Supplementary information for:

Achieving in vivo target depletion through the discovery and optimisation of benzimidazolone BCL6 degraders

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1. Supplementary experimental: protein production, purification and crystallography

1.1 BCL6 constructs used for assays and crystallography

A first construct of BCL6 BTB domain, which we will refer to as Trx-6His-HRV3C-BCL6, was obtained by sub-cloning the sequence coding for residues 5-129 of human BCL6, corresponding to its BTB domain, into a pET48b vector with N-terminal Thioredoxin and 6-Histidine tags, followed by a HRV-3C protease cleavage site. For the TR-FRET assay, the Trx-6His-HRV3C-BCL6 protein construct was used without cleaving the tag, as a 6His was needed to bind to the anti-6His-Terbium antibody. For the FP assay and crystallography with compounds 4, 5, 6, 11a, 11f, 23d and 25b, the tag was removed by HRV-3C protease treatment, generating a simpler BCL6 5-129 product.

For crystallography with compounds 17a, 25a and 1, the construct described above was modified to introduce a Flag Tag and a TEV cleavage site between the HRV3C and BCL6 sequences. This construct will be referred to as Flag-TEV-BCL6.

1.2 BCL6 expression

For both plasmid constructs described above, transformed BL21-AI E. coli cells were grown in LB media supplemented with 50 mg/L kanamycin at 37 ^{o}C until an OD600 nm of 0.6 was reached. Protein expression was then induced by addition of 0.2 mM IPTG and 0.2 % Arabinose. Expression was carried out at 18 ^{o}C for 18 hours. Cells were harvested by centrifugation (5500 g for 30 minutes at 4 ^{o}C) and stored at -80 ^{o}C.

1.3 BCL6 purification

Cells were re-suspended in a buffer composed of 20 mM Tris pH 8, 250 mM NaCl, 1 mM $MgCl_2$, 0.5 mM TCEP and 5 % glycerol, 1x cOmplete™ ULTRA protease inhibitors and 12.5 U/ml Benzonaze. Cells were lysed by sonication followed by centrifugation at 21,000 g for 45 minutes at 4 ^{o}C. The supernatant

was loaded onto a HisTrap FF column followed by on-column cleavage of the Trx-6His-HRV3C tag by addition of 2 mg of HRV-3C protease. The cleaved BCL6 5-129 BTB domain or Flag-TEV-BCL6 5-129 was then eluted and purified further by ResourceQ (for Flag-TEV-BCL6 construct only) and gel filtration using a HiLoad 26/60 Superdex75 column in a buffer containing 20 mM HEPES pH 7.5, 250 mM NaCl, 1 mM TCEP and 5 % glycerol. The final protein was assessed for purity and molar mass by SDS-PAGE and high-resolution mass spectrometry, respectively.

For the uncleaved Trx-6His-HRV3C-BCL6 protein construct to be used in TR-FRET, the protein was directly eluted from the HisTrap FF column without HRV-3C treatment, and submitted to Superdex75 gel filtration as described above.

1.4 BCL6 crystallisation

The purified BCL6 5-129 was crystallised in the presence of a tetra-peptide of sequence Ac-WVIP-NH_2. A stock solution of WVIP peptide at 100 mM in 100 % DMSO was added to a 2 mg/mL solution of purified BCL6 to a final concentration of 1 mM. This mixture was then concentrated to a final protein concentration of 4 mg/mL using a centrifugal concentrator with a 3 kDa molecular weight cut-off. Crystals were grown at 18 ^{o}C in hanging drops composed of 2 µL of the BCL6-BTB/WVIP complex plus 1 µL of a crystallisation solution consisting of 1 M K_2HPO_4, 0.7 M NaH_2PO_4, 75 mM sodium acetate buffer pH 4.5 and 2 % DMSO, against 350 µL of crystallisation solution. Crystals typically grew in 2 days, and compounds were soaked into crystals by addition of 0.5 µL of each compound (dissolved in DMSO to a final concentration of 10 to 200 mM) directly to crystallisation drops, followed by 10-120 minutes incubation. Crystals were then cryo-protected in a solution composed of the crystallisation reagent supplemented with 30 % ethylene glycol and cryo-cooled in liquid nitrogen.

The purified Flag-TEV-BCL6 5-129 was crystallised without any peptide, the supplementary Flag-TEV tag of this construct replacing the WVIP peptide in the crystal packing. The protein was concentrated to a final protein concentration of 10 mg/mL using a centrifugal concentrator with a 3 kDa molecular weight

cut-off. Crystals were grown at 18 ^{o}C in hanging drops composed of 1.5 µL of the Flag-TEV-BCL6 complex plus 1.5 µL of a crystallisation solution consisting of 0.1 M Tris pH 7.5 and 0.80 M Na/K Tartrate, against 300 µL of crystallisation solution. Crystals typically grew in 2 days, and compounds were soaked as described above for the other construct. Crystals were then cryo-protected in a solution composed of the crystallisation reagent supplemented with 30 % ethylene glycol and cryo-cooled in liquid nitrogen.

