken for 3 h at 37 ºC.

Table S3. Conditions and conversions for C2Am-Cys95 conjugation with **5e**[a]

5e concentration (μM)	C2Am-Cys95 concentration (μM)	Temperature (ºC)	DMF (%)	Time (h)	Conversion (%)
821 (82 eq)	10	25	10	3	42
3000 (300 eq)	10	25	10	3.5	>95[b]
8210 (821 eq)	10	25	10	3	>95[b]

[a] All reactions performed in 50 mM NaP_i pH 8.0

[b] No starting material detected

Table S4. Conditions and conversions for C2Am-Cys95 conjugation with **11**[a]

11 concentration (μM)	C2Am-Cys95 concentration (μM)	Temperature (ºC)	DMF (%)	Time (h)	Conversion (%)
10 (1 eq)	10	25	10	3	70
100 (10 eq)	10	25	10	3.5	88
821 (82 eq)	10	25	10	1	>95[b]

[a] All reactions performed in 50 mM NaP_i pH 8.0

[b] No starting material detected

Table S5. Conditions and conversions for anti-HER2 2Rb17c conjugation with **5e**[a]

5e concentration (μM)	2Rb17c concentration (μM)	Temperature (ºC)	DMF (%)	Time (h)	Conversion (%)
1000 (100 eq)	10	37	10	3	0
10,000 (1000 eq)	10	37	10	3	43
20,000 (2000 eq)	10	37	10	5	no signal detected[b]

[a] All reactions performed in 50 mM NaP_i pH 8.0

[b] No protein detected most likely due to precipitation from high small molecule concentration

Table S6. Conditions and conversions for anti-HER2 2Rb17c conjugation with **11**[a]

11 concentration (μM)	2Rb17c concentration (μM)	Temperature (ºC)	DMF (%)	Time (h)	Conversion (%)
100 (10 eq)	10	37	10	3	0
500 (50 eq)	10	37	10	3	>95[b]
1000 (100 eq)	10	37	10	3	>95[b]
2500 (250 eq)	10	37	10	5	>95[b]
5000 (500 eq)	10	37	10	5	>95[b]

[a] All reactions performed in 50 mM NaP_i pH 8.0

[b] No starting material detected

Kinase Panel Data

Biochemical potency data for all compounds were obtained at Thermo Fisher Scientific.

Selectivity of inhibitors **16**-**18** and **26** in a panel of either 140 or 403 human kinases run in the SelectScreen kinase panel at ThermoFisher Scientific at a single concentration of 1 μM.

Data for compound 16

Kinase Name	Activity flag	Mean Inhibition (%)
AAK1	Active	97.3194
ABL1	Not Active	55.4901
ABL2	Not Active	61.8084
ACVR1B	Not Active	-0.4109
AKT1	Not Active	2.8379
AKT2	Not Active	14.8043
ALK	Not Active	24.2618
AMPK A2/B1/G1	Not Active	21.8734
ARK5	Active	98.672
AurKB	Not Active	69.0011
AurKC	Not Active	43.6611
AXL	Not Active	32.944
BLK	Not Active	65.2312
BMX	Active	92.5245
bRAF	Not Active	-2.4128
BTK	Active	79.4305
CaMK1	Not Active	-4.9366
CAMK2b	Not Active	0.7558
CDK1:CB	Not Active	31.3551
CDK2:CA	Not Active	48.9856
CDK2:CE	Not Active	43.174
CDK7:Cyc H:MNAT1	Not Active	1.476
CDK8:CC	Not Active	16.5822
CDK9:CT	Active	84.1833
CHK1	Not Active	15.2969

cKIT	Not Active	12.4703
CLK1	Not Active	13.6522
CLK2	Not Active	72.7672
CLK3	Not Active	9.4084
CLK4	Active	79.7832
cRAF	Not Active	19.2453
CSF1R	Not Active	28.589
CSNK1g1	Not Active	3.9237
CSNK2a2	Not Active	19.8022
DDR2	Not Active	6.3062
DMPK	Not Active	52.2254
DNA-PK	Not Active	5.381
DYRK2	Active	76.3399
EEF2K	Not Active	3.5692
EGFR	Not Active	55.8212
EGFR	Active	86.2323
EPHA5	Not Active	15.4978
EPHB1	Not Active	9.3567
EphB4	Not Active	6.3578
ERBB2	Not Active	16.5016
ERBB4	Not Active	37.3806
FER	Not Active	34.105
FES	Not Active	12.5113
FGFR1	Not Active	45.0486
FGFR1	Not Active	16.7172
FGFR2	Not Active	17.3222
FGFR3	Not Active	22.2816
FGFR4	Not Active	-0.7964
FLT1	Not Active	14.964
FLT3	Not Active	72.9354
FRAP1	Not Active	-4.5895
FYN	Not Active	27.2795
GSG2	Not Active	46.2486
GSK3A	Not Active	67.6405
GSK3b	Active	82.1826

IGF1R	Not Active	-0.2694
IKKb	Not Active	13.2408
INSR	Not Active	1.1105
INSRR	Not Active	7.1835
IRAK1	Not Active	73.8139
IRAK4	Not Active	31.9774
JAK1	Not Active	44.5239
JAK2	Active	84.1656
JAK3	Active	101.1731
JNK1	Not Active	10.7556
KDR	Not Active	49.4142
LCK	Not Active	25.4086
LRRK2	Active	91.6952
LYN	Not Active	28.4673
MAP2K1	Not Active	5.8667
MAP3K7	Active	81.8058
MAP3K9	Active	97.8003
MAP4K1	Not Active	49.9383
MAP4K3	Not Active	31.932
MAPK1	Not Active	0.3303
MAPKAPK1A	Not Active	37.8916
MAPKAPK2	Not Active	3.5673
MAPKAPK5	Not Active	4.1439
MARK1	Not Active	23.2688
MARK2	Not Active	20.7635
MERTK	Not Active	19.7312
MINK	Active	79.2568
MKNK2	Active	84.2912
MSK1	Not Active	28.4299
MST1R	Not Active	21.502
MTK	Not Active	18.9717
MYLK	Not Active	12.5146
NEK2	Not Active	-10.8264
NTRK3	Not Active	65.4542
p38a	Not Active	2.9828

P70S6K	Not Active	7.5888
PAK1	Not Active	18.1142
PAK2	Not Active	8.1648
PAK4	Not Active	22.2334
PAK5	Not Active	6.8192
PDGFRb	Not Active	16.8232
PDPK1	Not Active	3.9148
PEAK1	Not Active	20.2232
PI3Kb	Not Active	3.4828
PI3Kd	Not Active	10.4414
PI3Kg	Not Active	18.2942
PI4KB	Not Active	69.9506
PIK3C2A	Not Active	7.8273
PIK3C3	Not Active	15.7651
PIK3CA:PIK3R1	Not Active	-8.3958
PIM2	Not Active	-0.6285
PKAa	Not Active	14.1084
PKCa	Not Active	43.6714
PKCe	Not Active	-7.9948
PKCt	Not Active	3.4876
PKG1	Not Active	2.7441
PLK1	Not Active	10.9429
PTK2	Not Active	28.6148
PTK6	Not Active	30.4769
RET	Not Active	33.607
RET	Not Active	32.832
RET	Not Active	31.4308
ROCK1	Not Active	15.8794
ROCK2	Not Active	4.1072
ROS1	Active	75.3877
SGK1	Not Active	40.3694
src	Not Active	26.4837
SRPK1	Not Active	3.0984
STK17A	Active	79.099
STK4	Not Active	30.1121

SYK	Active	83.3916
TBK1	Not Active	40.9645
TGFBR1	Not Active	25.5106
TNIK	Active	78.7459
TRKA	Not Active	53.7019
TRKB	Not Active	44.8247
TYRO3	Not Active	38.5508
ULK2	Not Active	9.791
YES	Not Active	36.5108
ZAP70	Not Active	11.8636

Data for compound 17

Kinase Name	**Activity flag**	**Mean Inhibition (%)**
AAK1	Active	92.5646
ABL1	Not Active	55.5999
ABL2	Not Active	60.1362
ACVR1B	Not Active	-4.0594
AKT1	Not Active	-1.654
AKT2	Not Active	9.493
ALK	Not Active	29.1124
AMPK A2/B1/G1	Not Active	15.374
ARK5	Active	95.4684
AurKB	Not Active	57.2626
AurKC	Not Active	26.7352
AXL	Not Active	30.0518
BLK	Not Active	26.5546
BMX	Not Active	53.9974
bRAF	Not Active	-3.4797
BTK	Not Active	27.285
CaMK1	Not Active	2.7998
CAMK2b	Not Active	-1.6204
CDK1:CB	Not Active	41.8964
CDK2:CA	Not Active	57.7548
CDK2:CE	Not Active	39.0004

CDK7:Cyc H:MNAT1	Not Active	-1.2371
CDK8:CC	Not Active	6.8319
CDK9:CT	Active	89.9563
CHK1	Not Active	6.7222
cKIT	Not Active	19.9857
CLK1	Not Active	25.379
CLK2	Not Active	51.9707
CLK3	Not Active	8.0609
CLK4	Active	86.4901
cRAF	Not Active	25.765
CSF1R	Not Active	36.8656
CSNK1g1	Not Active	0.9319
CSNK2a2	Not Active	23.4159
DDR2	Not Active	4.8183
DMPK	Not Active	25.0816
DNA-PK	Not Active	6.631
DYRK2	Not Active	69.5576
EEF2K	Not Active	7.4156
EGFR	Active	83.5024
EGFR	Not Active	40.0748
EPHA5	Not Active	13.0144
EPHB1	Not Active	10.5602
EphB4	Not Active	8.8425
ERBB2	Not Active	-0.0832
ERBB4	Not Active	13.1762
FER	Not Active	35.4818
FES	Not Active	17.8417
FGFR1	Not Active	-0.6718
FGFR1	Not Active	16.4125
FGFR2	Not Active	16.5932
FGFR3	Not Active	13.6832
FGFR4	Not Active	-3.5054
FLT1	Not Active	15.7129
FLT3	Active	76.175
FRAP1	Not Active	3.1522

FYN	Not Active	22.9266
GSG2	Not Active	42.7077
GSK3A	Not Active	61.5468
GSK3b	Active	77.1159
IGF1R	Not Active	7.2208
IKKb	Not Active	8.7254
INSR	Not Active	3.1558
INSRR	Not Active	5.6726
IRAK1	Active	76.276
IRAK4	Not Active	42.1748
JAK1	Not Active	32.2331
JAK2	Active	75.1496
JAK3	Active	76.4822
JNK1	Not Active	1.3049
KDR	Not Active	51.2813
LCK	Not Active	12.1115
LRRK2	Active	91.0334
LYN	Not Active	30.7276
MAP2K1	Not Active	7.6752
MAP3K7	Active	102.5824
MAP3K9	Active	110.1385
MAP4K1	Not Active	43.0356
MAP4K3	Not Active	29.4958
MAPK1	Not Active	2.3322
MAPKAPK1A	Not Active	41.3406
MAPKAPK2	Not Active	1.5246
MAPKAPK5	Not Active	2.2264
MARK1	Not Active	12.3413
MARK2	Not Active	7.9452
MERTK	Not Active	20.8866
MINK	Active	90.2628
MKNK2	Active	83.8075
MSK1	Not Active	17.404
MST1R	Not Active	18.8754
MTK	Not Active	11.8924

MYLK	Not Active	12.5824
NEK2	Not Active	-8.8048
NTRK3	Not Active	57.3368
p38a	Not Active	17.6671
P70S6K	Not Active	5.5026
PAK1	Not Active	12.0348
PAK2	Not Active	6.973
PAK4	Not Active	23.5696
PAK5	Not Active	6.8616
PDGFRb	Not Active	27.6824
PDPK1	Not Active	3.2782
PEAK1	Not Active	14.7216
PI3Kb	Not Active	-3.895
PI3Kd	Not Active	18.3563
PI3Kg	Not Active	-0.3296
PI4KB	Active	75.8411
PIK3C2A	Not Active	10.8018
PIK3C3	Not Active	8.4867
PIK3CA:PIK3R1	Not Active	17.7506
PIM2	Not Active	2.3057
PKAa	Not Active	7.4092
PKCa	Not Active	32.2446
PKCe	Not Active	-0.3923
PKCt	Not Active	7.095
PKG1	Not Active	6.651
PLK1	Not Active	10.7478
PTK2	Not Active	29.4016
PTK6	Not Active	32.7568
RET	Not Active	28.6842
RET	Not Active	25.2797
RET	Not Active	26.231
ROCK1	Not Active	7.2227
ROCK2	Not Active	-0.769
ROS1	Active	78.3821
SGK1	Not Active	42.4808

src	Not Active	33.6438
SRPK1	Not Active	1.8463
STK17A	Not Active	68.7291
STK4	Not Active	26.472
SYK	Active	84.3698
TBK1	Not Active	44.0277
TGFBR1	Not Active	11.6408
TNIK	Active	87.5815
TRKA	Not Active	47.9755
TRKB	Not Active	25.4362
TYRO3	Not Active	47.6342
ULK2	Not Active	4.6198
YES	Not Active	37.0873
ZAP70	Not Active	11.5708

Data for compound 18

Kinase Name	**Activity flag**	**Mean Inhibition (%)**
AAK1	Active	80.3862
ABL1	Not Active	7.1614
ABL2	Not Active	-7.0958
ACVR1B	Not Active	1.3127
AKT1	Not Active	4.1862
AKT2	Not Active	0.0474
ALK	Not Active	2.6958
AMPK A2/B1/G1	Not Active	2.9746
ARK5	Not Active	50.6194
AurKB	Not Active	5.4825
AurKC	Not Active	1.1874
AXL	Not Active	2.1918
BLK	Not Active	13.4886
BMX	Not Active	70.7044
bRAF	Not Active	-8.6975
BTK	Not Active	39.3948
CaMK1	Not Active	-15.5873

CAMK2b	Not Active	5.848
CDK1:CB	Not Active	11.8725
CDK2:CA	Not Active	13.3303
CDK2:CE	Not Active	4.8887
CDK7:Cyc H:MNAT1	Not Active	4.4466
CDK8:CC	Not Active	9.3184
CDK9:CT	Not Active	17.7407
CHK1	Not Active	6.8989
cKIT	Not Active	-2.4802
CLK1	Not Active	3.3321
CLK2	Not Active	22.8558
CLK3	Not Active	-2.2334
CLK4	Not Active	31.802
cRAF	Not Active	14.788
CSF1R	Not Active	20.2412
CSNK1g1	Not Active	1.1816
CSNK2a2	Not Active	10.6358
DDR2	Not Active	-15.5418
DMPK	Not Active	28.3686
DNA-PK	Not Active	18.8937
DYRK2	Not Active	44.4647
EEF2K	Not Active	4.1952
EGFR	Not Active	14.3406
EGFR	Not Active	7.3997
EPHA5	Not Active	1.731
EPHB1	Not Active	5.8652
EphB4	Not Active	1.2483
ERBB2	Not Active	-3.1664
ERBB4	Not Active	11.3787
FER	Not Active	7.2245
FES	Not Active	7.0928
FGFR1	Not Active	18.5759
FGFR1	Not Active	8.5845
FGFR2	Not Active	0.1979
FGFR3	Not Active	-4.8161

FGFR4	Not Active	-6.3695
FLT1	Not Active	0.545
FLT3	Not Active	37.9699
FRAP1	Not Active	-2.0508
FYN	Not Active	6.1576
GSG2	Not Active	-9.345
GSK3A	Not Active	6.4355
GSK3b	Not Active	9.9126
IGF1R	Not Active	9.9446
IKKb	Not Active	-2.1996
INSR	Not Active	5.3953
INSRR	Not Active	8.3017
IRAK1	Not Active	28.9156
IRAK4	Not Active	5.7262
JAK1	Not Active	2.8192
JAK2	Not Active	10.4032
JAK3	Active	91.3381
JNK1	Not Active	-2.4002
KDR	Not Active	7.2639
LCK	Not Active	8.7298
LRRK2	Not Active	62.8362
LYN	Not Active	7.2292
MAP2K1	Not Active	23.0239
MAP3K7	Not Active	8.709
MAP3K9	Active	78.0416
MAP4K1	Not Active	63.3246
MAP4K3	Not Active	13.7004
MAPK1	Not Active	-6.6124
MAPKAPK1A	Not Active	8.7053
MAPKAPK2	Not Active	-2.2108
MAPKAPK5	Not Active	4.0045
MARK1	Not Active	6.1203
MARK2	Not Active	3.6804
MERTK	Not Active	6.5516
MINK	Not Active	27.4689

MKNK2	Active	81.8623
MSK1	Not Active	13.4108
MST1R	Not Active	4.3346
MTK	Not Active	0.739
MYLK	Not Active	4.6767
NEK2	Not Active	8.4752
NTRK3	Not Active	3.2836
p38a	Not Active	1.0674
P70S6K	Not Active	-1.6506
PAK1	Not Active	2.7646
PAK2	Not Active	-5.2479
PAK4	Not Active	12.3944
PAK5	Not Active	-2.015
PDGFRb	Not Active	4.607
PDPK1	Not Active	8.3308
PEAK1	Not Active	1.6678
PI3Kb	Not Active	7.2992
PI3Kd	Not Active	-14.4975
PI3Kg	Not Active	-1.1335
PI4KB	Not Active	16.5089
PIK3C2A	Not Active	8.4876
PIK3C3	Not Active	-15.9787
PIK3CA:PIK3R1	Not Active	14.6996
PIM2	Not Active	-0.671
PKAa	Not Active	-1.9774
PKCa	Not Active	9.1996
PKCe	Not Active	0.8238
PKCt	Not Active	7.0026
PKG1	Not Active	6.8632
PLK1	Not Active	5.4516
PTK2	Not Active	6.0151
PTK6	Not Active	6.0857
RET	Not Active	17.1842
RET	Not Active	4.4412
RET	Not Active	6.2398

ROCK1	Not Active	4.3474
ROCK2	Not Active	15.2329
ROS1	Not Active	42.4392
SGK1	Not Active	2.0186
src	Not Active	12.9527
SRPK1	Not Active	2.5765
STK17A	Not Active	33.8272
STK4	Not Active	7.2365
SYK	Not Active	12.783
TBK1	Not Active	16.2422
TGFBR1	Not Active	26.431
TNIK	Not Active	18.5095
TRKA	Not Active	17.3486
TRKB	Not Active	1.0203
TYRO3	Not Active	7.291
ULK2	Not Active	19.2106
YES	Not Active	-2.8244
ZAP70	Not Active	1.3958

Data for compound 26

Kinase Name	**Activity flag**	**Mean Inhibition (%)**
AAK1	Not Active	-2.514
ABL1	Not Active	36.7956
ABL2	Not Active	25.7212
ACVR1	Not Active	0.1494
ACVR1B	Not Active	-12.1776
ACVR2A	Not Active	-20.6184
ACVR2B	Not Active	7.1074
ACVRL1	Not Active	9.6785
ADRBK1	Not Active	-1.8509
ADRBK2	Not Active	-4.4028
AKT1	Not Active	-1.6351
AKT2	Not Active	0.393
AKT3	Not Active	-1.8231

ALK	Not Active	0.7571
AMPK A1/B2/G3	Not Active	-1.533
AMPK A2/B1/G2	Not Active	-8.3562
AMPK A2/B1/G3	Not Active	-0.6813
AMPK A2/B2/G3	Not Active	0.4433
AMPK A1/B2/G2	Not Active	2.4598
AMPK A1/B1/G1	Not Active	-2.6216
AMPK A1/B1/G2	Not Active	-5.4346
AMPK A1/B1/G3	Not Active	15.1246
AMPK A1/B2/G1	Not Active	3.3355
AMPK A2/B2/G1	Not Active	-7.8911
AMPK A2/B2/G2	Not Active	-18.5772
AMPK A2/B1/G1	Not Active	1.4163
ANKK1	Not Active	6.217
ARK5	Not Active	-0.9867
AurKA	Not Active	-3.7077
AurKB	Not Active	20.774
AurKC	Not Active	25.6249
AXL	Not Active	4.4458
BLK	Not Active	26.4655
BMPR1A	Not Active	-7.8191
BMPR1B	Not Active	1.0099
BMPR2	Not Active	-8.474
BMX	Not Active	5.6165
bRAF	Not Active	67.7454
bRAF	Not Active	1.8286
BRSK1	Not Active	-1.6163
BRSK2	Not Active	13.0234
BTK	Not Active	-2.3674
CABC1	Not Active	1.7936
CaMK1	Not Active	-6.4542
CAMK1d	Not Active	1.2912
CAMK1G	Not Active	2.7656
CAMK2a	Not Active	-4.2435
CAMK2b	Not Active	-3.225

CAMK2d	Not Active	6.5317
CAMK2g	Not Active	-4.6494
CAMK4	Not Active	1.5763
CAMKK1	Not Active	-3.6928
CAMKK2	Not Active	6.2573
CASK	Not Active	-8.2405
CDC42BPa	Not Active	0.8111
CDC42BPb	Not Active	7.5027
CDC42BPG	Not Active	-4.537
CDC7	Not Active	12.7868
CDK1:CB	Not Active	-5.5598
CDK13:CCNK	Not Active	0.2454
CDK19	Not Active	-3.1541
CDK19:CCNC	Not Active	-2.5886
CDK2:CA	Not Active	11.9094
CDK2:CA	Not Active	1.5552
CDK2:CE	Not Active	6.9772
CDK2:Cyclin O	Not Active	10.4209
CDK3:Cyclin E	Not Active	8.0254
CDK4:CD1	Not Active	-3.4815
CDK4:CD3	Not Active	-1.854
CDK5	Not Active	1.2303
CDK5	Not Active	4.3012
CDK5	Not Active	-0.2273
CDK6:CycD1	Not Active	-6.0844
CDK7:Cyc H:MNAT1	Not Active	-18.701
CDK8:CC	Not Active	-4.3892
CDK9	Not Active	-7.7491
CDK9	Not Active	12.6031
CDK9:CT	Not Active	-7.7756
CDKL5	Not Active	-0.0132
CHK1	Not Active	21.3479
CHK2	Not Active	3.2003
cKIT	Not Active	48.5912
CLK1	Not Active	-1.2575

CLK2	Not Active	9.9227
CLK3	Not Active	-0.5732
CLK4	Not Active	2.0205
CSF1R	Active	92.1339
CSK	Not Active	38.3658
CSNK1A1	Not Active	12.8887
CSNK1A1L	Not Active	2.303
CSNK1d	Not Active	2.7856
CSNK1E	Not Active	8.772
CSNK1g1	Not Active	-1.596
CSNK1g2	Not Active	-3.9386
CSNK1g3	Not Active	-2.487
CSNK2A1	Not Active	2.4774
CSNK2a2	Not Active	6.412
DAPK1	Not Active	-16.9999
DAPK2	Not Active	11.3548
DAPK3	Not Active	-8.108
DCAMKL2	Not Active	-3.3181
DCLK1	Not Active	-6.7092
DDR1	Not Active	26.0986
DDR2	Not Active	28.761
DMPK	Not Active	-4.9986
DNA-PK	Not Active	5.1586
DYRK1A	Not Active	0.816
DYRK1b	Not Active	1.9828
DYRK2	Not Active	-3.2872
DYRK3	Not Active	-3.1968
DYRK4	Not Active	-1.007
EEF2K	Not Active	0.4815
EGFR	Not Active	1.5935
EIF2AK2	Not Active	-2.4795
EPHA1	Not Active	3.2162
EPHA3	Not Active	0.5311
EPHA4	Not Active	-0.9242
EPHA5	Not Active	-0.2279

EPHA6	Not Active	8.1605
EPHA7	Not Active	-17.0597
EPHA8	Not Active	1.1623
EPHB1	Not Active	1.9382
EPHB2	Not Active	-1.1874
EPHB3	Not Active	-2.4014
EphB4	Not Active	-5.9307
EPHRA2	Not Active	-18.3359
ERBB2	Not Active	-26.3594
ERBB4	Not Active	-0.6242
ERN1	Not Active	4.2634
ERN2	Not Active	0.9538
FER	Not Active	4.9372
FES	Not Active	-3.749
FGFR1	Not Active	0.7141
FGFR2	Not Active	-3.6502
FGFR3	Not Active	-13.1132
FGFR4	Not Active	-2.5773
FGR	Not Active	28.7353
FLT1	Not Active	-0.679
FLT3	Not Active	19.5915
FLT4	Not Active	7.1352
FRAP1	Not Active	-1.5702
FRK	Not Active	7.0623
FYN	Not Active	19.1797
FYN	Not Active	13.2957
GAK	Not Active	5.772
GRK1	Not Active	2.7402
GRK4	Not Active	-5.7218
GRK5	Not Active	-0.8492
GRK6	Not Active	-7.3992
GRK7	Not Active	-7.1098
GSG2	Not Active	2.1795
GSK3A	Not Active	3.083
GSK3b	Not Active	3.1896

HCK	Not Active	22.3389
HIPK1	Not Active	-4.4768
HIPK2	Not Active	-0.3725
HIPK3	Not Active	-2.5806
HIPK4	Not Active	3.1703
HUNK	Not Active	6.1858
IGF1R	Not Active	-2.3744
IKKa	Not Active	-7.5622
IKKb	Not Active	-1.6212
IKKe	Not Active	0.084
INSR	Not Active	-3.2737
INSRR	Not Active	-0.3296
IRAK1	Not Active	1.3789
IRAK3	Not Active	8.6365
IRAK4	Not Active	-5.9368
ITK	Not Active	5.5982
JAK1	Not Active	0.1819
JAK2	Not Active	-5.7933
JAK2	Not Active	-2.2972
JAK3	Not Active	-5.9852
JNK1	Not Active	-13.916
JNK1	Not Active	7.274
JNK3	Not Active	6.453
JNK3	Not Active	-0.9194
KDR	Not Active	58.3984
KSR2	Not Active	2.7424
LATS2	Not Active	-4.9043
LCK	Active	92.9366
LCK	Not Active	-2.7543
LIMK1	Not Active	39.4231
LIMK2	Not Active	14.9854
LRRK2	Not Active	3.6574
LRRK2	Not Active	0.9912
LTK	Not Active	-0.7886
LYN	Not Active	57.0578

LYN	Not Active	58.8141
MAP2K1	Not Active	-11.7041
MAP2K1	Not Active	-2.5137
MAP2K2	Not Active	6.1703
MAP2K2	Not Active	3.6096
MAP2K4	Not Active	9.4686
MAP2K5	Not Active	2.1753
MAP2K6	Not Active	3.551
MAP2K6	Not Active	8.0006
MAP3K10	Not Active	-4.7244
MAP3K11	Not Active	0.8229
MAP3K14	Not Active	0.1223
MAP3K2	Not Active	-8.8374
MAP3K3	Not Active	0.0502
MAP3K5	Not Active	-11.4194
MAP3K7	Not Active	10.4833
MAP3K8	Not Active	5.7642
MAP3K9	Not Active	-14.7382
MAP4K1	Not Active	17.783
MAP4K2	Not Active	12.8842
MAP4K3	Not Active	25.3408
MAP4K4	Not Active	36.3272
MAP4K5	Active	80.2741
MAPK1	Not Active	-6.4226
MAPK13	Not Active	-1.5718
MAPK15	Not Active	9.3096
MAPK3	Not Active	-2.602
MAPK7	Not Active	-3.0275
MAPK9	Not Active	1.7662
MAPK9	Not Active	4.0628
MAPKAP3	Not Active	1.0734
MAPKAPK1A	Not Active	5.7407
MAPKAPK1B	Not Active	-1.3214
MAPKAPK2	Not Active	-0.5722
MAPKAPK5	Not Active	-3.7368

MARK1	Not Active	3.3236
MARK2	Not Active	-3.6675
MARK3	Not Active	-1.7215
MARK4	Not Active	0.108
MASTL	Not Active	-5.5809
MATK	Not Active	2.2758
MELK	Not Active	1.2115
MERTK	Not Active	2.0088
MINK	Not Active	-28.2092
MKNK1	Not Active	1.7727
MKNK2	Not Active	2.5003
MLK4	Not Active	-8.0124
MSK1	Not Active	2.1011
MST1R	Not Active	0.4375
MST4	Not Active	5.7471
MTK	Not Active	4.1741
MUSK	Not Active	4.534
MYLK	Not Active	-0.0096
MYLK2	Not Active	-1.484
MYLK3	Not Active	11.6614
MYLK4	Not Active	13.0256
MYO3A	Not Active	-8.2777
MYO3B	Not Active	8.194
NEK1	Not Active	1.2561
NEK2	Not Active	-5.1718
NEK4	Not Active	-2.2358
NEK6	Not Active	-0.6284
NEK8	Not Active	-6.9331
NEK9	Not Active	-2.3288
NIM1	Not Active	-1.2894
NLK	Not Active	-4.1754
NTRK3	Not Active	-1.937
NUAK2	Not Active	-4.9216
p38a	Not Active	4.6111
p38a	Not Active	1.1482

p38b	Not Active	-1.3953
p38g	Not Active	-6.7944
P70S6K	Not Active	-10.8647
PAK1	Not Active	15.778
PAK2	Not Active	-2.7663
PAK3	Not Active	7.859
PAK4	Not Active	-2.0129
PAK5	Not Active	-12.744
PAK6	Not Active	3.8134
PASK	Not Active	6.9677
PCTK1	Not Active	8.201
PCTK2	Not Active	-1.161
PCTK3	Not Active	-3.9148
PDGFRa	Active	87.6201
PDGFRb	Active	97.8194
PDPK1	Not Active	-2.128
PDPK1	Not Active	2.0565
PEAK1	Not Active	-7.2694
PFTK1	Not Active	-9.0361
PHKG1	Not Active	-3.4148
PHKG2	Not Active	-10.2921
PI3Ka	Not Active	2.5011
PI3Kb	Not Active	-0.1373
PI3Kd	Not Active	-2.5212
PI3Kg	Not Active	32.6563
PI4K?	Not Active	-3.3356
PI4K2A	Not Active	-18.8804
PI4K3A	Not Active	8.492
PI4KB	Not Active	-31.2582
PIK3C2A	Not Active	-3.5343
PIK3C2B	Not Active	-1.1773
PIK3C2G	Not Active	-7.9012
PIK3C3	Not Active	-1.3341
PIK3CA:PIK3R1	Not Active	-3.65
PIK3CB: PIK3R2	Not Active	-4.9451

PIM1	Not Active	3.7972
PIM2	Not Active	-5.8398
PIM3	Not Active	6.4307
PIP5K1A	Not Active	7.8142
PIP5K1B	Not Active	0.4546
PIP5K1C	Not Active	8.4031
PIP5K2A	Not Active	7.718
PKAa	Not Active	1.5972
PKCa	Not Active	-1.5834
PKCb	Not Active	-1.4681
PKCb	Not Active	-6.944
PKCd	Not Active	5.164
PKCe	Not Active	-8.1972
PKCg	Not Active	-2.0944
PKCh	Not Active	4.9319
PKCi	Not Active	-0.1248
PKCRK2	Not Active	5.357
PKCt	Not Active	-10.0319
PKCz	Not Active	3.7496
PKD1	Not Active	4.9023
PKD2	Not Active	1.2898
PKG1	Not Active	8.9793
PKMYT1	Not Active	-16.7792
PKN1	Not Active	2.4695
PKX	Not Active	-3.094
PLK1	Not Active	3.847
PLK2	Not Active	0.7585
PLK3	Not Active	-2.836
PLK4	Not Active	8.0021
PRKACB	Not Active	-11.901
PRKACG	Not Active	2.5296
PRKD3	Not Active	4.7444
PRKG2	Not Active	0.916
PTK2	Not Active	3.7271
PTK2b	Not Active	4.5302

PTK6	Not Active	-3.7964
RET	Active	98.1885
RIPK2	Not Active	48.8754
RIPK3	Not Active	61.07
ROCK1	Not Active	10.7988
ROCK2	Not Active	-6.9814
ROS1	Not Active	-3.638
RPS6KA2	Not Active	2.3604
RPS6KA4	Not Active	-2.1533
RPS6KA6	Not Active	3.144
RPS6KB2	Not Active	7.5368
SBK1	Not Active	0.3238
SGK1	Not Active	-3.9972
SGK2	Not Active	2.3833
SGK3	Not Active	-3.7416
SIK1	Not Active	-1.6398
SIK3	Not Active	-9.9554
SLK	Not Active	-0.814
SNF1LK2	Not Active	-0.5124
SphK1	Not Active	-3.937
SphK2	Not Active	6.9198
src	Not Active	6.1794
SRMS	Not Active	-1.201
SRPK1	Not Active	-0.768
SRPK2	Not Active	-2.8148
STE20	Not Active	-3.5264
STK16	Not Active	8.3312
STK17A	Not Active	-14.4982
STK17B	Not Active	2.9058
STK23	Not Active	-4.0315
STK24	Not Active	3.6746
STK25	Not Active	2.4988
STK32B	Not Active	-3.2289
STK32C	Not Active	5.001
STK33	Not Active	0.0062

STK38	Not Active	-1.1223
STK38L	Not Active	-8.2522
STK39	Not Active	8.5262
STK4	Not Active	-5.8051
SYK	Not Active	0.5504
TAO2	Not Active	0.8342
TAO3	Not Active	4.9162
TAOK1	Not Active	2.1886
TBK1	Not Active	-2.248
TEC	Not Active	-6.9758
TESK1	Not Active	-4.7141
TESK2	Not Active	14.1032
TGFBR1	Not Active	9.5964
TGFbR2	Not Active	5.2548
TIE2	Not Active	6.1946
TLK1	Not Active	-6.0237
TLK2	Not Active	-3.7008
TNIK	Not Active	70.6957
TNK1	Not Active	8.6212
TNK2	Active	76.6878
TRKA	Not Active	6.9286
TRKB	Not Active	-4.9116
TSSK1A; TSSK1B	Not Active	-0.7173
TSSK2	Not Active	1.3973
TTK	Not Active	-10.3016
TXK	Not Active	6.6321
TYK2	Not Active	-0.5864
TYRO3	Not Active	0.3211
ULK1	Not Active	-10.1536
ULK2	Not Active	-1.6615
ULK3	Not Active	-11.1906
VRK2	Not Active	11.7068
WEE1	Not Active	-10.8813
WNK1	Not Active	-3.1211
WNK2	Not Active	3.8232

WNK3	Not Active	-18.605
YES	Not Active	6.4422
YSK4	Not Active	-9.3702
ZAK	Not Active	33.165
ZAP70	Not Active	0.5569

Synthetic Experimental Section

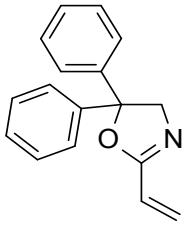
General Experimental

Air and/or moisture sensitive reactions were performed under an atmosphere of nitrogen. Dry organic solvents and starting materials were obtained from commercial sources and used as received unless otherwise specified. 4 Å molecular sieves were oven-dried prior to use.

Reactions were monitored by thin layer chromatography (TLC) using Merck silica gel 60 covered alumina plates F254 or by analytical UPLC. Visualisation of TLC plates was carried out under UV light and stained using potassium permanganate solution. Analytical UPLC was performed on CSH C18 reverse-phase silica, using a Waters XSelect CSH C18 column with dimensions 2.1 x 50mm and particle size 1.7 micron). Gradient analysis was employed using decreasingly polar mixtures as eluent, for example decreasingly polar mixtures of water (containing 0.1% formic acid or 0.1% ammonia) as solvent A and acetonitrile as solvent B. Normal phase flash column chromatography was performed using ultra performance Interchim puriflash 50 µm silica columns and carried out using Teledyne ISCO Combiflash Lumen system. Reverse phase column chromatography was carried out using preparative HPLC (Waters XSelect CSH C18 ODB column, 5µ silica, 30 mm diameter, 100 mm length) Interchim 4250 system.

All ^{1}H spectra were recorded on Bruker 400 MHz or 500 MHz spectrometers at rt. ^{1}H NMR data are reported as follows: chemical shifts in ppm relative to $CDCl_3$ (7.26) or d6-DMSO (2.50) on the δ scale, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, app. = apparent or a combination of thereof), coupling constant(s) J (Hz) and integration,. All ^{13}C NMR spectra were recorded on Bruker 400 MHz and Bruker 500 MHz spectrometers at 101 MHz and 126 MHz respectively. Data are reported as follows; chemical shifts in ppm relative to $CDCl_3$ (77.16) or d6-DMSO (39.52) on the δ scale. Low resolution mass spectra were recorded by UPLC methods on reverse-phase C18 silica with detection by Electrospray Mass Spectrometry using positive or negative ion electrospray (ESI+ or ESI-) and by UV absorbance recording a wavelength range of 220-320 nm. High resolution mass spectra were recorded on Thermo Scientific and Fusion Orbitrap MS using positive or negative ion electrospray (ESI+ or ESI-).

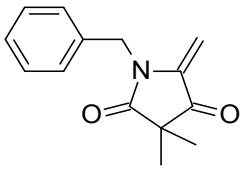
Experimental Procedures



5,5-diphenyl-2-vinyl-4H-oxazole (1): Acryloyl chloride (0.052 mL, 0.64 mmol) was added to 2-amino-1,1-diphenylethan-1-ol[a] (130 mg, 0.61 mmol) and triethylamine (0.212 mL, 1.52 mmol) in DCM (3 mL) at 0 °C over a period of 5 minutes under nitrogen. The resulting solution was warmed to rt and stirred at rt for 2.5 hours. The reaction mixture was quenched with saturated NH_4Cl (10 mL) and diluted with DCM (10 mL). The biphasic mixture was separated, and the organic layer washed with saturated brine (10 mL). The organic layer was dried over MgSO*4*, filtered and evaporated to afford crude material. The crude product was purified by preparative HPLC (Waters XSelect CSH C18 ODB column, 5µ silica, 30 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% NH_3) and MeCN as eluents. Fractions containing the desired compound were combined and diluted with DCM (50 mL) and saturated aq. NH_4Cl (50 mL). The biphasic mixture was separated and the organic phase dried over $MgSO_4$, filtered and evaporated to afford *N*-(2-hydroxy-2,2-diphenylethyl)acrylamide (57.1 mg) as a yellow solid.

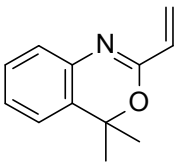
Methanesulfonic acid (0.03 mL, 0.46 mmol) was added to *N*-(2-hydroxy-2,2-diphenylethyl)acrylamide (25 mg, 0.09 mmol) in DCM (2.000 mL) at 25 °C under nitrogen. The resulting solution was stirred at 45 °C for 4 hours. The reaction mixture was quenched with saturated NaHCO*3* (10 mL), extracted with DCM (10 mL), the organic layer was dried over a phase separating cartridge, filtered and evaporated to afford crude material. The crude materal was loaded on to two 0.25 mm silica plates and purified by prep TLC (eluent 35% EtOAc in heptane). UV active material at Rf 0.40 was taken and dissolved in DCM, silica was filtered off and the solvent was removed under vacuum to afford 5,5-diphenyl-2-vinyl-4,5-dihydrooxazole (6.1 mg, 26%, over 2 steps) as a colorless wax. ^{1}H NMR (400 MHz, DMSO) 4.48 (s, 2H), 5.86 (dd, *J* = 10.4, 2.0 Hz, 1H), 6.28 (dd, *J* = 17.5, 2.0 Hz, 1H), 6.37 (dd, *J* = 17.5, 10.4 Hz, 1H), 7.27 – 7.32 (m, 2H), 7.35 – 7.45 (m, 8H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 161.1, 144.3, 128.5, 127.6, 126.7, 125.2, 124.8, 89.0, 67.9; HRMS (ESI+) calcd for $C_{17}H_{15}NO$ $[M+H]^+$ 250.1226, found 250.1232

[a]2-amino-1,1-diphenylethan-1-ol was made according to literature procedure[13]



1-benzyl-3,3-dimethyl-5-methylene-pyrrolidine-2,4-dione (3): Diisopropylamine (0.650 mL, 4.60 mmol) was added to 1-benzyl-3,3-dimethylpyrrolidine-2,4-dione (250 mg, 1.15 mmol)[b] in THF (20 mL) at 25 °C under nitrogen. The resulting solution was warmed to 65 °C and stirred for 30 minutes. Paraformaldehyde (207 mg, 2.30 mmol) in THF (20 mL) was added and the reaction stirred for 4 hours. Water (10 mL) was subsequently added and the solution heated at reflux for a further 17 hours. The reaction mixture was quenched with 2M HCl (5 mL), diluted with water (50 mL) and extracted with Et_2O (2 x 50 mL). The organic layers were combined and washed with saturated brine (100 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 50% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 1-benzyl-3,3-dimethyl-5-methylenepyrrolidine-2,4-dione (200 mg, 76%) as a colorless oil. ^{1}H NMR (400 MHz, DMSO) 1.21 (s, 6H), 4.71 (d, *J* = 2.1 Hz, 1H), 4.84 (s, 2H), 5.05 (d, *J* = 2.1 Hz, 1H), 7.24 – 7.31 (m, 3H), 7.31 – 7.4 (m, 2H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 199.7, 175.0, 140.1, 135.4, 128.6, 127.4, 127.0, 92.3, 44.7, 42.6, 20.1; HRMS (ESI+) calcd for $C_{14}H_{15}NO_2$ $[M+H]^+$ 230.1176, found 230.1176

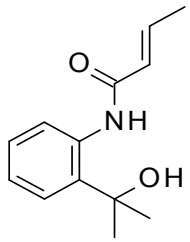
[b]1-benzyl-3,3-dimethylpyrrolidine-2,4-dione was made according to literature procedure[14]



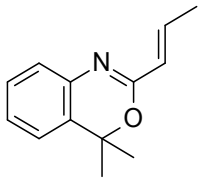
4,4-dimethyl-2-vinyl-3,1-benzoxazine (5a): Acryloyl chloride (0.24 mL, 2.91 mmol) was added dropwise to 2-(2-aminophenyl)propan-2-ol (440 mg, 2.91 mmol) and triethylamine (1.01 mL, 7.27 mmol) in DCM (13.5 mL) at 25 °C over a period of 5 minutes under nitrogen. The resulting solution was stirred at rt for 90 minutes. The reaction mixture was washed sequentially with saturated NH_4Cl (15 mL) and saturated $NaHCO_3$ (15 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude *N*-(2-(2-hydroxypropan-2-yl)phenyl)acrylamide (589 mg) as an orange oil, which was used directly without further purification.

Methanesulfonic acid (0.08 mL, 1.23 mmol) was added to crude *N*-(2-(2-hydroxypropan-2-yl)phenyl)acrylamide (50 mg) in DCM (5 mL) at 25 °C under nitrogen. The resulting solution was warmed to 45 °C and stirred for 3 hours. The reaction was cooled to rt and quenched with saturated $NaHCO_3$ (10 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford 4,4-dimethyl-2-vinyl-4H-benzo[d][1,3]oxazine (45.8 mg, quant., over 2 steps) as an orange oil. ^{1}H NMR (400 MHz, DMSO) 1.58 (s, 6H), 5.74 (dd, *J* = 10.4, 1.8 Hz, 1H), 6.14 (dd, *J* = 17.4, 1.8 Hz, 1H), 6.28 (dd, *J* = 17.4, 10.4 Hz, 1H), 7.07 – 7.14 (m, 1H), 7.18 – 7.24 (m, 1H), 7.25 – 7.31 (m, 2H); ^{13}C

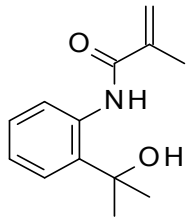
NMR (101 MHz, DMSO, 30 °C) 155.5, 137.9, 131.7, 131.4, 128.4, 126.9, 124.9, 124.4, 122.8, 77.6, 27.9; HRMS (ESI+) calcd for $C_{12}H_{13}NO$ $[M+H]^+$ 188.0679, found 188.0699.