1.5 Crystallographic data collection, processing and refinement

X-ray data were collected at Diamond Light Source, Harwell campus, Oxfordshire, UK, on beamlines I03, and I04-1, and at ESRF, Grenoble, France, on beamline ID30A-1. Crystals obtained with both BCL6 constructs belonged to the space group P 6_1 2 2 and diffracted to between 1.43 and 2.36 Å resolution. Datasets were integrated with XDS[1] or DIALS[2] and scaled and merged with AIMLESS[3]. Structures were solved by molecular replacement using PHASER[4-5] with a publicly available BCL6 structure[6] (PDB code 3BIM) with ligand and water molecules removed used as molecular replacement model. All protein/ligand structures were manually corrected and rebuilt in COOT[7] and refined with BUSTER[8] in iterative cycles. Ligand restraints were generated with GRADE[9] and MOGUL[10]. The quality of the structures was assessed with MOLPROBITY[11-12]. The data collection and refinement statistics are presented in Table S3.

2. Supplementary experimental: biological assay conditions

Cell lines were supplied by the German Collection of Microorganisms and Cell Cultures (DSMZ). Cell lines were authenticated by STR profiling using a GenePrint® 10 kit (Promega, Southampton, UK) and a 3730xl DNA analyser (Applied Biosystems, Warrington, UK). All STR profiles were >80% match (using ATCC or DSMZ matching algorithms) with the respective reference profile. Cells were routinely screened for *Mycoplasma*, using an in-house PCR-based assay (Universal Mycoplasma Detection Kit (30-1012K, ATCC, Manassas, VA, USA).

2.1 Fluorescence polarization (FP) assay

A modified BCOR peptide containing a C-terminal cysteine, labelled with Alexa Fluor 633 C5 maleimide (RSEIISTAPSSWVVPGP-Cys(AF633)-amide) was obtained from Cambridge Research Biochemical. Assay reactions of 10 µL final volume were performed in 384-well black Proxi Plus (Perkin Elmer) plates. Each complete reaction (a modified version from Ghetu[6] *et al* and Cerchietti[13] *et a*l) contained 20 mM Hepes/NaOH pH8.0, 150 mM NaCl, 0.5 mM tris(2-carboxyethyl)phosphine (TCEP), 0.05% Tween 20, 10 nM AF633-BCOR, 3 µM BCL6 BTB and 1-2% DMSO. Compounds and control (unlabelled BCOR peptide) were dispensed on Labcyte Echo550 acoustic dispenser. After 2 hours incubation at room temperature, plates were read on an Envision (Perkin Elmer) plate reader with an excitation and emission wavelengths of 620 nm and 688 nm, respectively. To determine the % inhibition from raw mP values on Dotmatics (Bishops Stortford, UK) or Prism (GraphPad Software, La Jolla, CA), test wells were normalised to control wells containing DMSO. We used as low signal control the signal obtained from the probe only in the absence of BCL6. IC_{50} were calculated using a sigmoidal dose-response (variable slope) four- parameter equation:

$$y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{1 + 10^{(\text{LogIC}_{50} - x)} * \text{Hill slope}}$$

2.2 TR-FRET assay

Assays were performed in a 384-well black Proxiplate (Perkin Elmer) containing 1 nM* Trx-6xHis-BCL6 (in house-produced, human BCL6 BTB domain covering amino-acid sequence 5-129), 300 nM BCOR-AF633 peptide (RSEIISTAPSSWVVPGP-Cys-AlexaFluor 633-amide, Cambridge Research Biochemical) and 0.5 nM anti-6xHis-Terbium cryptate (CisBio Bioassays, France), in assay buffer (25 mM Hepes pH8, 100 mM NaCl, 0.05% Tween20, 0.5 mM TCEP, 0.05% bovine serum albumin). Test compounds in DMSO or DMSO alone were added to the wells using an ECHO550 acoustic dispenser (Labcyte Inc) to give the appropriate test concentration in 0.7% v/v DMSO final. After 2 hours incubation at room temperature the plate was read on an Envision plate reader (Perkin Elmer) with 337 nm laser excitation, a first emission filter APC 665 nm and a second emission filter Europium 615 nm, or alternatively on a Pherastar FSX (BMG Labtech) plate reader equipped with 337 nm laser excitation filter, a first emission filter at 620 nm and a second emission filter at 665 nm. The % inhibition at each concentration was calculated by normalising FRET ratio to the appropriate high (DMSO with all reagents) and low (DMSO without BCL6) controls. IC_{50} values were determined using GraphPad Prism 6.0 or Dotmatics (Bishops Stortford, UK) software by fitting the normalised data to a sigmoidal four-parameter logistic fit equation.