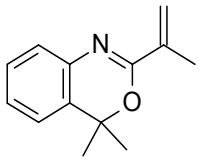
(*E*)-*N*-[2-(1-hydroxy-1-methyl-ethyl)phenyl]but-2-enamide: (*E*)-but-2-enoyl chloride (0.09 mL, 0.94 mmol) was added to 2-(2-aminophenyl)propan-2-ol (145 mg, 0.96 mmol) and triethylamine (0.33 mL, 2.37 mmol) in DCM (4.5 mL) at 25 °C under nitrogen. The resulting solution was stirred at rt for 90 minutes. The reaction mixture was diluted with DCM (10 mL), and washed sequentially with saturated NH_4Cl (20 mL) and saturated $NaHCO_3$ (20 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product. The crude product was purified by preparative HPLC (Waters XSelect CSH C18 ODB column, 5µ silica, 30 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford (*E*)-*N*-(2-(2-hydroxypropan-2-yl)phenyl)but-2-enamide (43.8 mg, 21%) as a white solid. ^{1}H NMR (400 MHz, DMSO) 1.53 (s, 6H), 1.88 (dd, *J* = 6.8, 1.7 Hz, 3H), 5.96 – 6.02 (m, 1H), 6.14 (s, 1H), 6.76 (dq, *J* = 15.2, 6.8 Hz, 1H), 7.02 (td, *J* = 7.8, 1.5 Hz, 1H), 7.22 (ddd, *J* = 8.3, 7.8, 1.5 Hz, 1H), 7.29 (dd, *J* = 7.8, 1.5 Hz, 1H), 8.20 (d, *J* = 8.3 Hz, 1H), 10.59 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 162.6, 139.5, 137.1, 127.1, 126.9, 125.4, 123.1, 121.6, 72.8, 30.0, 17.4; LRMS *m/z* (ESI-) $[M-H]^-$ 218.3



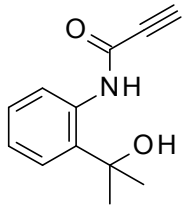
4,4-dimethyl-2-[(*E*)-prop-1-enyl]-3,1-benzoxazine (5b): Methanesulfonic acid (0.06 mL, 0.92 mmol) was added to (*E*)-N-(2-(2-hydroxypropan-2-yl)phenyl)but-2-enamide (41 mg, 0.19 mmol) in DCM (4.0 mL) under nitrogen. The resulting solution was warmed to 45 °C and stirred for 1 hour. The reaction mixture was diluted with DCM (5 mL) and washed with saturated $NaHCO_3$ (40 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford desired (*E*)-4,4-dimethyl-2-(prop-1-en-1-yl)-4H-benzo[d][1,3]oxazine (26.0 mg, 69%) as a white waxy solid. ^{1}H NMR (400 MHz, DMSO) 1.55 (s, 6H), 1.88 (dd, *J* = 6.9, 1.6 Hz, 3H), 5.99 (dq, *J* = 15.5, 1.6 Hz, 1H), 6.71 (dq, *J* = 15.5, 6.9 Hz, 1H), 7.05 – 7.08 (m, 1H), 7.15 – 7.2 (m, 1H), 7.23 – 7.28 (m, 2H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 155.7, 138.3, 138.1, 131.4, 128.3, 126.4, 125.8, 124.1, 122.7, 77.3, 27.8, 17.8; HRMS (ESI+) calcd for $C_{13}H_{15}NO$ $[M+H]^+$ 202.12264, found 202.12282.



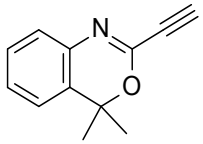
***N*-[2-(1-hydroxy-1-methyl-ethyl)phenyl]-2-methyl-prop-2-enamide**: Methacryloyl chloride (0.094 mL, 0.96 mmol) was added to 2-(2-aminophenyl)propan-2-ol (145 mg, 0.96 mmol) and triethylamine (0.33 mL, 2.37 mmol) in DCM (4.5 mL) under nitrogen. The resulting solution was stirred at rt for 1 hour. The reaction mixture was diluted with DCM (5 mL), and washed sequentially with saturated NH_4Cl (15 mL) and saturated $NaHCO_3$ (15 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford desired product. The crude product was purified by preparative HPLC (Waters XSelect CSH C18 ODB column, 5µ silica, 30 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford *N*-(2-(2-hydroxypropan-2-yl)phenyl)methacrylamide (108 mg, 51%) as a white solid. ^{1}H NMR (400 MHz, DMSO) 1.54 (s, 6H), 1.99 (dd, *J* = 1.4, 0.9 Hz, 3H), 5.51 – 5.54 (m, 1H), 5.8 – 5.83 (m, 1H), 6.24 (s, 1H), 6.99 – 7.08 (m, 1H), 7.21 – 7.26 (m, 1H), 7.29 (dd, *J* = 7.8, 1.4 Hz, 1H), 8.29 (dd, *J* = 8.2, 1.4 Hz, 1H), 11.05 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 164.6, 140.6, 137.1, 135.9, 127.2, 125.4, 123.1, 121.0, 119.9, 72.9, 30.0, 18.4; LRMS *m/z* (ESI-) $[M-H]^-$ 218.2



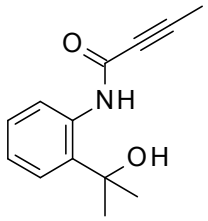
2-isopropenyl-4,4-dimethyl-3,1-benzoxazine (5c): Methanesulfonic acid (0.07 mL, 1.08 mmol) was added to *N*-(2-(2-hydroxypropan-2-yl)phenyl)methacrylamide (50 mg, 0.23 mmol) in DCM (5 mL) under nitrogen. The resulting solution was stirred at 45 °C for 1 hour. The reaction mixture was diluted with DCM (5 mL) and washed sequentially with saturated $NaHCO_3$ (40 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford desired product 4,4-dimethyl-2-(prop-1-en-2-yl)-4H-benzo[d][1,3]oxazine (43.2 mg, 94%) as a white waxy solid. ^{1}H NMR (400 MHz, DMSO) 1.56 (s, 6H), 2.00 (s, 3H), 5.52 – 5.56 (m, 1H), 5.95 (s, 1H), 7.11 – 7.15 (m, 1H), 7.19 – 7.24 (m, 1H), 7.25 – 7.31 (2H, m); ^{13}C NMR (101 MHz, DMSO, 30 °C) 156.6, 138.0, 137.9, 131.5, 128.3, 126.9, 124.6, 122.7, 121.2, 77.4, 27.7, 18.4; HRMS (ESI+) calcd for $C_{13}H_{15}NO$ $[M+H]^+$ 202.12264, found 202.12302.



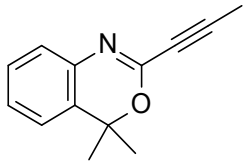
***N*-[2-(1-hydroxy-1-methyl-ethyl)phenyl]prop-2-ynamide**: Dicyclohexylmethanediimine (136 mg, 0.66 mmol) was added to 2-(2-aminophenyl)propan-2-ol (95 mg, 0.63 mmol) and propiolic acid (0.039 mL, 0.63 mmol) in DCM (3.3 mL) under nitrogen. The resulting solution was stirred at rt for 2 hours. The reaction mixture was filtered and solvent evaporated to afford crude product.The crude product was purified by preparative HPLC (Waters XSelect CSH C18 ODB column, 5µ silica, 30 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford *N*-(2-(2-hydroxypropan-2-yl)phenyl)propiolamide (67.8 mg, 53%) as a pale yellow waxy solid. ^{1}H NMR (400 MHz, $CDCl_3$) 1.54 (s, 6H), 4.39 (s, 1H), 6.30 (s, 1H), 7.04 – 7.14 (m, 1H), 7.25 (td, *J* = 7.9, 1.4 Hz, 1H), 7.29 – 7.37 (m, 1H), 8.03 – 8.11 (m, 1H), 11.1 – 11.25 (m, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 148.7, 136.3, 135.9, 127.3, 125.7, 124.2, 121.8, 78.5, 76.5, 72.9, 30.1; LRMS *m/z* (ESI-) $[M-H]^-$ 202.0



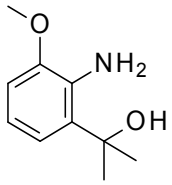
2-ethynyl-4,4-dimethyl-3,1-benzoxazine (5d): Methanesulfonic acid (0.1 mL, 1.54 mmol) was added to N-(2-(2-hydroxypropan-2-yl)phenyl)propiolamide (61 mg, 0.30 mmol) in DCM (6.5 mL) under nitrogen. The resulting solution was warmed to 45 °C and stirred for 1 hour. The reaction mixture was diluted with DCM (5 mL) and washed sequentially with saturated $NaHCO_3$ (25 mL) and saturated brine (10 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product.The crude product was purified by preparative HPLC (Waters XSelect CSH C18 ODB column, 5µ silica, 30 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 2-ethynyl-4,4-dimethyl-4H-benzo[d][1,3]oxazine (19.6 mg, 35%) as an amorphous orange solid. ^{1}H NMR (400 MHz, DMSO) 1.60 (s, 6H), 4.40 (s, 1H), 7.06 – 7.14 (m, 1H), 7.22 – 7.35 (m, 3H); ^{13}C NMR (126 MHz, DMSO, 27 °C) 142.4, 136.8, 131.2, 128.6, 128.1, 124.4, 123.1, 79.6, 79.1, 77.3, 28.1; HRMS (ESI+) calcd for $C_{12}H_{11}NO$ $[M+H]^+$ 186.09134, found 186.09149.



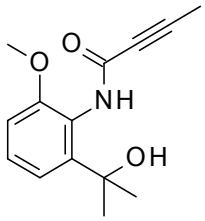
***N*-[2-(1-hydroxy-1-methyl-ethyl)phenyl]but-2-ynamide**: Dicyclohexylmethanediimine (136 mg, 0.66 mmol) was added to 2-(2-aminophenyl)propan-2-ol (95 mg, 0.63 mmol) and but-2-ynoic acid (52.8 mg, 0.63 mmol) in DCM (3.3 mL) under nitrogen. The resulting solution was stirred at rt for 2 hours. The reaction mixture was filtered and solvent evaporated to afford crude product. The crude product was purified by preparative HPLC (Waters XSelect CSH C18 ODB column, 5µ silica, 30 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford *N*-(2-(2-hydroxypropan-2-yl)phenyl)but-2-ynamide (128 mg, 94%) as a colorless waxy solid. ^{1}H NMR (400 MHz, DMSO) 1.53 (s, 6H), 2.03 (s, 3H), 6.23 (s, 1H), 7.01 – 7.1 (m, 1H), 7.18 – 7.27 (m, 1H), 7.27 – 7.34 (m, 1H), 8.08 (d, *J* = 8.0 Hz, 1H), 10.90 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 149.6, 136.2, 136.1, 127.2, 125.5, 123.8, 121.7, 83.8, 75.9, 72.8, 30.1, 3.0; LRMS *m/z* (ESI-) $[M-H]^-$ 216.2



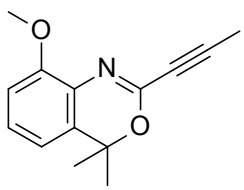
4,4-dimethyl-2-prop-1-ynyl-3,1-benzoxazine (5e): Methanesulfonic acid (0.06 mL, 0.92 mmol) was added to *N*-(2-(2-hydroxypropan-2-yl)phenyl)but-2-ynamide (39 mg, 0.18 mmol) in DCM (3.9 mL) under nitrogen. The resulting solution was warmed to 45 °C and stirred for 2 hours. The reaction mixture was diluted with DCM (5 mL) and washed sequentially with saturated $NaHCO_3$ (25 mL) and saturated brine (10 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 30% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 4,4-dimethyl-2-(prop-1-yn-1-yl)-4H-benzo[d][1,3]oxazine (23 mg, 64%) as a colorless oil which solidified on standing. ^{1}H NMR (400 MHz, DMSO) 1.57 (s, 6H), 2.05 (s, 3H), 7.05 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.2 – 7.31 (m, 3H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 143.1, 137.3, 131.1, 128.5, 127.5, 124.1, 122.9, 86.3, 79.1, 74.4, 28.0, 3.3; HRMS (ESI+) calcd for $C_{13}H_{13}NO$ $[M+H]^+$ 200.10699, found 200.10704.



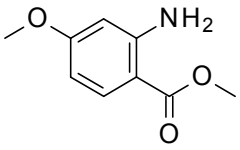
2-(2-amino-3-methoxy-phenyl)propan-2-ol: Methylmagnesium bromide (3M in Et_2O) (11.4 mL, 34.2 mmol) was added dropwise to methyl 2-amino-3-methoxybenzoate (1.98 g, 10.9 mmol) in THF (25 mL) over a period of 30 minutes under nitrogen. The resulting solution was stirred at rt for 18 hours. The reaction mixture was quenched with saturated NH_4Cl (25 mL), extracted with EtOAc (2 x 20 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford 2-(2-amino-3-methoxyphenyl)propan-2-ol (1.96 g, 99%) as a yellow oil which solidified on standing. ^{1}H NMR (400 MHz, DMSO) 1.50 (s, 6H), 3.76 (s, 3H), 5.07 (s, 2H), 5.20 (s, 1H), 6.49 (t, *J* = 7.9 Hz, 1H), 6.69 (dd, *J* = 7.9, 1.1 Hz, 1H), 6.73 (dd, *J* = 7.9, 1.1 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 146.9, 135.7, 131.0, 117.7, 115.1, 109.0, 72.1, 55.5, 29.2; LRMS *m/z* (ESI+) $[M\text{-}OH]^+$ 164.1



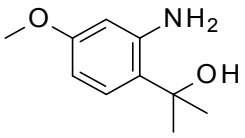
***N*-[2-(1-hydroxy-1-methyl-ethyl)-6-methoxy-phenyl]but-2-ynamide**: Dicyclohexylmethanediimine (2.45 g, 11.9 mmol) was added to 2-(2-amino-3-methoxyphenyl)propan-2-ol (1.96 g, 10.8 mmol) and 2-butynoic acid (0.918 g, 10.9 mmol) in DCM (55 mL) under nitrogen. The resulting suspension was stirred at rt for 2 hours. The reaction mixture was diluted with Et_2O (50 mL), filtered and the solvent removed under vacuum to afford the crude as a pale yellow solid. The crude product was purified by flash silica chromatography, elution gradient 40 to 60% EtOAc in heptane. Pure fractions were evaporated to dryness to afford *N*-(2-(2-hydroxypropan-2-yl)-6-methoxyphenyl)but-2-ynamide (1.59 g, 59%) as a white solid. ^{1}H NMR (400 MHz, DMSO) 1.42 (s, 6H), 2.01 (s, 3H), 3.29 (s, 1H), 3.71 (s, 3H), 5.20 (s, 1H), 6.9 – 6.98 (m, 1H), 7.15 – 7.25 (m, 2H), 9.38 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 155.3, 151.5, 146.7, 127.1, 122.5, 117.6, 110.5, 82.9, 76.0, 71.1, 55.7, 30.2, 3.0; LRMS *m/z* (ESI-) $[M\text{-}H]^-$ 246.1



8-methoxy-4,4-dimethyl-2-prop-1-ynyl-3,1-benzoxazine (*o*-MeO-5e): Methanesulfonic acid (0.41 mL, 6.31 mmol) was added to *N*-(2-(2-hydroxypropan-2-yl)-6-methoxyphenyl)but-2-ynamide (310 mg, 1.25 mmol) in DCM (27.5 mL) under nitrogen. The resulting solution was warmed to 45 °C and stirred for 3 hours. The reaction mixture was quenched with saturated $NaHCO_3$ (30 mL), the organic layer was dried over a phase separating cartridge, filtered and evaporated to afford colorless oil. The crude product was purified by flash silica chromatography, elution gradient 35 to 50% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 8-methoxy-4,4-dimethyl-2-(prop-1-yn-1-yl)-4H-benzo[d][1,3]oxazine (153 mg, 53%) as a colorless oil. ^{1}H NMR (400 MHz, DMSO) 1.54 (s, 6H), 2.04 (s, 3H), 3.77 (s, 3H), 6.83 (dd, *J* = 7.8, 1.1 Hz, 1H), 6.92 (dd, *J* = 8.3, 1.1 Hz, 1H), 7.18 (dd, *J* = 8.3, 7.8 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 152.2, 141.8, 132.2, 128.0, 126.6, 114.5, 111.5, 86.0, 78.6, 74.7, 55.6, 27.9, 3.3; HRMS (ESI+) calcd for $C_{14}H_{15}NO_2$ $[M+H]^+$ 230.1181, found 230.1182.

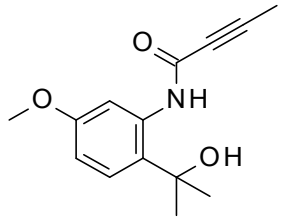


methyl 2-amino-4-methoxy-benzoate: A solution of methyl 4-methoxy-2-nitrobenzoate (1 g, 4.74 mmol) in EtOH (24 mL) was added slowly to 10% palladium on charcoal (100 mg, 4.74 mmol) under a positive flow of nitrogen. The resulting suspension was placed under a hydrogen atmosphere and stirred at rt for 17 hours. The reaction mixture was filtered over celite and washed with EtOH (3 x 30 mL). The solvent was removed under vacuum to afford the crude as a brown solid. The crude product was purified by flash silica chromatography, elution gradient 10 to 50% EtOAc in heptane. Pure fractions were evaporated to dryness to afford methyl 2-amino-4-methoxybenzoate (0.794 g, 93%) as a white solid. ^{1}H NMR (400 MHz, DMSO) 3.72 (s, 3H), 3.74 (s, 3H), 6.13 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.27 (d, *J* = 2.5 Hz, 1H), 6.68 (s, 2H), 7.62 (d, *J* = 9.0 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 167.4, 163.7, 153.3, 132.3, 103.6, 102.4, 98.6, 54.9, 51.0; LRMS *m/z* (ESI+) $[M+H]^+$ 182.0

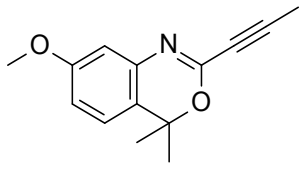


2-(2-amino-4-methoxy-phenyl)propan-2-ol: Methylmagnesium bromide (3M in Et_2O) (4.5 mL, 13.5 mmol) was added dropwise to methyl 2-amino-4-methoxybenzoate (785 mg, 4.33 mmol) in THF (10 mL) over a period of 20 minutes under nitrogen. The resulting solution was stirred at rt for 18 hours. The reaction mixture was quenched with saturated NH_4Cl (15 mL), extracted with EtOAc (2 x 20 mL), the organic layer was dried over a phase separating cartridge, filtered and evaporated to afford 2-(2-amino-4-methoxyphenyl)propan-2-ol (782 mg, quant.) as a yellow

oil which solidified on standing and was used without further purification. ^{1}H NMR (400 MHz, DMSO) 1.46 (s, 6H), 3.63 (s, 3H), 5.04 (s, 1H), 5.40 (s, 2H), 6.04 (dd, *J* = 8.5, 2.7 Hz, 1H), 6.18 (d, *J* = 2.7 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 158.7, 148.1, 125.9, 124.0, 101.2, 100.8, 71.7, 54.5, 29.4; LRMS *m/z* (ESI+) $[M\text{-}OH]^{+}$ 164.1

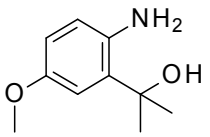


***N*-[2-(1-hydroxy-1-methyl-ethyl)-5-methoxy-phenyl]but-2-ynamide**: Dicyclohexylmethanediimine (971 mg, 4.70 mmol) was added to 2-(2-amino-4-methoxyphenyl)propan-2-ol (775 mg, 4.28 mmol) and 2-butynoic acid (363 mg, 4.32 mmol) in DCM (21 mL) under nitrogen. The resulting solution was stirred at rt for 2 hours. The reaction mixture was diluted with Et_2O (50 mL), filtered and the solvent removed under vacuum to afford the crude as a yellow oil. The crude product was purified by flash silica chromatography, elution gradient 5 to 50% EtOAc in heptane. Pure fractions were evaporated to dryness to afford *N*-(2-(2-hydroxypropan-2-yl)-5-methoxyphenyl)but-2-ynamide (927 mg, 88%) as a colorless oil which solidified on standing. ^{1}H NMR (400 MHz, DMSO) 1.50 (s, 6H), 2.03 (s, 3H), 3.71 (s, 3H), 6.16 (s, 1H), 6.63 (dd, *J* = 8.7, 2.7 Hz, 1H), 7.19 (d, *J* = 8.7 Hz, 1H), 7.77 (d, *J* = 2.7 Hz, 1H), 10.98 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 158.1, 149.6, 137.3, 128.2, 126.3, 108.5, 107.4, 83.8, 75.9, 72.6, 55.0, 30.3, 3.0; LRMS *m/z* (ESI-) $[M\text{-}H]^{-}$ 246.1

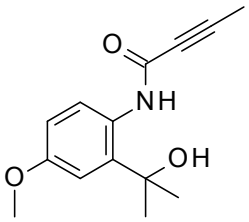


7-methoxy-4,4-dimethyl-2-prop-1-ynyl-3,1-benzoxazine (*m*-OMe-5e): Methanesulfonic acid (0.35 mL, 5.39 mmol) was added to *N*-(2-(2-hydroxypropan-2-yl)-5-methoxyphenyl)but-2-ynamide (260 mg, 1.05 mmol) in DCM (23 mL) under nitrogen. The resulting solution was warmed to 45 °C and stirred for 3 hours. The reaction mixture was quenched with saturated $NaHCO_3$ (25 mL), the organic layer was dried over a phase separating cartridge, filtered and evaporated to afford yellow oil. The crude product was purified by flash silica chromatography, elution gradient 30 to 40% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 7-methoxy-4,4-dimethyl-2-(prop-1-yn-1-yl)-4H-benzo[d][1,3]oxazine (188 mg, 78%) as a pale yellow oil. ^{1}H NMR (400 MHz, DMSO) 1.54 (s, 6H), 2.04 (s, 3H), 3.74 (s, 3H), 6.62 (d, *J* = 2.7 Hz, 1H), 6.79 (dd, *J* = 8.5, 2.7 Hz, 1H), 7.17 (d, *J* = 8.5 Hz, 1H); ^{13}C NMR (101 MHz,

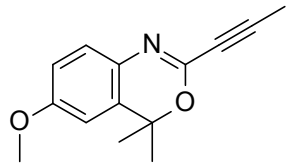
DMSO, 30 °C) 159.3, 143.5, 138.5, 123.9, 123.4, 113.1, 109.1, 86.4, 79.2, 74.4, 55.2, 28.2, 3.3; HRMS (ESI+) calcd for $C_{14}H_{15}NO_2$ $[M+H]^+$ 230.1181, found 230.1182.



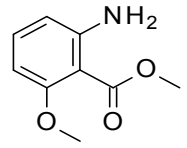
2-(2-amino-5-methoxy-phenyl)propan-2-ol: Methylmagnesium bromide (3M in Et_2O) (11.4 mL, 34.2 mmol) was added dropwise to methyl 2-amino-5-methoxybenzoate (2.0 g, 11.0 mmol) in THF (25 mL) over a period of 30 minutes under nitrogen. The resulting solution was stirred at rt for 20 hours. The reaction mixture was quenched with saturated NH_4Cl (25 mL), extracted with EtOAc (2 x 20 mL), the organic layer was dried over a phase separating cartridge, filtered and evaporated to afford brown oil. The crude product was purified by flash silica chromatography, elution gradient 5 to 60% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 2-(2-amino-5-methoxyphenyl)propan-2-ol (1.65 g, 82%) as a yellow oil. ^{1}H NMR (400 MHz, DMSO) 1.48 (s, 6H), 3.62 (s, 3H), 4.97 (s, 2H), 5.15 (s, 1H), 6.52 – 6.6 (m, 2H), 6.62 (d, *J* = 2.6 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 150.4, 140.5, 132.2, 116.8, 112.2, 112.0, 71.9, 55.3, 29.0; LRMS *m/z* (ESI+) $[M-OH]^+$ 164.1



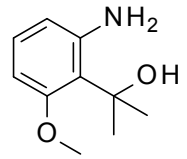
***N*-[2-(1-hydroxy-1-methyl-ethyl)-4-methoxy-phenyl]but-2-ynamide**: Dicyclohexylmethanediimine (2.02 g, 9.79 mmol) was added to 2-(2-amino-5-methoxyphenyl)propan-2-ol (1.61 g, 8.88 mmol) and 2-butynoic acid (0.752 g, 8.94 mmol) in DCM (45 mL) under nitrogen. The resulting suspension was stirred at rt for 1 hour. The reaction mixture was diluted with Et2O (50 mL), filtered and the solvent removed under vacuum to afford the crude as a yellow oil. The crude product was purified by flash silica chromatography, elution gradient 0 to 60% EtOAc in heptane. Pure fractions were evaporated to dryness to afford *N*-(2-(2-hydroxypropan-2-yl)-4-methoxyphenyl)but-2-ynamide (1.96 g, 89%) as a yellow foam. ^{1}H NMR (400 MHz, DMSO) 1.51 (s, 6H), 2.02 (s, 3H), 3.73 (s, 3H), 6.12 (s, 1H), 6.75 – 6.87 (m, 2H), 7.92 (d, *J* = 8.6 Hz, 1H), 10.60 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 155.4, 149.5, 138.5, 129.2, 123.5, 112.0, 111.3, 83.4, 76.0, 72.6, 55.2, 30.0, 3.0; LRMS *m/z* (ESI+) $[M-OH]^+$ 230.0



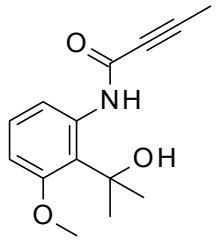
6-methoxy-4,4-dimethyl-2-prop-1-ynyl-3,1-benzoxazine (*p*-OMe-5e): Methanesulfonic acid (0.58 mL, 8.93 mmol) was added to *N*-(2-(2-hydroxypropan-2-yl)-4-methoxyphenyl)but-2-ynamide (440 mg, 1.78 mmol) in DCM (40 mL) under nitrogen. The resulting solution was warmed to 45 °C and stirred for 3 hours. The reaction mixture was quenched with saturated $NaHCO_3$ (50 mL), the organic layer was dried over a phase separating cartridge, filtered and evaporated to afford yellow oil. The crude product was purified by flash silica chromatography, elution gradient 10 to 50% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 6-methoxy-4,4-dimethyl-2-(prop-1-yn-1-yl)-4H-benzo[d][1,3]oxazine (237 mg, 58%) as a pale yellow oil. ^{1}H NMR (400 MHz, DMSO) 1.55 (s, 6H), 2.03 (s, 3H), 3.76 (s, 3H), 6.79 – 6.88 (m, 2H), 6.96 – 7.03 (m, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 158.6, 141.1, 132.5, 130.8, 125.4, 113.3, 108.8, 85.7, 78.8, 74.5, 55.4, 27.9, 3.3; HRMS (ESI+) calcd for $C_{14}H_{15}NO_2$ $[M+H]^+$ 230.1181, found 230.1182.



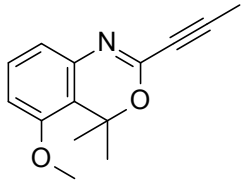
methyl 2-amino-6-methoxy-benzoate: A solution of methyl 2-methoxy-6-nitrobenzoate (1.01 g, 4.74 mmol) in EtOH (24 mL) was added slowly to 10% Palladium on charcoal (100 mg, 4.74 mmol) under a positive flow of nitrogen. The resulting suspension was placed under a hydrogen atmosphere and stirred at rt for 17 hours. The reaction mixture was filtered over celite and washed with EtOH (3 x 30 mL). The solvent was removed under vacuum to afford the crude as a yellow oil. The crude product was purified by flash silica chromatography, elution gradient 10 to 50% EtOAc in heptane. Pure fractions were evaporated to dryness to afford methyl 2-amino-6-methoxybenzoate (841 mg, 98%) as a pale yellow oil which solidified on standing. ^{1}H NMR (400 MHz, DMSO) 3.69 (s, 3H), 3.74 (s, 3H), 5.64 (s, 2H), 6.19 (dd, *J* = 8.2, 0.8 Hz, 1H), 6.33 (dd, *J* = 8.2, 0.8 Hz, 1H), 7.06 (t, *J* = 8.2 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 167.6, 158.8, 149.1, 131.9, 108.6, 104.7, 98.6, 55.5, 51.4; LRMS *m/z* (ESI+) $[M+H]^+$ 182.0



2-(2-amino-6-methoxy-phenyl)propan-2-ol: Methylmagnesium bromide (3M in Et_2O) (4.8 mL, 14.4 mmol) was added dropwise to methyl 2-amino-6-methoxybenzoate (839 mg, 4.63 mmol) in THF (10.7 mL) over a period of 30 minutes under nitrogen. The resulting solution was stirred at rt for 17 hours. The reaction mixture was quenched with saturated NH_4Cl (15 mL), extracted with EtOAc (2 x 20 mL), the organic layer was dried over a phase separating cartridge, filtered and evaporated to afford 2-(2-amino-6-methoxyphenyl)propan-2-ol (831 mg, 99%) as an orange oil which solidified on standing. ^{1}H NMR (400 MHz, DMSO) 1.55 (s, 6H), 3.65 (s, 3H), 5.24 (s, 1H), 5.76 (s, 2H), 6.14 (dd, *J* = 8.0, 1.2 Hz, 1H), 6.19 (dd, *J* = 8.0, 1.2 Hz, 1H), 6.79 (t, *J* = 8.0 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 157.4, 148.5, 126.7, 118.1, 110.5, 100.3, 74.1, 55.3, 30.7; LRMS *m/z* (ESI+) $[M\text{-}OH]^-$ 164.1

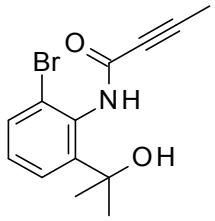


***N*-[2-(1-hydroxy-1-methyl-ethyl)-3-methoxy-phenyl]but-2-ynamide**: Dicyclohexylmethanediimine (1.04 g, 5.03 mmol) was added to 2-(2-amino-6-methoxyphenyl)propan-2-ol (829 mg, 4.57 mmol) and 2-butynoic acid (388 mg, 4.62 mmol) in DCM (23 mL) under nitrogen. The resulting suspension was stirred at rt for 3 hours. The reaction mixture was diluted with Et_2O (50 mL), filtered and the solvent removed under vacuum to afford a yellow solid. The crude product was purified by flash silica chromatography, elution gradient 0 to 50% EtOAc in heptane. Pure fractions were evaporated to dryness to afford *N*-(2-(2-hydroxypropan-2-yl)-3-methoxyphenyl)but-2-ynamide (195 mg, 17%) as a white solid. ^{1}H NMR (400 MHz, DMSO) 1.61 (s, 6H), 2.00 (s, 3H), 3.75 (s, 3H), 6.45 (s, 1H), 6.77 (dd, *J* = 8.3, 1.0 Hz, 1H), 7.15 (t, *J* = 8.3 Hz, 1H), 7.89 (d, *J* = 8.3 Hz, 1H), 12.11 (s, 1H); ^{13}C NMR (126 MHz, DMSO, 27 °C) 156.7, 149.5, 137.7, 127.3, 123.1, 114.4, 108.4, 83.0, 76.4, 75.4, 55.8, 30.7, 3.0; LRMS *m/z* (ESI-) $[M\text{-}H]^-$ 246.2



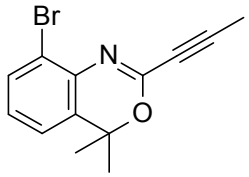
5-methoxy-4,4-dimethyl-2-prop-1-ynyl-3,1-benzoxazine (*m*'-OMe-5e): Methanesulfonic acid (0.25 mL, 3.85 mmol) was added to *N*-(2-(2-hydroxypropan-2-yl)-3-methoxyphenyl)but-2-ynamide (185 mg, 0.75 mmol) in DCM (16.5 mL) under nitrogen. The resulting solution was warmed to 45 °C and stirred for 3 hours. The reaction mixture was quenched with saturated $NaHCO_3$ (25 mL), the organic layer was dried over a phase separating cartridge, filtered

and evaporated to afford yellow oil. The crude product was purified by flash silica chromatography, elution gradient 25 to 40% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 5-methoxy-4,4-dimethyl-2-(prop-1-yn-1-yl)-4H-benzo[d][1,3]oxazine (109 mg, 64%) as a pale yellow oil. ^{1}H NMR (400 MHz, DMSO) 1.62 (s, 6H), 2.04 (s, 3H), 3.80 (s, 3H), 6.67 (dd, *J* = 7.8, 1.0 Hz, 1H), 6.92 (dd, *J* = 8.4, 1.0 Hz, 1H), 7.22 (dd, *J* = 8.4, 7.8 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 154.6, 142.7, 138.5, 128.9, 117.6, 117.5, 111.1, 86.1, 79.4, 74.3, 55.8, 28.3, 3.3; HRMS (ESI+) calcd for $C_{14}H_{15}NO_2$ $[M+H]^+$ 230.1181, found 230.1182.

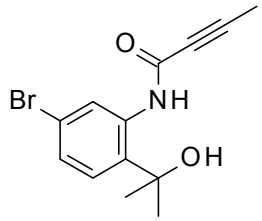


***N*-[2-bromo-6-(1-hydroxy-1-methyl-ethyl)phenyl]but-2-ynamide**: Methylmagnesium bromide (3M in Et_2O) (2.6 mL, 7.80 mmol) was added dropwise to methyl 2-amino-3-bromobenzoate (360 mg, 1.56 mmol) in THF (10 mL) at 0 °C under nitrogen. The resulting solution was warmed to rt and stirred for 30 minutes. The reaction mixture was quenched with water (10 mL), brine (20 mL) added and extracted with EtOAc (2 x 25 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford the crude as pale yellow oil (361 mg) which was used directly without further purification.

dicyclohexylmethanediimine (338 mg, 1.64 mmol) was added to 2-(2-amino-3-bromophenyl)propan-2-ol (359 mg) and but-2-ynoic acid (138 mg, 1.64 mmol) in DCM (8 mL) under nitrogen. The resulting solution was stirred at rt for 3 hours. The reaction was incomplete and further but-2-ynoic acid (138 mg, 1.64 mmol) and dicyclohexylmethanediimine (338 mg, 1.64 mmol) were added and the suspension was stirred at rt for a further 18 hours. The reaction mixture was diluted with Et_2O (20 mL), filtered and the solvent removed under vacuum to afford yellow oil. The crude product was purified by flash silica chromatography, elution gradient 0 to 100% EtOAc in heptane. Pure fractions were evaporated to dryness to afford *N*-(2-bromo-6-(2-hydroxypropan-2-yl)phenyl)but-2-ynamide (264 mg, 57%, over 2 steps) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO) 1.43 (s, 6H), 2.04 (s, 3H), 5.33 (s, 1H), 7.20 (t, *J* = 8.0 Hz, 1H), 7.57 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.67 (dd, *J* = 8.0, 1.4 Hz, 1H), 9.99 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 151.5, 149.2, 132.3, 131.1, 128.4, 125.7, 124.7, 83.9, 75.7, 71.2, 30.2, 3.1; LRMS *m/z* (ESI-) $[M-H]^-$ 294.0

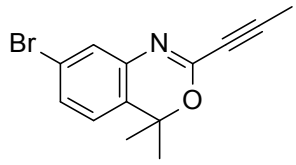


8-bromo-4,4-dimethyl-2-prop-1-ynyl-3,1-benzoxazine (*o*-Br-5e): Methanesulfonic acid (0.14 mL, 2.16 mmol) was added to *N*-(2-bromo-6-(2-hydroxypropan-2-yl)phenyl)but-2-ynamide (125 mg, 0.42 mmol) in DCM (9.2 mL) under nitrogen. The resulting solution was warmed to 45 °C and stirred for 2 hours. The reaction mixture was diluted with DCM (5 mL) and washed sequentially with saturated $NaHCO_3$ (25 mL) and saturated brine (10 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 25% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 8-bromo-4,4-dimethyl-2-(prop-1-yn-1-yl)-4H-benzo[d][1,3]oxazine (63.5 mg, 54%) as a colorless oil. ^{1}H NMR (400 MHz, DMSO) 1.59 (s, 6H), 2.08 (s, 3H), 7.15 (t, *J* = 7.8 Hz, 1H), 7.29 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.57 (dd, *J* = 7.8, 1.3 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 144.1, 135.4, 133.1, 132.3, 128.7, 122.7, 119.2, 87.9, 79.5, 74.3, 27.9, 3.4; HRMS (ESI+) calcd for $C_{13}H_{12}BrNO$ $[M+H]^+$ 278.0181, found 278.0176

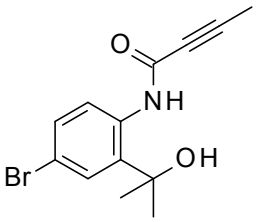


***N*-[5-bromo-2-(1-hydroxy-1-methyl-ethyl)phenyl]but-2-ynamide**: Methylmagnesium bromide (3M in Et_2O) (2.6 mL, 7.80 mmol) was added dropwise to methyl 2-amino-4-bromobenzoate (363 mg, 1.58 mmol) in THF (10 mL) at 0 °C under nitrogen. The resulting solution was warmed to rt and stirred for 45 minutes. The reaction mixture was quenched with water (10 mL), brine (20 mL) added and extracted with EtOAc (2 x 25 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford the crude (368 mg) as brown oil which was used directly without further purification.

Dicyclohexylmethanediimine (338 mg, 1.64 mmol) was added to 2-(2-amino-4-bromophenyl)propan-2-ol (364 mg) and but-2-ynoic acid (139 mg, 1.66 mmol) in DCM (8 mL) under nitrogen. The resulting solution was stirred at rt for 1 hour. The reaction mixture was diluted with Et_2O (20 mL), and washed sequentially with saturated $NaHCO_3$ (25 mL) and saturated brine (25 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 35% EtOAc in heptane. Pure fractions were evaporated to dryness to afford *N*-(5-bromo-2-(2-hydroxypropan-2-yl)phenyl)but-2-ynamide (259 mg, 55%, over 2 steps) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO) 1.52 (s, 6H), 2.04 (s, 3H), 6.38 (s, 1H), 7.25 (d, *J* = 1.0 Hz, 2H), 8.33 (s, 1H), 11.05 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 149.8, 137.7, 135.2, 127.7, 126.3, 123.6, 119.8, 84.7, 75.6, 72.8, 29.9, 3.0; LRMS *m/z* (ESI-) $[M-H]^-$ 294.0

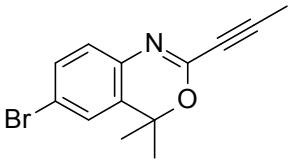


7-bromo-4,4-dimethyl-2-prop-1-ynyl-3,1-benzoxazine (*m*-Br-5e): Methanesulfonic acid (0.14 mL, 2.16 mmol) was added to *N*-(5-bromo-2-(2-hydroxypropan-2-yl)phenyl)but-2-ynamide (125 mg, 0.42 mmol) in DCM (9.2 mL) under nitrogen. The resulting solution was warmed to 45 °C and stirred for 2 hours. The reaction mixture was diluted with DCM (5 mL) and washed sequentially with saturated $NaHCO_3$ (25 mL) and saturated brine (10 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 25% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 7-bromo-4,4-dimethyl-2-(prop-1-yn-1-yl)-4H-benzo[d][1,3]oxazine (95 mg, 81%) as a colorless oil. ^{1}H NMR (400 MHz, DMSO) 1.57 (s, 6H), 2.06 (s, 3H), 7.2 – 7.28 (m, 2H), 7.42 (dd, *J* = 8.2, 2.1 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 144.2, 139.1, 130.3, 130.0, 126.4, 125.2, 121.0, 87.5, 79.4, 74.1, 27.9, 3.3; HRMS (ESI+) calcd for $C_{13}H_{12}BrNO$ $[M+H]^+$ 278.0181, found 278.0176

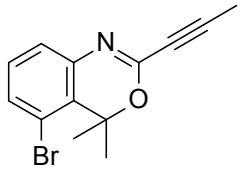


***N*-[4-bromo-2-(1-hydroxy-1-methyl-ethyl)phenyl]but-2-ynamide**: Methylmagnesium bromide (3M in Et_2O) (2.6 mL, 7.80 mmol) was added dropwise to methyl 2-amino-5-bromobenzoate (358 mg, 1.56 mmol) in THF (10 mL) at 0 °C under nitrogen. The resulting solution was warmed to rt and stirred for 30 minutes. The reaction mixture was quenched with water (10 mL), brine (20 mL) added and extracted with EtOAc (2 x 25 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford the crude as pale yellow solid (362 mg) which was used directly without further purification.

Dicyclohexylmethanediimine (338 mg, 1.64 mmol) was added to 2-(2-amino-5-bromophenyl)propan-2-ol (359 mg) and but-2-ynoic acid (138 mg, 1.64 mmol) in DCM (8 mL) under nitrogen. The resulting solution was stirred at rt for 1 hour. The reaction mixture was diluted with Et_2O (20 mL), and washed sequentially with saturated $NaHCO_3$ (25 mL) and saturated brine (25 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 35% EtOAc in heptane. Pure fractions were evaporated to dryness to afford *N*-(4-bromo-2-(2-hydroxypropan-2-yl)phenyl)but-2-ynamide (302 mg, 65%, over 2 steps) as a white solid. ^{1}H NMR (400 MHz, DMSO) 1.52 (s, 6H), 2.03 (s, 3H), 6.35 (s, 1H), 7.40 – 7.47 (2H, m), 8.03 (d, *J* = 9.3 Hz, 1H), 10.89 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 149.7, 138.9, 135.5, 130.0, 128.3, 123.7, 116.0, 84.4, 75.7, 72.7, 29.8, 3.0; LRMS *m/z* (ESI-) $[M-H]^-$ 294.0



6-bromo-4,4-dimethyl-2-prop-1-ynyl-3,1-benzoxazine (*p*-Br-5e): Methanesulfonic acid (0.09 mL, 1.39 mmol) was added to *N*-(4-bromo-2-(2-hydroxypropan-2-yl)phenyl)but-2-ynamide (85 mg, 0.29 mmol) in DCM (6.3 mL) at 25 °C under nitrogen. The resulting solution was warmed to 45 °C and stirred for 2 hours. The reaction mixture was diluted with DCM (5 mL) and washed sequentially with saturated $NaHCO_3$ (25 mL) and saturated brine (10 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 25% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 6-bromo-4,4-dimethyl-2-(prop-1-yn-1-yl)-4H-benzo[d][1,3]oxazine (15.6 mg, 20%) as a colorless oil. ^{1}H NMR (400 MHz, DMSO) 1.58 (s, 6H), 2.05 (s, 3H), 7.00 (d, *J* = 8.3 Hz, 1H), 7.46 (dd, *J* = 8.3, 2.2 Hz, 1H), 7.51 (d, *J* = 2.2 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 143.5, 136.6, 133.2, 131.5, 126.2, 125.9, 119.6, 87.2, 79.0, 74.2, 27.9, 3.3; HRMS (ESI+) calcd for $C_{13}H_{12}BrNO$ $[M+H]^+$ 278.0181, found 278.0176

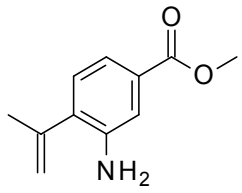


5-bromo-4,4-dimethyl-2-prop-1-ynyl-3,1-benzoxazine (*m*'-Br-5e): Methylmagnesium bromide (3M in Et_2O) (2.6 mL, 7.80 mmol) was added dropwise to methyl 2-amino-6-bromobenzoate (0.363 g, 1.58 mmol) in THF (10 mL) at 0 °C under nitrogen. The resulting solution was warmed to rt and stirred for 30 minutes. The reaction mixture was quenched with water (10 mL), brine (20 mL) added and extracted with EtOAc (2 x 25 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford the crude as yellow oil (368 mg) which was used directly without further purification.