*10 nM was used for early examples, indicated with an asterisk in supplementary table S2

2.3 NanoBRET assay

A cellular nano-Bioluminescence Resonance Energy Transfer (nanoBRET) assay (Promega NanoBRET Nano-Glo Detection System, catalogue number N1662) was used to detect inhibition of the BCL6-SMRT (also called NCOR2) corepressor protein-protein interaction. DNA encoding full length BCL6 and SMRT were inserted into pFC32K.NanoLuc and pFC14K.HaloTag vectors (Promega) to produce C-terminal tagged fusion proteins BCL6-nanoLuc and SMRT-HaloTag, respectively. HEK293T cells were plated ($5x10^5$) in T75 tissue culture flask and bulk transfected 48 hours later with Fugene 6

(Promega cat.# E2691) reagent and 18 µg total DNA plasmids encoding BCL6-nanoLuc as donor and SMRT-HaloTag as acceptor, at a donor:acceptor DNA ratio of 1:25. At 24 hr post-transfection, HEK293T cells were collected and stored in liquid nitrogen in 90% FBS (PAN Biotech UK) and 10% DMSO. At the time of assay, compounds (100nL/well) and NanoBRET 618 ligand (10nL of 1mg/ml stock solution per well) were dispensed in a dry 384-well NUNC white assay plate (ThermoScientific NUNC cat.#10080681) using Echo550 acoustic dispensing (Labcyte Inc.). Frozen transfected HEK293T cells were thawed, centrifuged and freezing medium was replaced by phenol red-free OptiMEM+4% FBS (Life Technology). The cell density was adjusted to $3x10^5$ cells/ml and 20 µL (6000 cells) were plated in each well containing test compounds (0.0125-50 µM) in DMSO or DMSO alone and 0.5 µg/ml NanoBRET 618 fluorescence ligand, in 0.55% v/v DMSO final concentration. Cells were incubated for 6 hr at 37 °C / 5% CO_2 then NanoBRET furimazine substrate (Promega) was added to give a final concentration of 10 µM. After a short centrifugation the plates were read on an Envision (Perkin Elmer) plate reader equipped with a LUM/D600 Dual mirror, Lum 450/40 nm bandpass and D605 nm longpass filters, with a 0.2 sec reading to determine the BRET ratio. Alternatively, plates were read on Pherastar FSX (BMG Labtech) equipped with BRET module LP610 nm (1^{st} emission filter) / 450-80 nm (2^{nd} emission filter). The % inhibition at each test concentration was calculated by normalising the BRET ratio to the appropriate high and low controls. The compound IC_{50}s were determined using Graphpad Prism 6.0 or Dotmatics software by fitting the normalised data to a sigmoidal four-parameter logistic fit equation.

2.4 Immunofluorescence-based BCL6 degradation assay

DC_{50} values (compound concentration at which 50% of endogenous BCL6 protein is degraded) were determined in SUDHL-4 cells (American Type Culture Collection) in an immunofluorescence-based assay using an InCell2200 high content imaging system (GE Healthcare). Briefly, 40 µL of lymphoma suspension cells cultured in RPMI 1640-10% FBS (Sigma-Aldrich or PAN Biotech UK Ltd) were plated on fibronectin (Sigma catalogue F1141) -coated 384 well Cell Carrier Ultra plate (Perkin Elmer catalogue 6057300) at $1.2x10^4$ cells/well. After 20 hours cell culture at 37 ºC/CO_2 incubator, compounds were

dispensed in the cell culture plate using ECHO550 acoustic dispenser (Labcyte, Inc.), as 8 point-concentration response (ranging from 5 nM to 10 μM) in 0.67% final DMSO concentration. Cells were incubated with compound for 2 hours at 37 ºC/CO_2 incubator followed by fixation in 4.5% formaldehyde (37% formaldehyde solution, Sigma catalogue F8775) at room temperature for 15 min. After fixing, cells were washed in 1xTBS (Tris Buffer Saline) using a Power Washer 384 (Tecan Group Ltd). Blocking and cell permeabilisation were performed by incubating the fixed cells for 1 hour at room temperature in 1xTBS, 5% BSA, 1% Triton X100, followed by three washes on PW384 plate washer. Primary and secondary antibodies were prepared in 1xTBS, 1% BSA, 0.2% Triton X100. BCL6 expression was detected by incubating the cells for 1h30 with BCL6 rabbit polyclonal antibody (Sigma Catalogue HPA004899) at 1:250, 0.8μg/ml, followed by 1 hour in chicken anti-Rabbit Alexa 488 conjugated antibody (Life Technology) at 1:500. After incubation in each antibody solution, cells were washed four times in 1xTBS-0.05% Tween20 on PW384 plate washer. Cells were finally incubated for 60 min with nuclear staining RedDot2 dye (Biotium) at 0.5x the stock concentration in 1xTBS. BCL6 expression in the absence or presence of compound was detected on InCell2200 with 20x objective and quantified on InCell Analyser 3.7.2 workstation (GE Healthcare). The % response at each concentration was calculated by normalising BCL6 expression in the presence of compound to the appropriate high (DMSO) and low (DMSO with 7 μM **CCT369260**) controls. The compound DC_{50} values were determined using GraphPad Prism 6.0 or Dotmatics (Bishops Stortford, UK) software by fitting the normalised data to a sigmoidal four-parameter logistic fit equation.