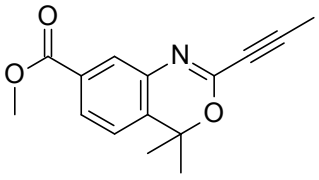
Dicyclohexylmethanediimine (0.338 g, 1.64 mmol) was added to 2-(2-amino-6-bromophenyl)propan-2-ol (364 mg) and but-2-ynoic acid (139 mg, 1.66 mmol) in DCM (8 mL) under nitrogen. The resulting solution was stirred at rt for 1 hour. The reaction mixture was diluted with Et_2O (20 mL), and washed sequentially with saturated $NaHCO_3$ (25 mL) and saturated brine (25 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 35% EtOAc in heptane. Fractions containing product were evaporated to dryness to afford impure *N*-(3-bromo-2-(2-hydroxypropan-2-yl)phenyl)but-2-ynamide (85 mg) as a pale yellow solid.

Methanesulfonic acid (0.07 mL, 1.08 mmol) was added to *N*-(3-bromo-2-(2-hydroxypropan-2-yl)phenyl)but-2-ynamide (80 mg) in DCM (4.4 mL) under nitrogen. The resulting solution was warmed to 45 °C and stirred for 2 hours. The reaction mixture was diluted with DCM (5 mL) and washed sequentially with saturated $NaHCO_3$ (25 mL) and

saturated brine (10 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 35% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 5-bromo-4,4-dimethyl-2-(prop-1-yn-1-yl)-4H-benzo[d][1,3]oxazine (26.5 mg, 6%, over 3 steps) as a white waxy solid. ^{1}H NMR (400 MHz, DMSO) 1.79 (s, 6H), 2.06 (s, 3H), 7.09 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.20 (t, *J* = 7.9 Hz, 1H), 7.48 (dd, *J* = 7.9, 1.3 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 142.8, 139.5, 133.4, 130.0, 129.2, 124.9, 117.5, 87.4, 80.9, 73.9, 28.0, 3.3; HRMS (ESI+) calcd for $C_{13}H_{12}BrNO$ $[M+H]^+$ 278.0181, found 278.0176



methyl 3-amino-4-isopropenyl-benzoate: Pd(dppf)Cl_2 (71 mg, 0.09 mmol) was added to a degassed solution of methyl 3-amino-4-bromobenzoate (200 mg, 0.87 mmol), 4,4,5,5-tetramethyl-2-(prop-1-en-2-yl)-1,3,2-dioxaborolane (0.33 mL, 1.74 mmol) and cesium carbonate (850 mg, 2.61 mmol) in 1,4-dioxane (4 mL) and water (0.4 mL) under nitrogen. The resulting solution was warmed to 100 °C and stirred for 18 hours. The reaction mixture was diluted with EtOAc (40 mL) and washed sequentially with saturated $NaHCO_3$ (50 mL) and saturated brine (50 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product.The crude product was purified by flash silica chromatography, elution gradient 0 to 40% EtOAc in heptane. Pure fractions were evaporated to dryness to afford methyl 3-amino-4-(prop-1-en-2-yl)benzoate (33.7 mg, 20%) as a pale yellow oil. ^{1}H NMR (400 MHz, DMSO) 1.99 – 2.08 (m, 3H), 3.81 (s, 3H), 5.03 (dd, *J* = 1.7, 0.9 Hz, 1H), 5.08 (s, 2H), 5.28 (dq, *J* = 1.7 Hz, 1H), 7.03 (d, *J* = 7.9 Hz, 1H), 7.12 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.32 (d, *J* = 1.7 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 166.5, 144.9, 142.5, 131.9, 128.8, 128.1, 116.6, 115.7, 115.3, 51.8, 22.8; LRMS *m/z* (ESI+) $[M+H]^+$ 192.1

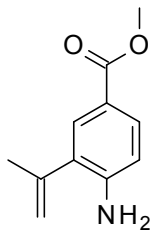


methyl 4,4-dimethyl-2-prop-1-ynyl-3,1-benzoxazine-7-carboxylate (*m*-CO_2Me-5e):

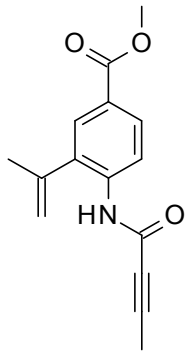
Dicyclohexylmethanediimine (37.2 mg, 0.18 mmol) was added to methyl 3-amino-4-(prop-1-en-2-yl)benzoate (30.1 mg, 0.16 mmol) and but-2-ynoic acid (15.2 mg, 0.18 mmol) in DCM (1 mL) under nitrogen. The resulting solution was stirred at rt for 2 hours. The reaction mixture was diluted with Et_2O (20 mL) and washed sequentially with saturated $NaHCO_3$ (25 mL) and saturated brine (25 mL). The organic layer was dried with a phase separating cartridge, filtered

and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 40% EtOAc in heptane. Fractions containing product were evaporated to dryness to afford crude methyl 3-(but-2-ynamido)-4-(prop-1-en-2-yl)benzoate (49.0 mg) as a pale yellow waxy solid.

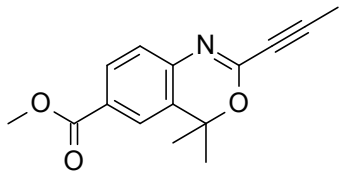
Methanesulfonic acid (0.05 mL, 0.77 mmol) was added to methyl 3-(but-2-ynamido)-4-(prop-1-en-2-yl)benzoate (49 mg) in DCM (3.5 mL) under nitrogen. The resulting solution was warmed to 45 °C and stirred for 30 minutes. The reaction mixture was quenched with saturated $NaHCO_3$ (10 mL) and extracted with DCM (2 x 10 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford pale yellow oil. The crude product was purified by flash silica chromatography, elution gradient 0 to 30% EtOAc in heptane. Pure fractions were evaporated to dryness to afford methyl 4,4-dimethyl-2-(prop-1-yn-1-yl)-4*H*-benzo[d][1,3]oxazine-7-carboxylate (20.7 mg, 50%, over 2 steps) as a colorless oil which solidified on standing. ^{1}H NMR (400 MHz, DMSO) 1.61 (s, 6H), 2.07 (s, 3H), 3.85 (s, 3H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.55 (d, *J* = 1.7 Hz, 1H), 7.81 (dd, *J* = 8.0, 1.7 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 165.5, 144.0, 137.7, 135.8, 130.1, 128.1, 124.4, 123.7, 87.4, 79.4, 74.2, 52.2, 27.9, 3.4; HRMS (ESI+) calcd for $C_{15}H_{15}NO_3$ $[M+H]^+$ 258.1130, found 258.1135



methyl 4-amino-3-isopropenyl-benzoate: $Pd(dppf)Cl_2$ (106 mg, 0.13 mmol) was added to a degassed solution of methyl 4-amino-3-bromobenzoate (300 mg, 1.30 mmol), 4,4,5,5-tetramethyl-2-(prop-1-en-2-yl)-1,3,2-dioxaborolane (0.49 mL, 2.61 mmol) and cesium carbonate (1.27 g, 3.91 mmol) in 1,4-dioxane (6 mL) and water (0.6 mL) under nitrogen. The resulting solution was warmed to 80 °C and stirred for 4 hours. The reaction mixture was diluted with EtOAc (40 mL), and washed sequentially with saturated $NaHCO_3$ (50 mL) and saturated brine (50 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product.The crude product was purified by flash silica chromatography, elution gradient 0 to 40% EtOAc in heptane. Pure fractions were evaporated to dryness to afford methyl 4-amino-3-(prop-1-en-2-yl)benzoate (103 mg, 41%) as an orange oil. ^{1}H NMR (400 MHz, DMSO) 2 – 2.02 (m, 3H), 3.74 (s, 3H), 4.99 (dq, *J* = 1.7, 0.9 Hz, 1H), 5.26 (dq, *J* = 3.1, 1.7 Hz, 1H), 5.65 (s, 2H), 6.68 (d, *J* = 8.5 Hz, 1H), 7.50 (d, *J* = 2.1 Hz, 1H), 7.57 (dd, *J* = 8.5, 2.1 Hz, 1H); ^{13}C NMR (126 MHz, DMSO, 27 °C) 166.3, 149.5, 142.4, 129.7, 129.5, 126.5, 116.0, 115.8, 113.8, 51.2, 23.0; LRMS *m/z* (ESI+) $[M+H]^+$ 192.1.



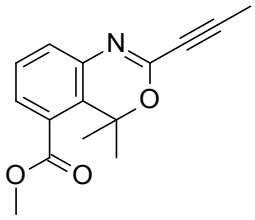
methyl 4-(but-2-ynoylamino)-3-isopropenyl-benzoate: Dicyclohexylmethanediimine (122 mg, 0.59 mmol) was added to methyl 4-amino-3-(prop-1-en-2-yl)benzoate (98 mg, 0.51 mmol) and but-2-ynoic acid (49.5 mg, 0.59 mmol) in DCM (3.4 mL) under nitrogen. The resulting solution was stirred at rt for 2 hours. The reaction was incomplete and further but-2-ynoic acid (49.5 mg, 0.59 mmol) and dicyclohexylmethanediimine (122 mg, 0.59 mmol) were added and the solution was stirred at rt for a further 5 hours. The reaction mixture was diluted with Et_2O (25 mL) and washed sequentially with saturated $NaHCO_3$ (25 mL) and saturated brine (25 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 40% EtOAc in heptane. Pure fractions were evaporated to dryness to afford methyl 4-(but-2-ynamido)-3-(prop-1-en-2-yl)benzoate (128 mg, 97%) as a pale yellow waxy solid. ^{1}H NMR (400 MHz, DMSO) 1.99 – 2.06 (m, 6H), 3.84 (s, 3H), 4.93 – 5 (m, 1H), 5.26 (dq, *J* = 1.5 Hz, 1H), 7.61 (d, *J* = 8.3 Hz, 1H), 7.77 (d, *J* = 2.1 Hz, 1H), 7.84 (dd, *J* = 8.3, 2.1 Hz, 1H), 9.96 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$, 27 °C) 166.6, 151.0, 141.9, 137.8, 132.6, 129.8, 129.4, 125.6, 120.2, 118.2, 85.3, 75.4, 52.2, 24.6, 4.0; LRMS *m/z* (ESI+) $[M+H]^+$ 258.1.



methyl 4,4-dimethyl-2-prop-1-ynyl-3,1-benzoxazine-6-carboxylate (*p*-CO_2Me-5e):

Methanesulfonic acid (0.16 mL, 2.46 mmol) was added to methyl 4-(but-2-ynamido)-3-(prop-1-en-2-yl)benzoate (123 mg, 0.48 mmol) in DCM (10.5 mL) under nitrogen. The resulting solution was warmed to 45 °C and stirred for 90 minutes. The reaction mixture was quenched with saturated $NaHCO_3$ (25 mL) and extracted with DCM (2 x 15 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford yellow oil. The crude product was purified by flash silica chromatography, elution gradient 0 to 30% EtOAc in heptane. Pure fractions were evaporated to dryness to afford methyl 4,4-dimethyl-2-(prop-1-yn-1-yl)-4*H*-benzo[d][1,3]oxazine-6-carboxylate (85 mg, 69%) as a white powder. ^{1}H NMR (400 MHz, DMSO) 1.62 (s, 6H), 2.07 (s, 3H), 3.85 (s, 3H), 7.17

(d, *J* = 8.2 Hz, 1H), 7.81 (d, *J* = 1.8 Hz, 1H), 7.87 (dd, *J* = 8.2, 1.8 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 165.5, 145.0, 141.4, 131.3, 129.8, 128.3, 124.4, 124.1, 88.0, 79.6, 74.2, 52.1, 27.9, 3.4; HRMS (ESI+) calcd for $C_{15}H_{15}NO_3$ $[M+H]^+$ 258.1130, found 258.1135

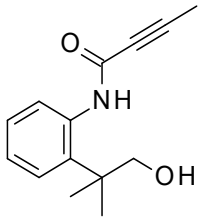


methyl 4,4-dimethyl-2-prop-1-ynyl-3,1-benzoxazine-5-carboxylate (*o'*-CO_2Me-5e):

$Pd(dppf)Cl_2$ (142 mg, 0.22 mmol) was added to a degassed solution of 4,4,5,5-tetramethyl-2-(prop-1-en-2-yl)-1,3,2-dioxaborolane (0.82 mL, 4.35 mmol), cesium carbonate (2.12 g, 6.52 mmol) and methyl 3-amino-2-bromobenzoate (500 mg, 2.17 mmol) in a mixture of dioxane (11 mL) and water (1.1 mL) under nitrogen. The resulting solution was stirred at 95 °C for 2 hours. The reaction mixture was diluted with DCM (25 mL) and washed sequentially with saturated NaHCO3 (25 mL) and saturated brine (25 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 40% EtOAc in heptane. Fractions containing product were evaporated to dryness to afford impure methyl 3-amino-2-(prop-1-en-2-yl)benzoate (245 mg) as a brown oil which was used immediately in the next reaction.

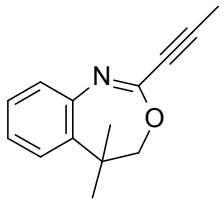
Dicyclohexylmethanediimine (300 mg, 1.46 mmol) was added to methyl 3-amino-2-(prop-1-en-2-yl)benzoate (242 mg) and but-2-ynoic acid (122 mg, 1.46 mmol) in DCM (8.5 mL) under nitrogen. The resulting solution was stirred at rt for 2 hours. The reaction mixture was diluted with Et_2O (20 mL), filtered and evaporated to afford crude product (88 mg) as a pale pink waxy solid which was used without further purification.

Methanesulfonic acid (0.10 mL, 1.54 mmol) was added to methyl 3-(but-2-ynamido)-2-(prop-1-en-2-yl)benzoate (80 mg) in DCM (6.9 mL) under nitrogen. The resulting solution was stirred at 45 °C for 40 minutes. The reaction mixture was quenched with saturated $NaHCO_3$ (20 mL) and extracted with DCM (2 x 20 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford pale yellow oil. The crude product was purified by flash silica chromatography, elution gradient 0 to 30% EtOAc in heptane. Pure fractions were evaporated to dryness to afford methyl 4,4-dimethyl-2-(prop-1-yn-1-yl)-4*H*-benzo[d][1,3]oxazine-5-carboxylate (34.4 mg, 6%, over 3 steps) as a white waxy solid. ^{1}H NMR (400 MHz, DMSO) 1.61 (s, 6H), 2.07 (s, 3H), 3.86 (s, 3H), 7.23 (dd, *J* = 7.5, 1.7 Hz, 1H), 7.31 – 7.44 (m, 2H); ^{13}C NMR (126 MHz, DMSO, 27 °C) 169.5, 143.8, 138.6, 129.4, 128.7, 128.7, 127.5, 127.0, 87.7, 79.7, 74.0, 52.8, 26.7, 3.4; HRMS (ESI+) calcd for $C_{15}H_{15}NO_3$ $[M+H]^+$ 258.1130, found 258.1135

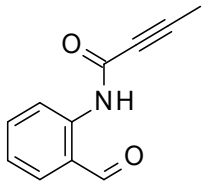


***N*-[2-(2-hydroxy-1,1-dimethyl-ethyl)phenyl]but-2-ynamide**: Dicyclohexylmethanediimine (1.25 g, 6.06 mmol) was added to a solution of 2-(2-aminophenyl)-2-methylpropan-1-ol (920 mg, 5.57 mmol) and but-2-ynoic acid (472 mg, 5.61 mmol) in DCM (28 mL) at rt under nitrogen. The resulting solution was stirred at rt for 1 hour. The reaction mixture was filtered and the solvent removed under vaccum to afford the crude as a brown oil. The crude product was purified by flash silica chromatography, elution gradient 25 to 60% EtOAc in heptane. Pure fractions were evaporated to dryness to afford *N*-(2-(1-hydroxy-2-methylpropan-2-yl)phenyl)but-2-ynamide (898 mg, 70%) as a pale yellow foam. ^{1}H NMR (400 MHz, DMSO) 1.31 (s, 6H), 2.02 (s, 3H), 3.51 (d, *J* = 4.5 Hz, 2H), 5.93 (t, *J* = 4.5 Hz, 1H), 7.14 (td, *J* = 7.7, 1.5 Hz, 1H), 7.20 (td, *J* = 7.7, 1.4 Hz, 1H), 7.38 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.44 (dd, *J* = 7.7, 1.4 Hz, 1H), 10.83 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 150.7, 140.3, 135.7, 127.5, 126.9, 126.3, 125.5, 83.6, 75.9, 71.6, 25.6, 3.1; LRMS *m/z* (ESI+) $[M+H]^+$ 231.2

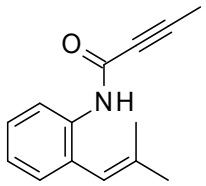
2-(2-aminophenyl)-2-methylpropan-1-ol was synthesised according to literature procedure.[15]



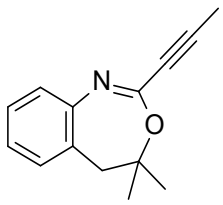
5,5-dimethyl-2-prop-1-ynyl-4H-3,1-benzoxazepine (6): Methanesulfonyl chloride (0.075 mL, 0.97 mmol) was added dropwise to *N*-(2-(1-hydroxy-2-methylpropan-2-yl)phenyl)but-2-ynamide (210 mg, 0.91 mmol) and triethylamine (0.38 mL, 2.72 mmol) in DCM (17 mL) under nitrogen. The resulting solution was stirred at rt for 1 hour. The reaction mixture was quenched with half saturated $NaHCO_3$ (20 mL), the organic layer was dried over a phase separating cartridge, filtered and evaporated to afford brown oil. The crude product was purified by flash silica chromatography, elution gradient 15 to 30% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 5,5-dimethyl-2-(prop-1-yn-1-yl)-4,5-dihydrobenzo[d][1,3]oxazepine (63.2 mg, 33%) as a white solid. ^{1}H NMR (400 MHz, DMSO) 1.31 (s, 6H), 2.09 (s, 3H), 3.99 (s, 2H), 7.08 (td, *J* = 7.4, 1.2 Hz, 1H), 7.19 (td, *J* = 7.8, 1.2 Hz, 1H), 7.27 – 7.3 (m, 1H), 7.92 (d, *J* = 7.8 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 150.5, 141.7, 140.3, 127.3, 124.5, 122.5, 116.1, 89.5, 74.5, 63.1, 42.9, 28.0, 3.4; HRMS (ESI+) calcd for $C_{14}H_{15}NO$ $[M+H]^+$ 214.1232, found 214.1227.



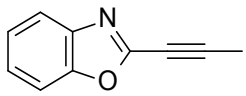
***N*-(2-formylphenyl)but-2-ynamide**: Manganese(IV) oxide (1.61 g, 18.5 mmol) was added to *N*-(2-(hydroxymethyl)phenyl)but-2-ynamide (350 mg, 1.85 mmol) in DCM (12.5 mL) under nitrogen. The resulting suspension was stirred at rt for 17 hours. The reaction mixture was filtered through celite and washed with DCM (50 mL). The solvent was removed under vacuum to afford *N*-(2-formylphenyl)but-2-ynamide (329 mg, 95%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO) 2.08 (s, 3H), 7.37 (td, *J* = 7.5, 1.1 Hz, 1H), 7.69 (ddd, *J* = 8.3, 7.5, 1.6 Hz, 1H), 7.90 (dd, *J* = 7.5, 1.6 Hz, 1H), 8.10 (d, *J* = 8.3 Hz, 1H), 9.98 (s, 1H), 11.14 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 194.3, 151.1, 138.8, 135.4, 133.5, 124.6, 121.4, 121.3, 85.9, 75.2, 3.1; LRMS *m/z* (ESI+) $[M+H]^+$ 188.2



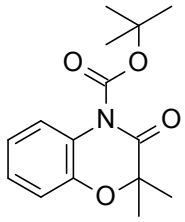
***N*-[2-(2-methylprop-1-enyl)phenyl]but-2-ynamide**: *n*-Butyllithium (1.6 M in hexane) (2.24 mL, 3.58 mmol) was added dropwise to isopropyltriphenylphosphonium iodide (1.62 g, 3.75 mmol) in THF (10 mL) at 0 °C under nitrogen. The resulting solution was stirred at for 30 minutes. A solution of *N*-(2-formylphenyl)but-2-ynamide (319 mg, 1.70 mmol) in THF (8 mL) was subsequently added and the resultant suspension warmed to rt and stirred for 3.5 hours. The reaction mixture was quenched with saturated NH_4Cl (25 mL)and extracted with EtOAc (2 x 20 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford orange oil. The crude product was purified by flash silica chromatography, elution gradient 0 to 30% EtOAc in heptane. Pure fractions were evaporated to dryness to afford *N*-(2-(2-methylprop-1-en-1-yl)phenyl)but-2-ynamide (182 mg, 50%) as an orange oil which solidified upon standing. ^{1}H NMR (400 MHz, DMSO) 1.67 (s, 3H), 1.87 (d, *J* = 1.3 Hz, 3H), 2.02 (s, 3H), 6.21 (s, 1H), 7.1 – 7.29 (m, 3H), 7.41 (d, *J* = 7.5 Hz, 1H), 9.74 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 150.9, 136.1, 134.6, 132.5, 129.8, 126.5, 125.3, 125.2, 121.3, 83.9, 75.8, 26.2, 19.2, 3.2; LRMS *m/z* (ESI+) $[M+H]^+$ 214.2



4,4-dimethyl-2-prop-1-ynyl-5H-3,1-benzoxazepine (7): Methanesulfonic acid (0.23 mL, 3.54 mmol) was added to *N*-(2-(2-methylprop-1-en-1-yl)phenyl)but-2-ynamide (151 mg, 0.71 mmol) in DCM (15.5 mL) under nitrogen. The resulting solution was warmed to 45 °C and stirred for 1.5 hours. The reaction mixture was quenched with saturated $NaHCO_3$ (25 mL) and extracted with DCM (2 x 15 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford pale yellow oil. The crude product was purified by flash silica chromatography, elution gradient 0 to 20% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 4,4-dimethyl-2-(prop-1-yn-1-yl)-4,5-dihydrobenzo[d][1,3]oxazepine (83 mg, 55%) as a colorless oil. ^{1}H NMR (400 MHz, DMSO) 1.24 (s, 6H), 1.98 (s, 3H), 2.97 (s, 2H), 7.08 – 7.17 (m, 3H), 7.21 – 7.27 (m, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 143.0, 138.5, 132.3, 129.6, 127.3, 127.2, 125.4, 86.4, 80.8, 77.5, 45.3, 28.0, 3.1; HRMS (ESI+) calcd for $C_{14}H_{15}NO$ $[M+H]^+$ 214.1232, found 214.1227.