2.5 Mesoscale Discovery (MSD) Degradation Assay

An MSD assay was developed for screening of compounds to determine the degradation of endogenous BCL6 in OCI-Ly1 cells. Briefly, test compounds in DMSO or DMSO alone to a total of 1332.5 nL/well were dispensed to a 96-well Nunc™ Edge 2 plate (ThermoFisher Scientific, 267544) using an Echo® 550 acoustic dispenser (Labcyte Inc) to give the appropriate test concentration in 0.67% v/v DMSO final. OCI-Ly1 passaged in Iscove's Modified Dulbecco's Medium (IMDM, ThermoFisher Scientific,

12440053) supplemented with 10% heat-inactivated fetal bovine serum (Sera Plus, PAN Biotech, P30-3702) were added to the compound/DMSO at a density of 2.5 x 10^5 cells/mL in 200 µL media/ well. After 2 hours incubation at 37 °C + 5% CO_2 the plate was centrifuged at 300 x g for 5 minutes. Using a BioTek 405TS plate washer for all wash and aspiration steps, media was removed and cells were rinsed with PBS, centrifuged as before and PBS removed. Cells were lysed in 50 µL ice-cold lysis buffer at pH 7.4 containing 50 mM Tris-HCl, 150 mM NaCl, 1 % Triton X-100 (v/v), 1 mM PMSF, 1 mM activated sodium vanadate, 1 mM EDTA, phosphatase inhibitors 2 and 3 (1:50 dilution) and protease inhibitor cocktail (1:100 dilution). The plate was shaken briefly and incubated on ice for 5-10 minutes before being frozen overnight at -20 °C or continuing the assay. A 96-well MSD standard bind plate (MSD, L15XA-3) which was coated overnight with 1 µg/mL anti-human BCL6 goat antibody in PBS (R&D Systems, AF5046) was blocked in 3% BSA in TBS + 0.1% Tween-20 for 1 hour with shaking. Wells were emptied thoroughly before 40 µL/well of lysate was transferred from the cell plate. The MSD plate was incubated at RT for 1 hour with shaking then washed three times with TBS + 0.1 % Tween-20. Antibodies were prepared in TBS + 0.1% Tween-20 + 1% BSA. Anti-human BCL6 rabbit antibody (Cell Signalling Technology, 14895S) was added at 1:100 dilution at 25 µL/well. The plate was incubated and washed as before. MSD Sulfo-tag anti-rabbit detection antibody (MSD, R32AB-1) at 1:1000 dilution at 25 µL/well was added. The plate was incubated and washed as before and MSD Gold read buffer (MSD R92TG) at 1:1 dilution with water at 150 µL/well was added. The plate was read within 10 minutes on the MSD Quickplex reader. The % inhibition at each concentration was calculated by normalising the electrochemiluminescent signal to the appropriate high (DMSO) and low (CCT369260 at 2 µM) controls. DC_{50} values (compound concentration at which 50% of endogenous BCL6 protein is degraded) were determined using GraphPad Prism 6.0 or Dotmatics (Bishops Stortford, UK) software by fitting the normalised data to a sigmoidal four-parameter logistic fit equation.

2.6 Cell proliferation assay

Cells were seeded in 96-well culture plates at a density of 2000 cells/well in RPMI-1640 medium (Sigma-Aldrich) supplemented with 10% FBS (Gibco). Compounds were initially dispensed into 96-well U-bottom plates using an Echo 550 acoustic dispenser (Labcyte Inc.), then diluted in RPMI-1640 medium and transferred onto the cells. Cells were treated with 8 compound concentrations in duplicate, ranging from 1.07 nM to 10 μM, in a final DMSO concentration of 0.1% and final volume of 100 μl. Cells were incubated with compound for 14 days, with medium changes at days 3, 7 and 10 carried out as follows: fresh 96-well cell culture plates were prepared containing 100 μl medium plus compound at the assay concentrations (white plates were used on day 10 to optimise luminescence measurement). Assay plates containing cells were vortexed to mix and cell density in one control well was counted using a Coulter Z2 cell counter (Beckman Coulter). The volume of medium containing 2000 cells in the control well was calculated and this volume of cells was transferred from every well of the assay plates to the corresponding well of the fresh plates containing compound. After 14 days, CellTiter Glo reagent (Promega) was added to the medium in each well of the assay plate at a ratio of 1:2, mixed on a plate shaker, then incubated at room temperature for 10 minutes. Luminescence was measured using an Envision plate reader (Perkin Elmer) and the relative luminescence at each compound concentration, compared to DMSO alone, was calculated. GI50 were determined using a 4-parameter curve fit in Dotmatics (Bishops Stortford, UK).

2.7 17-day cell proliferation curves

Cell lines were seeded in RPMI-1640 medium (Sigma-Aldrich) supplemented with 10% FBS (Gibco) and treated with 1 μM CCT369260 or 0.01% DMSO vehicle control. Every three or four days, cell density was measured using a Coulter Z2 cell counter (Beckman Coulter) and cells were returned to the starting density in fresh medium with CCT369260 or DMSO control. Cumulative cell number was calculated from the cell density measured at each time point and normalised to the starting cell density.

3. Supplementary experimental: physicochemical assays

3.1 NMR solubility assay

9 µL of 10 mM DMSO stock solution was pipetted into one well of a 384 deepwell plate (Greiner, part-no. 781270), then 171 µL of HEPES buffer (20 mM HEPES [Sigma Aldrich, cat-no. H3375-250G], 150 mM NaCl, 0.5 mM TCEP, 10% D_2O) was pipetted into the same well and mixed by up-down pipetting 3 times to create a 0.5 mM solution or suspension containing 5% DMSO. It was then separated by centrifugation (1000 rpm for 1 min, Eppendorf 5810C). The plate was then sealed and incubated at room temperature for 20 hours, without shaking. The plate was centrifuged again for 1 minute at 1000 rpm on Eppendorf 5810R before 165 µL of the supernatant was transferred to a 3 mm NMR tube (Bruker, Part No. Z112272) using liquid handler SamplePro Tube SJ S (Bruker). The concentration of the solubilized compound in solubility sample is measured by quantitative ^{1}H-NMR using a single external standard (200 µM caffeine (Sigma, C1778) in PBS (pH 7.4) with 1% DMSO-d6).

The detail of the NMR method is as following: NMR data was collected on a Bruker Avance Neo 600 spectrometer equipped with a 5 mm TCI-CryoProbe. The ^{1}H spectrum was referenced to the internal deuterated solvent. The operating frequency for ^{1}H was 600 MHz. All NMR data were acquired at the temperature of 298 K. All data were acquired and processed using Bruker Topspin 4.0. The quantitative ^{1}H-NMR spectrum was acquired using a Bruker standard 1D lc1pngppsf2 pulse sequence with 32 scans. The sweep width was 6.2 ppm with O1P set to 8.8 ppm, and the FID contained 16k time-domain data points. Relaxation delay was set to 20 sec. Water signal was suppressed. [14-16]

3.2 HPLC solubility assay

10 µL of 10 mM DMSO stock solution was pipetted into a micro centrifuge tube (1.5 mL, Sarstedt part-no. 72.690.001) containing 990 µL of PBS buffer (pH 7.4, Sigma Aldrich, cat-no. 79382) and mixed for 5 seconds on vortex mixer (Grant-bio vortex mixer) to create 100 µM solution with 1% DMSO. Following

shaking of the suspension on a Bohdan Shaker at 500 rpm for 2 hours at room temperature (20 °C), it was separated by centrifugation (14000 rpm for 15 min, Eppendorf 5415C). 200 µL of the supernatant was transferred to a 2 mL Agilent vial containing 50 µL of DMSO (Sigma Aldrich, cat-no. 41640-100ML) and mixed for 5 seconds to avoid precipitation from the saturated solution.

The concentration of the solubilized compound in solubility sample is measured by HPLC with UV detection using an external standard which was prepared by pipetting 10 µL of the same batch of compound DMSO stock used in solubility sample preparation to 990 µL of DMSO.

The detail of the HPLC method is as following: chromatographic separation at 30°C is carried out over a 5 minute gradient elution method from 90:10 to 10:90 water:methanol (both modified with 0.1% formic acid) at a flow rate of 1.5 mL/min. Calibration curve is prepared by injecting 0.5, 2.5, and 5 µL of compound external standard. Compound solubility value is obtained by injecting 6.25 and 62.5 µL of compound solubility sample.