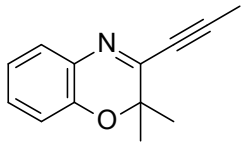
2-prop-1-ynyl-1,3-benzoxazole (8): trimethyl(prop-1-yn-1-yl)silane (0.26 ml, 1.79 mmol) and TBAF (1M in THF) (1.8 ml, 1.80 mmol) were added to 2-chlorobenzo[d]oxazole (250 mg, 1.63 mmol), $Pd(PPh_3)_4$ (94 mg, 0.08 mmol), copper(I) iodide (93 mg, 0.49 mmol) and triethylamine (0.68 ml, 4.88 mmol) in toluene (13 mL) under nitrogen. The resulting solution was stirred at rt for 24 hours. The reaction mixture was diluted with DCM (20 mL) and washed with saturated NH_4Cl (50 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 30% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 2-(prop-1-yn-1-yl)benzo[d]oxazole (33.6 mg, 13%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO) 2.23 (s, 3H), 7.42 (td, *J* = 7.7, 1.3 Hz, 1H), 7.45 – 7.51 (m, 1H), 7.71 (ddd, *J* = 8.1, 1.3, 0.6 Hz, 1H), 7.76 (ddd, *J* = 7.7, 1.3, 0.6 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 149.4, 146.7, 140.1, 126.5, 125.2, 119.9, 110.7, 93.6, 68.3, 3.8; HRMS (ESI+) calcd for $C_{10}H_7ON$ $[M+H]^+$ 158.0606, found 158.0601.



***tert*-butyl 2,2-dimethyl-3-oxo-1,4-benzoxazine-4-carboxylate**: Di-*tert*-butyl dicarbonate (1.92 g, 8.80 mmol) was added to 2,2-dimethyl-2H-benzo[b][1,4]oxazin-3(4H)-one (1.30 g, 7.34 mmol), triethylamine (1.03 mL, 7.41 mmol) and *N*,*N*-dimethylpyridin-4-amine (0.090 g, 0.73 mmol) in DCM (23 mL) under nitrogen. The resulting solution was stirred at rt for 18 hours. The reaction mixture was diluted with DCM (15 mL) and washed sequentially with saturated $NaHCO_3$ (2 x 50 mL) and saturated brine (50 mL). The organic layer was dried with a phase separating cartridge,

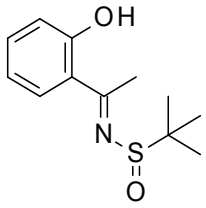
filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 20% EtOAc in heptane. Pure fractions were evaporated to dryness to afford *tert*-butyl 2,2-dimethyl-3-oxo-2,3-dihydro-4H-benzo[b][1,4]oxazine-4-carboxylate (1.85 g, 91%) as a colorless oil. ^{1}H NMR (400 MHz, CDCl3) 1.51 (s, 6H), 1.62 (s, 9H), 6.96 – 7.08 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$, 30 °C) 167.9, 150.5, 143.1, 126.7, 125.2, 122.5, 118.3, 116.8, 85.8, 78.2, 27.8, 23.5; LRMS *m/z* (ESI-) $[M+H]^+$ 278.0

2,2-dimethyl-2H-benzo[b][1,4]oxazin-3(4H)-one was synthesised according to literature procedure.[16]

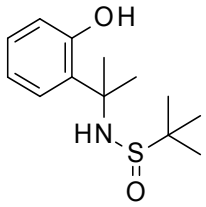


2,2-dimethyl-3-prop-1-ynyl-1,4-benzoxazine (9): Prop-1-yn-1-ylmagnesium bromide (0.5 M in THF) (3.6 mL, 1.80 mmol) was added dropwise to *tert*-butyl 2,2-dimethyl-3-oxo-2,3-dihydro-4H-benzo[b][1,4]oxazine-4-carboxylate (450 mg, 1.62 mmol) in THF (7 mL) at -78 °C under nitrogen. The resulting solution was stirred at -78 °C for 2 hours before gradual warming to rt and stirring for 1 hour. The reaction mixture was quenched with water (15 mL) and extracted with EtOAc (2 x 15 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford crude *tert*-butyl (2-((2-methyl-3-oxohex-4-yn-2-yl)oxy)phenyl)carbamate (475 mg) as a pale yellow oil which was used directly in the next reaction.

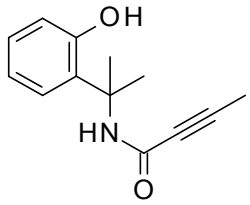
Methanesulfonic acid (0.045 mL, 0.69 mmol) was added to crude *tert*-butyl (2-((2-methyl-3-oxohex-4-yn-2-yl)oxy)phenyl)carbamate (120 mg, 0.38 mmol) in DCM (7.5 mL) at 0 °C under nitrogen. The resulting solution was stirred at 0 °C for 1 hour. The reaction mixture was quenched with saturated $NaHCO_3$ (15 mL) and extracted with DCM (2 x 10 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford yellow oil. The crude product was purified by flash silica chromatography, elution gradient 0 to 30% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 2,2-dimethyl-3-(prop-1-yn-1-yl)-2H-benzo[b][1,4]oxazine (40.6 mg, 54%, over 2 steps) as a yellow oil which solidified on standing. ^{1}H NMR (400 MHz, DMSO) 1.44 (s, 6H), 2.12 (s, 3H), 6.87 (dd, *J* = 8.0, 1.3 Hz, 1H), 6.98 (td, *J* = 7.7, 1.3 Hz, 1H), 7.12 – 7.21 (m, 1H), 7.23 (dd, *J* = 7.7, 1.6 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 152.7, 144.9, 132.4, 129.4, 126.6, 122.0, 116.2, 93.9, 76.7, 74.3, 24.0, 4.0; HRMS (ESI+) calcd for $C_{13}H_{13}NO$ $[M+H]^+$ 200.1075, found 200.1082



***N*-[1-(2-hydroxyphenyl)ethylidene]-2-methyl-propane-2-sulfinamide**: Tetraethoxytitanium (5.96 mL, 28.4 mmol) was added dropwise to 1-(2-hydroxyphenyl)ethan-1-one (1.8 mL, 14.9 mmol) and 2-methylpropane-2-sulfinamide (3.62 g, 29.9 mmol) in THF (30 mL) under nitrogen. The resulting solution was warmed to 65 °C and stirred for 90 minutes. The reaction was cooled to rt and diluted with EtOAc (30 mL), quenched with water (50 mL) and extracted with EtOAc (2 x 25 mL). The combined organic layers were dried over a phase separating cartridge, filtered and evaporated to afford yellow oil. The crude product was purified by flash silica chromatography, elution gradient 20 to 50% EtOAc in heptane. Pure fractions were evaporated to dryness to afford *N*-(1-(2-hydroxyphenyl)ethylidene)-2-methylpropane-2-sulfinamide (1.33 g, 37%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO) 1.22 (s, 9H), 2.78 (s, 3H), 6.91 – 7 (m, 2H), 7.42 – 7.51 (m, 1H), 7.79 (dd, *J* = 8.3, 1.3 Hz, 1H), 12.93 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 182.2, 160.4, 134.6, 130.3, 120.0, 119.0, 117.6, 56.3, 21.5, 20.7; LRMS *m/z* (ESI+) $[M+H]^+$ 240.3

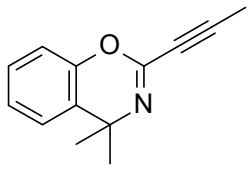


***N*-[1-(2-hydroxyphenyl)-1-methyl-ethyl]-2-methyl-propane-2-sulfinamide**: Methylmagnesium bromide (3.5 mL, 10.5 mmol) was added dropwise to *N*-(1-(2-hydroxyphenyl)ethylidene)-2-methylpropane-2-sulfinamide (500 mg, 2.09 mmol) in THF (10.5 mL) at -10 °C under nitrogen. The resulting solution was stirred at -10 °C for 30 minutes before warming to rt and stirring for 4 hours. The reaction mixture was quenched with half sat. aq. NH_4Cl (20 mL) and extracted with EtOAc (2 x 25 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford *N*-(2-(2-hydroxyphenyl)propan-2-yl)-2-methylpropane-2-sulfinamide (534 mg, quant.) as a yellow solid. ^{1}H NMR (400 MHz, DMSO) 1.10 (s, 9H), 1.58 (s, 3H), 1.63 (s, 3H), 5.55 (s, 1H), 6.76 (td, *J* = 7.6, 1.3 Hz, 1H), 6.80 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.07 (ddd, *J* = 8.0, 7.6, 1.6 Hz, 1H), 7.27 (dd, *J* = 7.6, 1.6 Hz, 1H), 9.74 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 154.7, 132.6, 127.8, 126.0, 118.9, 116.3, 57.2, 54.7, 28.6, 28.4, 22.3; LRMS *m/z* (ESI-) $[M-H]^-$ 254.2



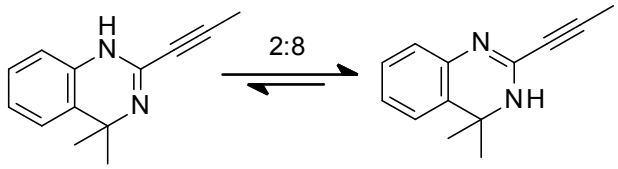
***N*-[1-(2-hydroxyphenyl)-1-methyl-ethyl]but-2-ynamide**: Hydrogen chloride (2M in Et_2O) (2.1 mL, 8.40 mmol) was added to *N*-(2-(2-hydroxyphenyl)propan-2-yl)-2-methylpropane-2-sulfinamide (520 mg, 2.04 mmol) in MeOH (2 mL) under nitrogen. The resulting solution was stirred at rt for 1 hours. Heptane was added (50 mL) and the precipitate was collected by filtration, washed with heptane (20 mL) and dried under vacuum to afford 2-(2-aminopropan-2-yl)phenol.HCl (253 mg, 66%) as a white solid, which was used without further purification.

Dicyclohexylmethanediimine (289 mg, 1.40 mmol) was added to 2-(2-aminopropan-2-yl)phenol.HCl (250 mg, 1.33 mmol), but-2-ynoic acid (112 mg, 1.33 mmol) and *N*-ethyl-*N*-isopropylpropan-2-amine (0.23 mL, 1.33 mmol) in DCM (8 mL) at 25 °C under nitrogen. The resulting solution was stirred at rt for 2 hours. 1H-benzo[d][1,2,3]triazol-1-ol hydrate (206 mg, 1.35 mmol) was added and the resultant stirred for 3 hours. The reaction mixture was diluted with DCM (25 mL) and washed sequentially with saturated $NaHCO_3$ (2 x 30 mL) and saturated brine (50 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 30 to 60% EtOAc in heptane. Fractions containing product were evaporated to dryness to afford a mixture of product and starting material as a colorless oil. The oil was dissolved in DCM (20 mL) and washed with 2M aq. HCl (30 mL), passed through a phase separator and the solvent removed under vacuum to afford *N*-(2-(2-hydroxyphenyl)propan-2-yl)but-2-ynamide (112 mg, 39%, over 2 steps) as a white foam. ^{1}H NMR (400 MHz, DMSO) 1.61 (s, 6H), 1.93 (s, 3H), 6.69 (td, *J* = 7.8, 1.2 Hz, 1H), 6.74 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.00 (td, *J* = 7.8, 1.6 Hz, 1H), 7.06 (dd, *J* = 7.8, 1.6 Hz, 1H), 8.28 (s, 1H), 9.31 (s, 1H); ^{13}C NMR (126 MHz, DMSO, 27 °C) 154.4, 151.4, 131.1, 127.3, 126.7, 118.3, 116.2, 80.5, 76.7, 55.4, 27.1, 3.0; LRMS *m/z* (ESI+) $[M+H]^+$ 218.2

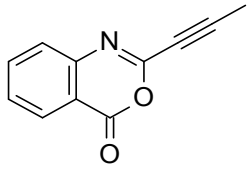


4,4-dimethyl-2-prop-1-ynyl-1,3-benzoxazine (10): Trifluoromethanesulfonic anhydride (0.085 mL, 0.51 mmol) was added dropwise to *N*-(2-(2-hydroxyphenyl)propan-2-yl)but-2-ynamide (99.8 mg, 0.46 mmol) in DCM (4.6 mL) at 0 °C under nitrogen. The resulting solution was stirred for 20 minutes before addition of triethylamine (0.15 mL, 1.06 mmol), warming to rt and stirring for 1 hour. The reaction mixture was quenched with saturated $NaHCO_3$ (20 mL), extracted with EtOAc (2 x 10 mL), the organic layer was dried over a phase separating cartridge, filtered and

evaporated to afford brown oil. The crude product was purified by flash silica chromatography, elution gradient 0 to 30% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 4,4-dimethyl-2-(prop-1-yn-1-yl)-4H-benzo[e][1,3]oxazine (13.9 mg, 15%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO) 1.43 (s, 6H), 2.02 (s, 3H), 6.94 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.16 (td, *J* = 7.5, 1.3 Hz, 1H), 7.24 (ddd, *J* = 8.1, 7.5, 1.6 Hz, 1H), 7.34 (dd, *J* = 7.5, 1.6 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 147.0, 136.4, 128.1, 127.3, 125.6, 125.2, 114.8, 84.1, 73.0, 53.1, 32.1, 3.1; HRMS (ESI+) calcd for $C_{13}H_{13}NO$ $[M+H]^+$ 200.1075, found 200.1082.

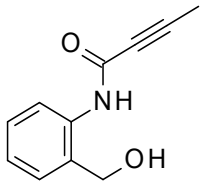


4,4-dimethyl-2-prop-1-ynyl-3,4-dihydroquinazoline (11): Dicyclohexylmethanediimine (295 mg, 1.43 mmol) was added to 2-(2-aminopropan-2-yl)aniline (215 mg, 1.43 mmol) and but-2-ynoic acid (120 mg, 1.43 mmol) in DCM (8.9 mL) under nitrogen. The resulting solution was stirred at rt for 3 hours. The reaction mixture was diluted with Et_2O (25 mL), and washed with saturated $NaHCO_3$ (25 mL) and saturated brine (25 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 20 to 70% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 4,4-dimethyl-2-(prop-1-yn-1-yl)-3,4-dihydroquinazoline (152 mg, 54%) as a yellow oil which solidified on standing. ^{1}H NMR (400 MHz, DMSO) 1.32 (s, 1.2H), 1.40 (s, 4.8H), 1.99 (s, 0.6H), 2.01 (s, 2.4H), 6.74 (dd, *J* = 7.9, 1.2 Hz, 0.2H), 6.83 (dd, *J* = 7.8, 1.3 Hz, 0.8H), 6.93 (td, *J* = 7.5, 1.2 Hz, 0.2H), 6.98 (td, *J* = 7.4, 1.3 Hz, 0.8H), 7.04 – 7.17 (m, 2H), 7.72 (s, 0.8H), 9.59 (s, 0.2H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 141.9, 140.8, 136.0, 135.5, 130.9, 127.3, 126.8, 124.5, 124.5, 123.4, 123.2, 122.7, 112.9, 84.0, 82.3, 76.2, 75.6, 54.5, 51.8, 31.2, 3.3, 3.2; HRMS (ESI+) calcd for $C_{13}H_{14}N_2$ $[M+H]^+$ 199.12297 found 199.12346

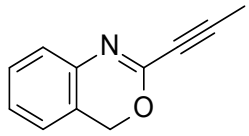


2-prop-1-ynyl-3,1-benzoxazin-4-one (12): Dicyclohexylmethanediimine (790 mg, 3.83 mmol) was added to a solution of but-2-ynoic acid (307 mg, 3.65 mmol) in DCM (18 mL) and the resultant stirred at rt for 10 minutes. 2-aminobenzoic acid (500 mg, 3.65 mmol) was subsequently added and the resultant suspension stirred for 2 hours. The reaction mixture was filtered and the solvent removed under vacuum to afford crude amide as a yellow solid which was used directly.

The crude was dissolved in DCM (50 mL), acetic anhydride (3.45 mL, 36.5 mmol) added and the resultant solution heated to 45 °C for 1 hour. The reaction mixture was quenched with saturated $NaHCO_3$ (100 mL), extracted with DCM (2 x 50 mL), the organic layer was dried over a phase separating cartridge, filtered and evaporated to afford yellow oil. The crude product was purified by flash silica chromatography, elution gradient 0 to 30% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 2-(prop-1-yn-1-yl)-4H-benzo[d][1,3]oxazin-4-one (113 mg, 17%, over 2 steps) as a white solid. ^{1}H NMR (400 MHz, DMSO) 2.18 (s, 3H), 7.57 – 7.68 (m, 2H), 7.93 (ddd, *J* = 7.9, 7.4, 1.5 Hz, 1H), 8.11 (dd, *J* = 7.9, 1.1 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 158.4, 145.8, 142.5, 136.8, 129.2, 128.0, 126.6, 117.9, 90.7, 72.6, 3.6; HRMS (ESI+) calcd for $C_{11}H_7NO_2$ $[M+H]^+$ 186.0555, found 186.0545.



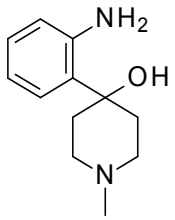
***N*-[2-(hydroxymethyl)phenyl]but-2-ynamide**: Dicyclohexylmethanediimine (880 mg, 4.26 mmol) was added to (2-aminophenyl)MeOH (500 mg, 4.06 mmol) and but-2-ynoic acid (341 mg, 4.06 mmol) in DCM (20 mL) at 0 °C under nitrogen. The resulting suspension was warmed to rt and stirred for 2 hours. The reaction mixture was filtered and the solvent evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 20 to 60% EtOAc in heptane. Pure fractions were evaporated to dryness to afford *N*-(2-(hydroxymethyl)phenyl)but-2-ynamide (593 mg, 77%) as a white solid. ^{1}H NMR (400 MHz, DMSO) 2.04 (s, 3H), 4.49 (d, *J* = 5.5 Hz, 2H), 5.34 (t, *J* = 5.5 Hz, 1H), 7.18 (t, *J* = 7.4 Hz, 1H), 7.21 – 7.27 (m, 1H), 7.41 (d, *J* = 7.4 Hz, 1H), 7.47 (d, *J* = 7.4 Hz, 1H), 9.88 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 150.8, 134.9, 134.4, 127.3, 127.0, 125.3, 124.1, 84.1, 75.6, 60.1, 3.1; LRMS *m/z* (ESI-) found 188.1



2-prop-1-ynyl-4H-3,1-benzoxazine (13): Methanesulfonyl chloride (0.09 mL, 1.11 mmol) was added dropwise to *N*-(2-(hydroxymethyl)phenyl)but-2-ynamide (200 mg, 1.06 mmol) and triethylamine (0.44 mL, 3.17 mmol) in DCM (11 mL) at 0 °C under nitrogen. The resulting solution was stirred at rt for 18 hours. The reaction mixture was washed sequentially with water (2 x 30 mL) and saturated brine (30 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude benzyl chloride.

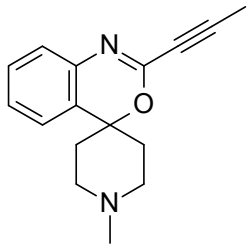
Sodium hydride (46.5 mg, 1.16 mmol) was added to a solution of crude benzyl chloride in DMF (25 mL) at 0 °C under nitrogen. The resulting solution was warmed to rt and stirred for 7 hours. The reaction mixture was quenched with saturated brine (10 mL) and extracted with EtOAc (25 mL). The organic layer was washed with water (2 x 50 mL) and

brine (50 mL) and dried over a phase separating cartridge, filtered and evaporated to afford orange oil. The crude product was purified by flash silica chromatography, elution gradient 0 to 50% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 2-(prop-1-yn-1-yl)-4H-benzo[d][1,3]oxazine (5.2 mg, 3%, over 2 steps) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) 2.04 (s, 3H), 5.27 (s, 2H), 6.88 – 6.99 (m, 1H), 7.13 – 7.22 (m, 2H), 7.22 – 7.31 (m, 1H); 13C NMR (126 MHz, $CDCl_3$, 27 °C) 144.8, 138.9, 129.2, 127.4, 124.8, 123.9, 122.1, 86.7, 73.9, 66.8, 4.3; HRMS (ESI+) calcd for $C_{11}H_9NO$ $[M+H]^+$ 172.0762, found 172.0759.