4. Supplementary experimental: in vitro DMPK assays

4.1 Microsomal clearance assay

Microsomal clearance was determined in female CD1 mice, female Sprague–Dawley rats, and mixed gender human liver microsomes obtained from BioIVT (Peterborough, U.K.) following incubation of 1 μM compound at 37 °C in 0.5 mg/mL microsomal protein, 3 mmol/L $MgCl_2$, 1 mmol/L NADPH, 2.5 mmol/L and 10 mmol/L phosphate buffer (pH 7.4) (all purchased from Sigma-Aldrich, Gillingham, U.K). Reactions were started by addition of the cofactors following 10 min preincubation of microsomes with test compound and were terminated at −1, 0, 5, 10, 15, and 30 min with three volumes of ice-cold methanol containing internal standard. Samples were centrifuged at 2800*g* for 30 min at 4 °C and the supernatants analyzed. Control incubations were prepared as above with omission of cofactors. Compound measurements were performed by LCMS on an Agilent quadrupole time-of-flight instrument (Agilent 6510) following separation with a 3 min gradient of 0.1% formic acid in methanol on a Kinetex C18, 50 × 2.1 mm 2.6 μm UPLC column (Phenomenex, Macclesfield, UK).

5. Supplementary experimental: in vivo PK and PD experiments

All procedures were in accordance with UK Home Office regulations under the Animals (Scientific Procedures) Act 1986, approved by The Institute of Cancer Research's Ethics Committee and in accordance with published guidelines[17]. Mice were allowed access to food and water *ad libitum*.

5.1 *In vivo* pharmacokinetic studies

All experiments were conducted in accordance with the UKCC guidelines for animal experimentations[17]. Animals were adapted to laboratory conditions for at least 1 week prior to dosing and were allowed food and water *ad libitum*. Compounds were administered iv or po (mouse, 0.1 mL/10 g in 10% DMSO, 5% tween 20 in saline); Blood samples were collected in heparinised capillaries from the tail vein at 8 time points over the 24 h post dose and frozen on collection together with a standard curve and quality controls spiked in control blood. Samples were reconstituted in a Water:MeOH mixture containing internal standard as previously described (Roberts et al, 2016). Following centrifugation, extracts were analyzed by multiple reaction monitoring of precursor and product ions by ESI-LCMS/MS on either a Waters (Milford, MA, USA) Xevo TQ-S or Sciex (Framingham, MA, USA) QTrap6500 following gradient separation with 0.1% formic acid and methanol on a Phenomenex (Macclesfield, UK) Kinetex C18 UPLC column (50 × 2.1 mm, 2.6 μM). Quantitation was carried out with an external calibration. Quality controls were included and were within 20% of nominal concentration.

Pharmacokinetic parameters were derived from noncompartmental analysis using Phoenix Pharsight Non compartmental analysis (model 200 and 201) version 6.3.

5.2 *In vivo* pharmacodynamic (PK/PD) studies

5.2.1 Preparation of OCI-Ly1 Cells

B cell lymphoma cells, OCI-Ly1 were grown in Iscove's Modified Dulbecco's Medium (IMDM) (#12440053, ThermoFisher Scientific, UK) supplemented with 15% fetal bovine serum (Sera Plus, #P30-

3702, PAN Biotech UK Limited. Cells were maintained at a density between 0.5-2 x 10^6 cells/mL and near-saturated cultures were subcultured 1:4 every 3-4 days.

5.2.2 Preparation of OCI-Ly1 xenograft model in mouse

To inject cells to mice a vehicle for injection was prepared containing 7 ml of serum–free IMDM and 7 mL of Matrigel®, (#354234, Corning® B.V., Netherlands) both chilled to 4°C. A total of 7 x10^8 cells were prepared as a single cell suspension in the 14 mL chilled vehicle. Cells were delivered to 60, six-week-old, female SCID mice (NOD.CB17-*Prkdc*^{scid}/J, Charles River, UK) at 10^7 per 200uL, subcutaneously, single site.

Twenty days post injection 48 tumour-bearing mice (between 0.5 and 0.8 cm^3) were selected for the study and grouped as follows:

1. CCT369260 15 mg/kg per os (n=24)

   Mice were then grouped n=3 for the following time points:

0.5, 2, 4, 6, 10, 12, 16 & 24 hours

2. Vehicle per os (n=24)

   Mice were then grouped n=3 for the following time points:

0.5, 2, 4, 6, 10, 12, 16 & 24 hours

5.2.3 Preparation of vehicle and compound for dosing

Complete vehicle was prepared in saline for injection (Fresenius Kabi, UK) containing 10% DMSO (#D2650, Sigma-Aldrich, UK) and 5% TWEEN® 20 (#P1379).

CCT369260 was dissolved in a pre-determined volume of DMSO (#D2650, Sigma-Aldrich, UK) and then divided equally into aliquots dedicated to each time point. To ensure compound solubility a pre-

determined volume of incomplete vehicle (saline containing 5% TWEEN® 20) was added, just prior to administration, at a final concentration of 1.5 mg/mL (final 10% DMSO). The aliquot was briefly vortexed. Both compound and vehicle were delivered orally (0.2 mL per 20 g mouse) using a gavage needle - mice were individually weighed and dosing volumes adjusted accordingly.