4-(2-aminophenyl)-1-methyl-piperidin-4-ol: *n*-Butyllithium (1.6 M in hexanes) (1.1 mL, 1.76 mmol) was added dropwise to *tert*-butyl (2-bromophenyl)carbamate (191 mg, 0.70 mmol) in THF (2 mL) at -78 °C under nitrogen. The resulting solution was stirred at -78 °C for 30 minutes. 1-methylpiperidin-4-one (0.09 mL, 0.73 mmol) was added dropwise and the solution warmed to rt and stirred for 1 hour. The reaction mixture was quenched with saturated NH_4Cl (10 mL) and extracted with EtOAc (2 x 20 mL). The aqueous phase was adjusted to pH 8 using saturated $NaHCO_3$ and washed with DCM (2 x 50 mL). The combined organic layers were dried over a phase separating cartridge, filtered and evaporated to afford crude 1'-methylspiro[benzo[d][1,3]oxazine-4,4'-piperidin]-2(1H)-one (210 mg) as a yellow oil which was used without further purification in the next step.

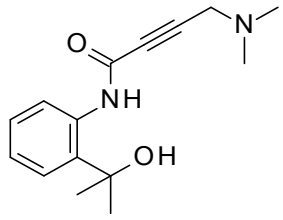
Sodium hydroxide (2M in H_2O) (1.5 mL, 3.00 mmol) was added to crude 1'-methylspiro[benzo[d][1,3]oxazine-4,4'-piperidin]-2(1H)-one (210 mg) in EtOH (3 mL). The resulting solution was stirred at rt for 2 hours, warmed to 50 °C and stirred for 1 hour, then further heated to 80 °C and stirred for 16 hours. The solvent was removed under vacuum and the residue suspended in MeCN (10 mL) and filtered. The solution was purified by preparative HPLC (Waters XSelect CSH C18 ODB column, 5µ silica, 30 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 4-(2-aminophenyl)-1-methylpiperidin-4-ol (40.1 mg, 27%, over 2 steps) as a pale yellow waxy solid. ^{1}H NMR (400 MHz, DMSO) 1.78 – 1.85 (m, 2H), 1.99 – 2.06 (m, 2H), 2.17 (s, 3H), 2.35 – 2.41 (m, 2H), 2.49 – 2.53 (m, 2H), 4.96 (s, 1H), 5.35 (s, 2H), 6.49 (ddd, *J* = 7.8, 7.3, 1.3 Hz, 1H), 6.60 (dd, *J* = 7.9, 1.3 Hz, 1H), 6.91 (ddd, *J* = 7.9, 7.3, 1.5 Hz, 1H), 7.01 (dd, *J* = 7.8, 1.5 Hz, 1H); ^{13}C NMR (126 MHz, DMSO, 27 °C) 147.4, 130.7, 127.2, 124.8, 116.2, 115.7, 69.9, 51.0, 46.1, 34.8; LRMS *m/z* (ESI+) $[M+H]^+$ 207.2



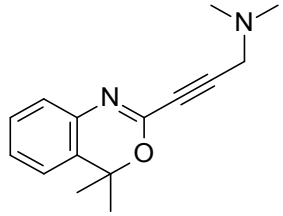
1'-methyl-2-prop-1-ynyl-spiro[3,1-benzoxazine-4,4'-piperidine] (14):

Dicyclohexylmethanediimine (39.8 mg, 0.19 mmol) was added to 4-(2-aminophenyl)-1-methylpiperidin-4-ol (39 mg, 0.19 mmol) and but-2-ynoic acid (16.2 mg, 0.19 mmol) in DCM (1 mL) under nitrogen. The resulting solution was stirred at rt for 2 hours. The reaction was incomplete and further but-2-ynoic acid (16.2 mg, 0.19 mmol) and dicyclohexylmethanediimine (39.8 mg, 0.19 mmol) were added and the solution was stirred at rt for a further 45 minutes. The reaction mixture was diluted with DCM (2 mL) and filtered through a syringe filter. The solvent was removed under vacuum to afford crude amide as a white foam.

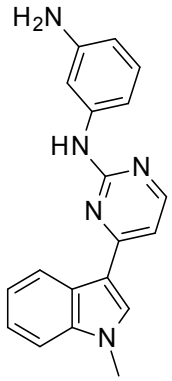
To a solution of the crude intermediate in DCM (4 mL) was added methanesulfonic acid (0.06 mL, 0.92 mmol). The resultant solution was warmed to 45 °C and stirred for 30 minutes. The reaction mixture was quenched with saturated $NaHCO_3$ (15 mL) and extracted with DCM (2 x 10 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford yellow oil. The crude product was purified by preparative HPLC (Waters XSelect CSH C18 ODB column, 5µ silica, 30 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 1'-methyl-2-(prop-1-yn-1-yl)spiro[benzo[d][1,3]oxazine-4,4'-piperidine] (21 mg, 44%, over 2 steps) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) 1.97 – 2.09 (m, 7H), 2.32 (s, 3H), 2.43 (td, *J* = 11.7, 3.4 Hz, 2H), 2.64 – 2.72 (m, 2H), 6.98 – 7.05 (m, 1H), 7.09 – 7.15 (m, 2H), 7.16 – 7.21 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$, 30 °C) 143.8, 138.5, 130.1, 128.8, 127.7, 125.1, 122.4, 86.0, 77.7, 74.6, 50.7, 46.3, 36.0, 4.4; HRMS (ESI+) calcd for $C_{16}H_{18}N_2O$ $[M+H]^+$ 255.1497 found 255.1491



4-(dimethylamino)-*N*-[2-(1-hydroxy-1-methyl-ethyl)phenyl]but-2-ynamide: HATU (249 mg, 0.65 mmol) was added to 2-(2-aminophenyl)propan-2-ol (90 mg, 0.60 mmol) and 4-(dimethylamino)but-2-ynoic acid (76 mg, 0.60 mmol) in DMF (3 mL) under nitrogen. The resulting solution was stirred at rt for 15 minutes. The reaction mixture was diluted with DCM (20 mL) and washed sequentially with half saturated brine (20 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product. The crude product was purified by preparative HPLC (Waters XSelect CSH C18 ODB column, 5µ silica, 30 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 4-(dimethylamino)-*N*-(2-(2-hydroxypropan-2-yl)phenyl)but-2-ynamide (31.5 mg, 20 %) as a pale yellow oil. ^{1}H NMR (500 MHz, CD_3CN) 1.61 (s, 6H), 2.27 (s, 6H), 3.41 (s, 2H), 7.04 – 7.12 (m, 1H), 7.23 – 7.28 (m, 1H), 7.29 – 7.35 (m, 1H), 8.15 (d, *J* = 8.1 Hz, 1H), 10.71 (s, 1H); ^{13}C NMR (126 MHz, CD_3CN, 27°C) 150.9, 137.5, 136.7, 128.6, 126.7, 125.0, 123.2, 82.4, 81.7, 74.8, 48.0, 44.2, 30.5; LRMS *m/z* (ESI-) $[M-H]^-$ 259.1

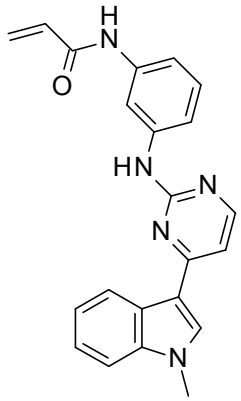


3-(4,4-dimethyl-3,1-benzoxazin-2-yl)-*N*,*N*-dimethyl-prop-2-yn-1-amine (15): Methanesulfonic acid (0.03 ml, 0.46 mmol) was added to 4-(dimethylamino)-*N*-(2-(2-hydroxypropan-2-yl)phenyl)but-2-ynamide (22 mg, 0.08 mmol) in DCM (1.9 ml) under nitrogen. The resulting solution was warmed to 45 °C and stirred for 30 minutes. The reaction mixture was quenched with saturated $NaHCO_3$ (15 mL) and extracted with DCM (2 x 15 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford orange oil. The crude product was purified by preparative HPLC (Waters XSelect CSH C18 ODB column, 5µ silica, 30 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 3-(4,4-dimethyl-4H-benzo[d][1,3]oxazin-2-yl)-*N*,*N*-dimethylprop-2-yn-1-amine (13.9 mg, 68 %) as a pale yellow oil. ^{1}H NMR (500 MHz, CD_3CN) 1.62 (s, 6H), 2.27 (s, 6H), 3.44 (s, 2H), 7.06 – 7.13 (m, 1H), 7.19 – 7.3 (m, 3H); ^{13}C NMR (126 MHz, CD_3CN, 27°C) 144.4, 138.7, 132.4, 129.6, 128.8, 125.6, 123.8, 85.0, 80.5, 80.3, 48.4, 44.4, 28.7; LRMS *m/z* (ESI+) $[M+H]^+$ 243.2; HRMS (ESI+) calcd for $C_{15}H_{18}N_2O$ $[M+H]^+$ 243.1497 found 243.1504

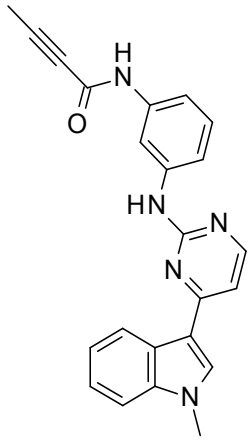


***N*3-[4-(1-methylindol-3-yl)pyrimidin-2-yl]benzene-1,3-diamine (21)**: 3-Nitroaniline (567 mg, 4.10 mmol), 3-(2-chloropyrimidin-4-yl)-1-methyl-1*H*-indole (1.01 g, 4.10 mmol) and 4-methylbenzenesulfonic acid (777 mg, 4.51 mmol) were suspended in EtOH (10 mL) and sealed into a microwave tube. The reaction was heated to 150 °C for 1 hour in the microwave reactor and cooled to rt. The reaction mixture was filtered and washed with MeOH (50 mL). The crude gum was triturated with MeOH to give a solid which was collected by filtration and dried under vacuum to afford crude 4-(1-methyl-1*H*-indol-3-yl)-N-(3-nitrophenyl)pyrimidin-2-amine (1.50 g) as a pale yellow solid which was used directly without further purification.

Iron (576 mg, 10.3 mmol) was added to crude 4-(1-methyl-1*H*-indol-3-yl)-*N*-(3-nitrophenyl)pyrimidin-2-amine (890 mg) and ammonia hydrochloride (64.4 mg, 1.20 mmol) in a mixture of EtOH (43.5 mL) and water (14.5 mL). The resulting suspension was warmed to 80 °C and stirred for 2.5 hours. The reaction mixture was filtered through celite and the solvent removed under vacuum. The crude residue was taken in DCM (50 mL), washed with saturated aq. $NaHCO_3$ (100 mL) and passed through a phase separator. The solvent was removed under vacuum to afford *N*1-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)benzene-1,3-diamine (524 mg, 68%, over 2 steps) as a brown foam. ^{1}H NMR (500 MHz, DMSO) 3.88 (s, 3H), 4.93 (s, 2H), 6.22 (d, *J* = 7.0 Hz, 1H), 6.9 – 6.99 (m, 2H), 7.11 – 7.17 (m, 2H), 7.19 (t, *J* = 7.4 Hz, 1H), 7.26 (t, *J* = 7.4 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 8.26 – 8.32 (m, 2H), 8.64 (d, *J* = 8.0 Hz, 1H), 9.07 (s, 1H); ^{13}C NMR (126 MHz, DMSO, 27 °C) 162.0, 160.2, 156.7, 148.7, 141.4, 137.6, 132.7, 128.6, 125.6, 122.5, 122.1, 120.8, 112.6, 110.2, 107.8, 107.7, 106.8, 105.1, 33.0; LRMS *m/z* (ESI+) $[M+H]^+$ 316.2



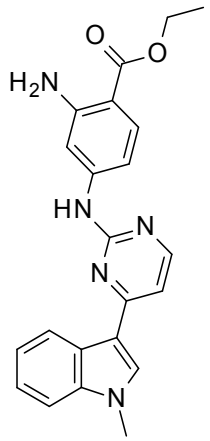
***N*-[3-[[4-(1-methylindol-3-yl)pyrimidin-2-yl]amino]phenyl]prop-2-enamide (16)**: Acryloyl chloride (0.07 mL, 0.87 mmol) was added dropwise to *N*1-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)benzene-1,3-diamine (260 mg, 0.82 mmol) and triethylamine (0.29 mL, 2.08 mmol) in DCM (5 mL) under nitrogen. The resulting solution was stirred at rt for 1 hour. The reaction mixture was quenched with saturated NaHCO*3* (15 mL) and extracted with DCM (3 x 10 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford brown waxy solid. The crude product was purified by flash silica chromatography, elution gradient 40 to 90% EtOAc in heptane. Pure fractions were evaporated to dryness to afford *N*-(3-((4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide (57.0 mg, 19 %) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO) 3.90 (s, 3H), 5.75 (dd, *J* = 10.1, 2.0 Hz, 1H), 6.28 (dd, *J* = 17.0, 2.0 Hz, 1H), 6.50 (dd, *J* = 17.0, 10.1 Hz, 1H), 7.13 – 7.3 (m, 5H), 7.43 (dt, *J* = 6.6, 2.0 Hz, 1H), 7.53 (d, *J* = 8.2 Hz, 1H), 8.32 (s, 1H), 8.35 (d, *J* = 5.3 Hz, 1H), 8.44 (s, 1H), 8.54 (d, *J* = 7.7 Hz, 1H), 9.41 (s, 1H), 10.08 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 163.0, 161.9, 160.0, 157.0, 141.2, 139.0, 137.6, 133.2, 132.1, 128.4, 126.5, 125.5, 122.1, 122.1, 120.8, 114.8, 112.8, 112.5, 110.6, 110.3, 107.2, 33.0; HRMS (ESI+) calcd for $C_{22}H_{19}N_5O$ $[M+H]^+$ 370.1668, found 370.1672



***N*-[3-[[4-(1-methylindol-3-yl)pyrimidin-2-yl]amino]phenyl]but-2-ynamide (17)**:

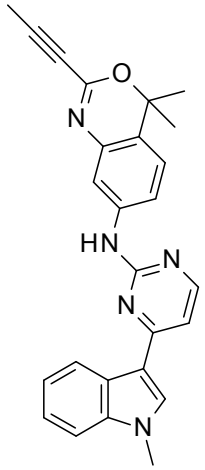
Dicyclohexylmethanediimine (187 mg, 0.91 mmol) was added to *N*1-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)benzene-1,3-diamine (260 mg, 0.82 mmol) and 2-butynoic acid (70.1 mg, 0.83 mmol) in DCM (5 mL) at 25 °C under nitrogen. The resulting solution was stirred at rt for 1 hour. The solvent was removed under vacuum to afford the crude as a brown wax. The crude product was purified by flash silica chromatography, elution gradient 40 to 90% EtOAc in heptane. Fractions containing product were evaporated to dryness to afford *N*-(3-((4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)amino)phenyl)but-2-ynamide (177 mg, 54%) as a pale yellow solid. 1H NMR (400 MHz, DMSO) 2.05 (s, 3H), 3.89 (s, 3H), 7.13 (d, *J* = 8.3 Hz, 1H), 7.17 – 7.3 (m, 4H), 7.46 – 7.56 (m, 2H), 8.18 (t, *J* = 2.1 Hz, 1H), 8.3 – 8.4 (m, 2H), 8.56 (d, *J* = 7.9 Hz, 1H), 9.41 (s, 1H), 10.52 (s, 1H); 13C NMR (101 MHz, DMSO, 30 °C) 161.9, 160.0, 156.9,

150.4, 141.2, 138.5, 137.6, 133.0, 128.4, 125.5, 122.3, 122.1, 120.8, 115.2, 113.0, 112.5, 111.1, 110.3, 107.2, 83.8, 76.0, 32.9, 3.2; HRMS (ESI+) calcd for $C_{23}H_{19}N_5O$ $[M+H]^+$ 382.1668, found 382.1684



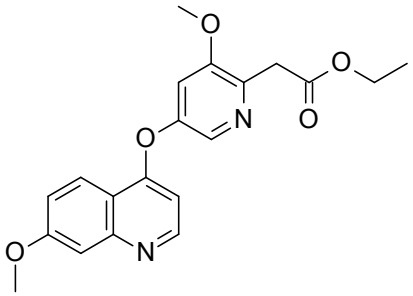
ethyl 2-amino-4-[[4-(1-methylindol-3-yl)pyrimidin-2-yl]amino]benzoate (22): Ethyl 4-amino-2-nitrobenzoate (200 mg, 0.95 mmol), 3-(2-chloropyrimidin-4-yl)-1-methyl-1*H*-indole (232 mg, 0.95 mmol) and 4-methylbenzenesulfonic acid (180 mg, 1.05 mmol) were suspended in EtOH (1.9 mL) and sealed into a microwave tube. The reaction was heated to 150 °C for 1 hour in the microwave reactor and cooled to rt. The solvent was removed under vacuum and the crude residue purified by flash silica chromatography, elution gradient 0 to 5% MeOH in DCM. Fractions containing products were evaporated to dryness to afford a 2:1 mixture of ethyl 4-((4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)amino)-2-nitrobenzoate and *p*TSA (317 mg) as a brown solid which was used directly in the next reaction without further purification.

Iron (257 mg, 4.61 mmol) was added to methyl 4-((4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)amino)-2-nitrobenzoate (310 mg) and ammonia hydrochloride (28.8 mg, 0.54 mmol) in a mixture of EtOH (16 mL) and water (5 mL). The resulting suspension was stirred at 80 °C for 2 hours. The reaction mixture was filtered through celite and the solvent removed under vacuum to afford a brown residue. The residue was dissolved in DCM (20 mL) and passed through a phase separator. The solvent was removed under vacuum to afford ethyl 2-amino-4-((4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)amino)benzoate (228 mg, 62%, over 2 steps) as a yellow foam. ^{1}H NMR (500 MHz, DMSO) 1.30 (t, *J* = 7.1 Hz, 3H), 3.89 (s, 3H), 4.23 (q, *J* = 7.1 Hz, 2H), 6.65 (s, 2H), 6.96 (dd, *J* = 8.9, 2.0 Hz, 1H), 7.2 – 7.25 (m, 1H), 7.25 – 7.31 (m, 2H), 7.47 (d, *J* = 2.0 Hz, 1H), 7.54 (d, *J* = 8.1 Hz, 1H), 7.66 (d, *J* = 8.9 Hz, 1H), 8.33 (s, 1H), 8.38 (d, *J* = 5.4 Hz, 1H), 8.64 (d, *J* = 7.9 Hz, 1H), 9.54 (s, 1H). (s, 1H); ^{13}C NMR (126 MHz, DMSO, 27 °C) 167.2, 162.2, 159.7, 156.6, 152.4, 145.9, 137.7, 133.1, 131.1, 125.5, 122.3, 122.3, 120.9, 112.3, 110.4, 108.0, 107.3, 103.8, 102.7, 59.3, 33.0, 14.4; LRMS *m/z* (ESI-) $[M-H]^-$ 377.2.