5.2.4 Tissue sampling

Blood (~1 mL) was taken from anaesthetized mice, via intracardiac puncture, using a 27 gauge needle and a syringe pre-coated with heparin (200 Units/mL), The heparinized blood was microcentrifuged for 2 minutes and ~200 uL of plasma was aspirated to a labelled tube, frozen on dry-ice and dedicated to pharmacokinetic analysis (PK). Each tumour was excised, divided into two halves, weighed and then snap frozen in liquid nitrogen, dedicating one half to PK analysis and the other half to pharmacodynamic analysis (PD). All frozen material was permanently stored in a -80°C freezer.

5.2.5 *Tumour lysis and Wes method*

Tumours were lysed in lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100 (v/v), 1 mM PMSF, 1 mM activated sodium vanandate, 1 mM EDTA, phosphatase inhibitors 2 and 3 (1:50m dilution) and protease cocktail (1:100 dilution)) using reinforced homogenizing tubes containing metal beads using a Precellys 24 with cryolys cooling at 6000 rpm, 2 × 20 s (Stretton Scientific). Lysates were incubated on ice for 15 minutes, spun at 14000 rpm at 4 °C for 10 minutes and supernatants collected and aliquoted. Samples were stored at -80 ^{o}C until use. Protein concentration was determined by diluting 1:10 with lysis buffer and measuring on Direct Detect® spectrometer according to manufacturer's instructions.

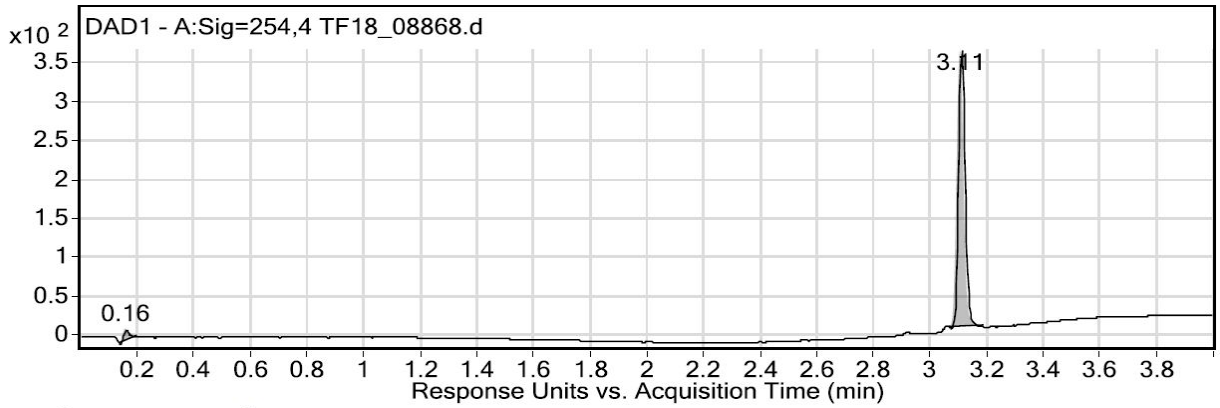
Protein lysates were diluted to 0.0875 mg/ml with 0.1x sample buffer and run on Wes 12-230 kDa kit according to manufacturer's instructions (ProteinSimple) with multiplexing BCL6 (CST14895 at 1:150 dilution) and GAPDH (CST 2118 at 1:50 dilution). Data was analysed using Compass for SW software. BCL6 data was normalised to GAPDH and transferred to GraphPad Prism 7.