4,4-dimethyl-N-[4-(1-methylindol-3-yl)pyrimidin-2-yl]-2-prop-1-ynyl-3,1-benzoxazin-7-amine (18):
Methylmagnesium bromide (3M in Et_2O) (2.58 mL, 7.74 mmol) was added dropwise to ethyl 2-amino-4-((4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)amino)benzoate (600 mg, 1.55 mmol) in THF (5 mL) at 0 °C under nitrogen. The resulting solution was warmed to rt and stirred for 2.5 hours. The reaction mixture was quenched with water (40 mL) and extracted with DCM (3 x 50 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford 2-(2-amino-4-((4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)amino)phenyl)propan-2-ol (314 mg) as a yellow solid which was used in the next reaction without further purification.
Dicyclohexylmethanediimine (176 mg, 0.85 mmol) was added to 2-(2-amino-4-((4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)amino)phenyl)propan-2-ol (370 mg) and 2-butynoic acid (65.9 mg, 0.78 mmol) in DCM (10 mL) at 25 °C under nitrogen. The resulting solution was stirred at rt for 18 hours. The reaction mixture was diluted with Et2O (50 mL), filtered and the solvent removed under vacuum to afford crude *N*-(2-(2-hydroxypropan-2-yl)-5-((4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)amino)phenyl)but-2-ynamide (285 mg) as a yellow solid.
Methanesulfonic acid (0.16 mL, 2.46 mmol) was added to *N*-(2-(2-hydroxypropan-2-yl)-5-((4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)amino)phenyl)but-2-ynamide (279 mg) in DCM (10.7 mL) at 25 °C under nitrogen. The resulting solution was warmed to 45 °C and stirred for 1.5 hours. The reaction mixture was quenched with saturated $NaHCO_3$ (25 mL) and the organic layer was dried over a phase separating cartridge, filtered and evaporated to afford yellow foam. The crude product was purified by preparative HPLC (Waters XSelect CSH C18 ODB column, 5µ silica, 30 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 4,4-dimethyl-*N*-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)-2-(prop-1-yn-1-yl)-4*H*-benzo[d][1,3]oxazin-7-amine (143 mg, 38%, over 3 steps) as a cream solid. ^{1}H NMR (400 MHz, DMSO) 1.59 (s, 6H), 2.07 (s, 3H), 3.90 (s, 3H), 7.16 – 7.25 (m, 3H), 7.28 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 7.54 (d, *J* = 8.2 Hz, 1H), 7.62 (d, *J* = 2.2 Hz, 1H), 7.68 (dd, *J* = 8.2, 2.2 Hz, 1H), 8.31 (s, 1H), 8.34 (d, *J* = 5.4 Hz, 1H), 8.64 (d, *J* = 7.8 Hz, 1H), 9.44 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30 °C) 162.2, 160.0, 156.7, 143.1, 141.0, 137.6,

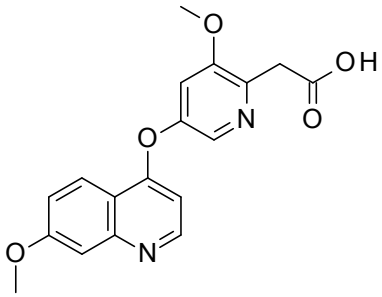
137.5, 132.8, 125.5, 123.9, 122.8, 122.5, 122.2, 120.8, 118.0, 114.7, 112.4, 110.2, 107.4, 86.0, 79.1, 74.6, 33.0, 28.3, 3.3; HRMS (ESI+) calcd for $C_{26}H_{23}N_5O$ $[M+H]^+$ 422.1981, found 422.1970



ethyl 2-[3-methoxy-5-[(7-methoxy-4-quinolyl)oxy]-2-pyridyl]acetate:

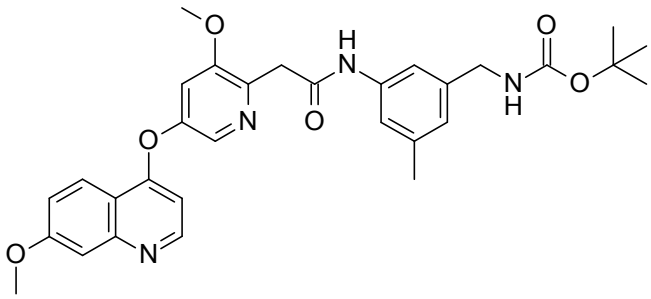
4-Dimethylaminopyridine (3.01 g, 24.6 mmol) was added to 4-chloro-7-methoxyquinoline (1.59 g, 8.21 mmol) and ethyl 2-(5-hydroxy-3-methoxypyridin-2-yl)acetate[a] (1.73 g, 8.21 mmol) in chlorobenzene (17 ml) under nitrogen. The resulting suspension was warmed to 135 °C and stirred for 18 hours. The solvent was removed under vacuum to afford brown solid. The crude product was absorbed on to silica and purified by flash silica chromatography, elution gradient 0 to 5% 1M NH_3/MeOH in DCM. Fractions containing product were evaporated to dryness to afford ethyl 2-(3-methoxy-5-((7-methoxyquinolin-4-yl)oxy)pyridin-2-yl)acetate (2.62 g, 87 %) as a pink solid. ^{1}H NMR (400 MHz, DMSO) 1.19 (t, *J* = 7.1 Hz, 3H), 3.81 (s, 2H), 3.82 (s, 3H), 3.95 (s, 3H), 4.11 (q, *J* = 7.1 Hz, 2H), 6.57 (d, *J* = 5.2 Hz, 1H), 7.31 (dd, *J* = 9.1, 2.5 Hz, 1H), 7.43 (d, *J* = 2.5 Hz, 1H), 7.52 (d, *J* = 2.3 Hz, 1H), 8.08 (d, *J* = 2.3 Hz, 1H), 8.22 (d, *J* = 9.1 Hz, 1H), 8.65 (d, *J* = 5.2 Hz, 1H); ^{13}C NMR (101 MHz, DMSO, 30°C) 169.8, 160.8, 160.5, 154.5, 151.8, 151.3, 150.7, 141.9, 132.5, 122.7, 118.9, 115.1, 111.9, 107.4, 103.0, 60.2, 56.2, 55.5, 38.4, 14.0, 13.9; LRMS *m/z* (ESI+) $[M+H]^+$ 369.2

[a] ethyl 2-(5-hydroxy-3-methoxypyridin-2-yl)acetate was obtained from an in-house building block collection and previously made according to literature procedure[17]

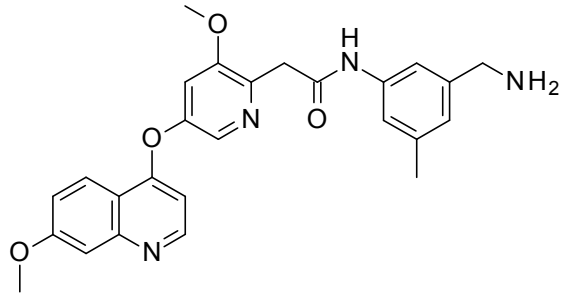


2-[3-methoxy-5-[(7-methoxy-4-quinolyl)oxy]-2-pyridyl]acetic acid: Lithium hydroxide monohydrate (446 mg, 10.6 mmol) was added to ethyl 2-(3-methoxy-5-((7-methoxyquinolin-4-yl)oxy)pyridin-2-yl)acetate (2.61 g, 7.08 mmol) in a mixture of THF (16.5 ml) and water (11 ml). The resulting solution was stirred at rt for 2 hours. THF was removed under vacuum and the resulting solution acidified to pH 3 by dropwise addition of 2M HCl until pH 8, followed by

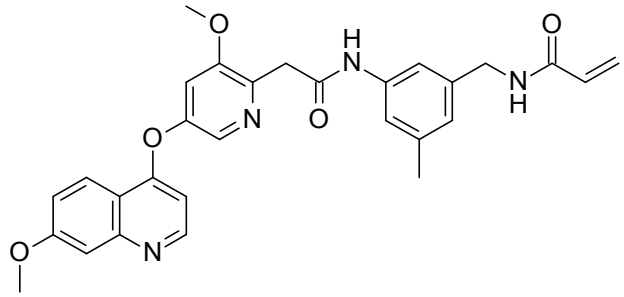
addition of 1M citric acid. The precipitate was collected by filtration, washed with water (3 x 20 mL) and dried under vacuum to afford 2-(3-methoxy-5-((7-methoxyquinolin-4-yl)oxy)pyridin-2-yl)acetic acid (2.32 g, 96%) as a cream solid. ^{1}H NMR (400 MHz, DMSO) 3.29 (s, 2H), 3.82 (s, 3H), 3.95 (s, 3H), 6.57 (d, *J* = 5.2 Hz, 1H), 7.31 (dd, *J* = 9.1, 2.5 Hz, 1H), 7.43 (d, *J* = 2.5 Hz, 1H), 7.51 (d, *J* = 2.3 Hz, 1H), 8.07 (d, *J* = 2.3 Hz, 1H), 8.22 (d, *J* = 9.1 Hz, 1H), 8.65 (d, *J* = 5.2 Hz, 1H), 12.34 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30°C) 171.3, 160.8, 160.5, 154.5, 151.8, 151.3, 150.5, 142.4, 132.4, 122.7, 118.9, 115.1, 111.8, 107.4, 103.0, 56.1, 55.5, 38.5; LRMS *m/z* (ESI+) $[M+H]^+$ 341.1



***tert*-butyl *N*-[[3-[[2-[3-methoxy-5-[(7-methoxy-4-quinolyl)oxy]-2-pyridyl]acetyl]amino]-5-methyl-phenyl]methyl]carbamate**: O-(7-Azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (275 mg, 0.72 mmol) was added to *tert*-butyl 3-amino-5-methylbenzylcarbamate (114 mg, 0.48 mmol), 2-(3-methoxy-5-((7-methoxyquinolin-4-yl)oxy)pyridin-2-yl)acetic acid (164 mg, 0.48 mmol) and DIPEA (0.21 ml, 1.21 mmol) in DMF (2 ml) at rt under nitrogen. The resulting mixture was stirred at rt for 16 hours. Water (20 mL) and EtOAc (50 mL) were added and the layers were separated. The aqueous layer was extracted with EtOAc (2 x 50 mL) and the combined organic layers were washed with saturated brine (20 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give the crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 100%, 3:1 EtOAc:EtOH in heptane. Pure fractions were evaporated to dryness to afford *tert*-butyl 3-(2-(3-methoxy-5-((7-methoxyquinolin-4-yl)oxy)pyridin-2-yl)acetamido)-5-methylbenzylcarbamate (224 mg, 83%) as a white solid. ^{1}H NMR (500 MHz, DMSO) 1.40 (s, 9H), 2.25 (s, 3H), 3.83 (s, 3H), 3.86 (s, 2H), 3.95 (s, 3H), 4.05 (d, *J* = 6.0 Hz, 2H), 6.56 (d, *J* = 5.2 Hz, 1H), 6.74 (s, 1H), 7.26 (s, 1H), 7.29 – 7.39 (m, 3H), 7.44 (d, *J* = 2.5 Hz, 1H), 7.52 (d, *J* = 2.2 Hz, 1H), 8.09 (d, *J* = 2.2 Hz, 1H), 8.23 (d, *J* = 9.1 Hz, 1H), 8.66 (d, *J* = 5.2 Hz, 1H), 10.06 (s, 1H); ^{13}C NMR (126 MHz, DMSO, 27°C) 167.7, 160.8, 160.7, 155.7, 154.6, 151.9, 151.3, 150.3, 143.4, 140.6, 139.2, 137.6, 132.5, 122.7, 122.4, 118.9, 118.0, 115.1, 114.9, 111.8, 107.4, 102.9, 77.7, 56.2, 55.5, 43.4, 40.6, 28.3, 21.2; LRMS *m/z* (ESI+) $[M+H]^+$ 559.3

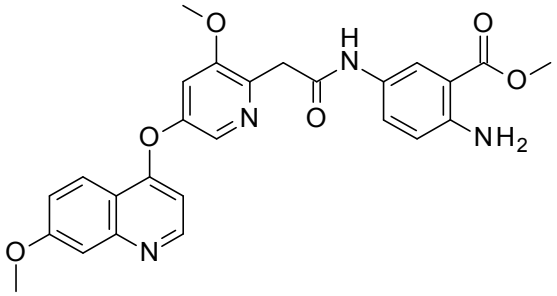


***N*-[3-(aminomethyl)-5-methyl-phenyl]-2-[3-methoxy-5-[(7-methoxy-4-quinolyl)oxy]-2-pyridyl]acetamide**: 2,2,2-trifluoroacetic acid (1 mL, 13.1 mmol) was added to *tert*-butyl (3-(2-(3-methoxy-5-((7-methoxyquinolin-4-yl)oxy)pyridin-2-yl)acetamido)-5-methylbenzyl)carbamate (260 mg, 0.47 mmol) in DCM (4 mL) under nitrogen. The resulting solution was stirred at rt for 90 minutes. The solvent was removed under vacuum and the resultant residue was diluted with EtOAc (50 mL), and washed sequentially with saturated Na_2CO_3 (100 mL) and saturated brine (50 mL). The organic layer was dried with a phase separating cartridge. The resulting solution was evaporated to dryness and the residue was azeotroped with heptane to afford desired *N*-(3-(aminomethyl)-5-methylphenyl)-2-(3-methoxy-5-((7-methoxyquinolin-4-yl)oxy)pyridin-2-yl)acetamide (178 mg, 83 %) as a cream solid. ^{1}H NMR (400 MHz, DMSO) 2.25 (s, 3H), 3.64 (s, 2H), 3.82 (s, 3H), 3.85 (s, 2H), 3.95 (s, 3H), 6.56 (d, *J* = 5.2 Hz, 1H), 6.84 (s, 1H), 7.28 – 7.36 (m, 3H), 7.43 (d, *J* = 2.5 Hz, 1H), 7.50 (d, *J* = 2.2 Hz, 1H), 8.09 (d, *J* = 2.3 Hz, 1H), 8.23 (d, *J* = 9.2 Hz, 1H), 8.65 (d, *J* = 5.2 Hz, 1H), 9.99 (s, 1H); ^{13}C NMR (126 MHz, DMSO, 27°C) 167.6, 160.8, 160.7, 154.6, 151.9, 151.3, 150.3, 144.7, 143.4, 139.1, 137.4, 132.5, 122.7, 122.5, 118.9, 117.5, 115.1, 115.0, 111.8, 107.4, 102.9, 56.2, 55.5, 45.7, 40.5, 21.2; LRMS *m/z* (ESI+) $[M+H]^+$ 459.3

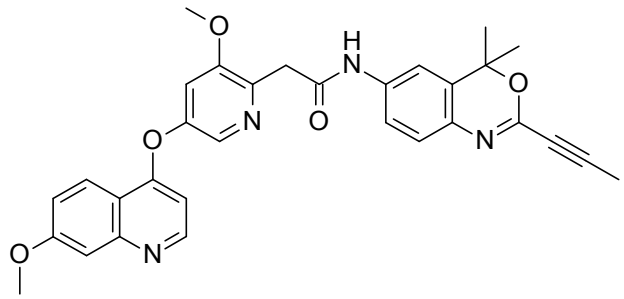


***N*-[[3-[[2-[3-methoxy-5-[(7-methoxy-4-quinolyl)oxy]-2-pyridyl]acetyl]amino]-5-methyl-phenyl]methyl]prop-2-enamide (25)**: A solution of acryloyl chloride (13 mg, 0.14 mmol) in DCM (0.5 mL) was added dropwise to a stirred suspension of *N*-(3-(aminomethyl)-5-methylphenyl)-2-(3-methoxy-5-((7-methoxyquinolin-4-yl)oxy)pyridin-2-yl)acetamide (66 mg, 0.14 mmol) and *N*-ethyl-*N*-isopropylpropan-2-amine (0.03 mL, 0.14 mmol) in DCM (5 mL) at 0 °C. The resulting solution was stirred at 0 °C for 60 minutes. The solvent was removed under vacuum and the residue purified by flash silica chromatography, elution gradient 0 to 60% 3:1 EtOAc:EtOH in heptane. Pure fractions were

evaporated to dryness to afford *N*-(3-(2-(3-methoxy-5-((7-methoxyquinolin-4-yl)oxy)pyridin-2-yl)acetamido)-5-methylbenzyl)acrylamide (55.0 mg, 75%) as a white foam. ^{1}H NMR (500 MHz, DMSO) 2.25 (s, 3H), 3.82 (s, 3H), 3.85 (s, 2H), 3.94 (s, 3H), 4.27 (d, *J* = 6.0 Hz, 2H), 5.62 (dd, *J* = 10.2, 2.2 Hz, 1H), 6.13 (dd, *J* = 17.1, 2.2 Hz, 1H), 6.28 (dd, *J* = 17.1, 10.2 Hz, 1H), 6.55 (d, *J* = 5.2 Hz, 1H), 6.77 (s, 1H), 7.29 (s, 1H), 7.31 (dd, *J* = 9.1, 2.5 Hz, 1H), 7.38 (s, 1H), 7.43 (d, *J* = 2.5 Hz, 1H), 7.50 (d, *J* = 2.2 Hz, 1H), 8.09 (d, *J* = 2.2 Hz, 1H), 8.22 (d, *J* = 9.1 Hz, 1H), 8.56 (t, *J* = 5.9 Hz, 1H), 8.65 (d, *J* = 5.2 Hz, 1H), 10.08 (s, 1H). ; ^{13}C NMR (126 MHz, DMSO, 27°C) 167.8, 164.5, 160.8, 160.7, 154.6, 151.9, 151.3, 150.3, 143.3, 139.7, 139.3, 137.8, 132.5, 131.7, 125.4, 122.9, 122.7, 118.9, 118.1, 115.2, 115.1, 111.8, 107.4, 102.9, 56.2, 55.5, 42.2, 40.5, 21.2; HRMS (ESI+) calcd for $C_{29}H_{28}N_4O_5$ $[M+H]^+$ 513.2138, found 513.2121



methyl 2-amino-5-[[2-[3-methoxy-5-[(7-methoxy-4-quinolyl)oxy]-2-pyridyl]acetyl]amino]benzoate: HATU (614 mg, 1.62 mmol) was added to methyl 2,5-diaminobenzoate (293 mg, 1.76 mmol), 2-(3-methoxy-5-((7-methoxyquinolin-4-yl)oxy)pyridin-2-yl)acetic acid (500 mg, 1.47 mmol) and *N*-ethyl-*N*-isopropylpropan-2-amine (0.77 ml, 4.41 mmol) in DMF (10 ml) under nitrogen. The resulting solution was stirred at rt for 3 hours. The reaction mixture was diluted with DCM (200 mL) and washed with saturated brine (2 x 200 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude product. The crude product was purified by preparative HPLC (Waters XSelect CSH C18 ODB column, 5µ silica, 30 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford methyl 2-amino-5-(2-(3-methoxy-5-((7-methoxyquinolin-4-yl)oxy)pyridin-2-yl)acetamido)benzoate (523 mg, 73 %) as a beige solid. ^{1}H NMR (400 MHz, DMSO) 3.79 (s, 3H), 3.81 (s, 2H), 3.83 (s, 3H), 3.95 (s, 3H), 6.46 (s, 2H), 6.57 (d, *J* = 5.2 Hz, 1H), 6.74 (d, *J* = 8.9 Hz, 1H), 7.32 (dd, *J* = 9.1, 2.5 Hz, 1H), 7.41 – 7.47 (m, 2H), 7.50 (d, *J* = 2.3 Hz, 1H), 8.03 (d, *J* = 2.5 Hz, 1H), 8.09 (d, *J* = 2.3 Hz, 1H), 8.23 (d, *J* = 9.1 Hz, 1H), 8.66 (d, *J* = 5.2 Hz, 1H), 9.85 (s, 1H); ^{13}C NMR (101 MHz, DMSO, 30°C) 167.6, 167.1, 160.8, 160.6, 154.6, 151.8, 151.3, 150.3, 147.7, 143.4, 132.4, 127.5, 127.0, 122.7, 121.0, 118.9, 116.6, 115.1, 111.7, 108.1, 107.4, 102.9, 56.2, 55.5, 51.3; LRMS *m/z* (ESI+) $[M+H]^+$ 489.3



***N*-(4,4-dimethyl-2-prop-1-ynyl-3,1-benzoxazin-6-yl)-2-[3-methoxy-5-[(7-methoxy-4-quinolyl)oxy]-2-pyridyl]acetamide (26)**: Methyl 2-amino-5-(2-(3-methoxy-5-((7-methoxyquinolin-4-yl)oxy)pyridin-2-yl)acetamido)benzoate (435 mg, 0.89 mmol) was dissolved in THF (8 ml) and methylmagnesium bromide (3M in Et2O) (1.187 ml, 3.56 mmol) added dropwise. The resultant solution was stirred at rt for 2 hours. The reaction mixture was quenched with water (10 mL) and extracted with EtOAc (2 x 50 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford crude *N*-(4-amino-3-(2-hydroxypropan-2-yl)phenyl)-2-(3-methoxy-5-((7-methoxyquinolin-4-yl)oxy)pyridin-2-yl) acetamide (438 mg) as a brown waxy solid.

Dicyclohexylmethanediimine (193 mg, 0.93 mmol) was added to *N*-(4-amino-3-(2-hydroxypropan-2-yl)phenyl)-2-(3-methoxy-5-((7-methoxyquinolin-4-yl)oxy)pyridin-2-yl) acetamide (435 mg) and but-2-ynoic acid (79 mg, 0.93 mmol) in DCM (4.0 ml) under nitrogen. The resulting solution was stirred at rt for 1 hour. The reaction mixture was diluted with DCM (10 mL) and washed with saturated $NaHCO_3$ (10 mL). The organic layer was dried with a phase separating cartridge, filtered and evaporated to afford crude *N*-[4-[[2-[3-methoxy-5-[(7-methoxy-4-quinolyl)oxy]-2-pyridyl]acetyl]amino]-2-(1-methoxy-1-methyl-ethyl)phenyl]but-2-ynamide (512 mg) which was used directly without further purification. Methanesulfonic acid (0.289 ml, 4.45 mmol) was added to crude *N*-[4-[[2-[3-methoxy-5-[(7-methoxy-4-quinolyl)oxy]-2-pyridyl]acetyl]amino]-2-(1-methoxy-1-methyl-ethyl)phenyl]but-2-ynamide (510 mg) in DCM (20 ml) under nitrogen. The resulting solution was warmed to 45 ^{o}C and stirred for 1 hour. The reaction mixture was quenched with saturated $NaHCO_3$ (25 mL) and extracted with DCM (2 x 50 mL). The organic layer was dried over a phase separating cartridge, filtered and evaporated to afford orange oil. The crude product was purified by preparative HPLC (Waters XSelect CSH C18 ODB column, 5µ silica, 30 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford *N*-(4,4-dimethyl-2-(prop-1-yn-1-yl)-4H-benzo[d][1,3]oxazin-6-yl)-2-(3-methoxy-5-((7-methoxyquinolin-4-yl)oxy)pyridin-2-yl)acetamide (255 mg, 53 %, over 3 steps) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO) 1.55 (s, 6H), 2.05 (s, 3H), 3.83 (s, 3H), 3.88 (s, 2H), 3.95 (s, 3H), 6.57 (d, *J* = 5.2 Hz, 1H), 7.03 (d, *J* = 8.5 Hz, 1H), 7.32 (dd, *J* = 9.1, 2.6 Hz, 1H), 7.41 – 7.49 (m, 2H), 7.52 (d, *J* = 2.3 Hz, 1H), 7.61 (d, *J* = 2.2 Hz, 1H), 8.10 (d, *J* = 2.3 Hz, 1H), 8.23 (d, *J* = 9.1 Hz, 1H), 8.66 (d, *J* = 5.2 Hz, 1H), 10.26 (s, 1H); ^{13}C NMR (126 MHz, DMSO, 27°C) 167.9, 160.8, 160.6, 154.6, 151.9, 151.3, 150.4, 143.1, 141.9, 138.7, 132.8, 132.5, 131.5, 124.7, 122.7,

118.9, 118.8, 115.1, 113.1, 111.8, 107.4, 102.9, 86.2, 78.8, 74.5, 56.2, 55.5, 40.5, 27.9, 3.4; HRMS (ESI+) calcd for $C_{31}H_{28}N_4O_5$ $[M+H]^+$ 537.2138, found 537.2120

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