6. Analytical data for CCT369260

6.1 LCMS method

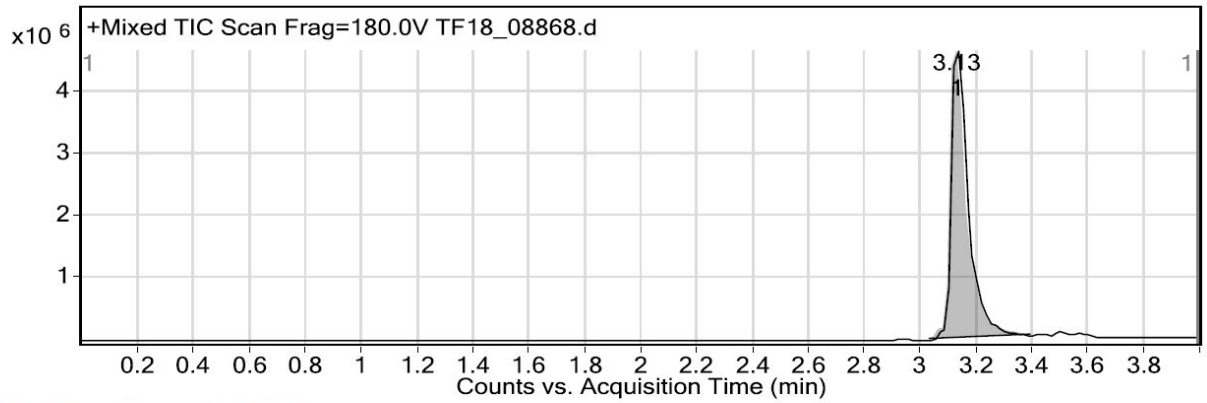
LC/MS and HRMS analysis for compounds shown below was performed on an Agilent 1200 series HPLC and diode array detector coupled to a 6210 time of flight mass spectrometer with dual multimode APCI/ESI source. Analytical separation was carried out at 30 ^{o}C on a Merck Chromolith Flash column (RP-18e, 25 x 2 mm) using a flow rate of 0.75 mL/min in a 4 minute gradient elution with detection at 254 nm. The mobile phase was a mixture of methanol (solvent A) and water (solvent B), both containing formic acid at 0.1%. Gradient elution was as follows: 5:95 (A/B) to 100:0 (A/B) over 2.5 min, 100:0 (A/B) for 1 min, and then reversion back to 5:95 (A/B) over 0.1 min, finally 5:95 (A/B) for 0.4 min.

6.2 HPLC and LCMS traces for CCT369260



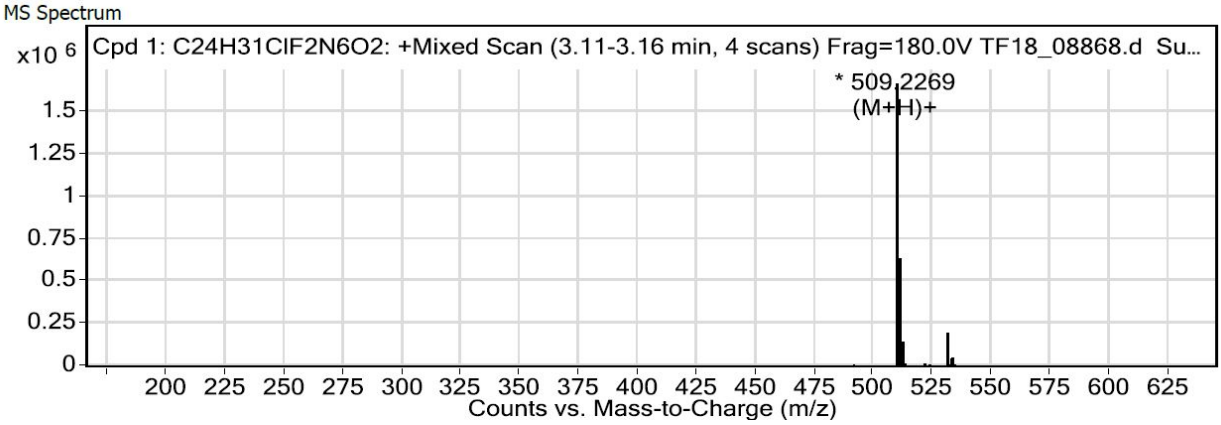
User Chromatogram Peak List

Peak #	RT (mins)	Area	Area Sum %
1	0.16	23.83	3.88
2	3.11	589.73	96.12



User Chromatogram Peak List

Peak #	RT (mins)	Area	Area Sum %	Base Peak m/z
1	3.13	19954505	100	509.2368



6.3 qNMR method

Sample Preparation

Around 2 mg of in-house compound or caffeine (Sigma reference standard, C1778) was accurately weighed (Mettler Toledo XP26 micro balance) and dissolved in 500 µL of deuterated DMSO (Euriso-top, D034T). 2 samples were prepared for each compound. 160 uL of the solution was transferred to 3 mm NMR tube (Bruker Z112272), 3 tubes were prepared from the same sample.

NMR experiment

NMR data was collected on a Bruker Avance NEO spectrometer equipped with 600MHz magnet and 5 mm TCI Cryoprobe. The ^{1}H spectrum was referenced to the internal deuterated solvent. The operating frequency for ^{1}H was 600 MHz. All NMR data were acquired at the temperature of 298 K. All data were acquired and processed using Bruker Topspin 4.0. The quantitative 1H-NMR spectrum (@qHNMR3) was acquired using a Bruker standard 1D zg pulse sequence with 32 scans. The sweep width was 19.8 ppm, and the FID contained 64k time-domain data points. Relaxation delay was set to 20 sec. The average value of the integrals in the aromatic region (7-9 ppm) was used to obtain the absolute concentration of the compound. Quantitative Data was analysed using Topspin 4.0.5, Eretic function. For each sample, three NMR tubes were prepared as described above, and the averaged value was used to obtain the purity value for each sample. The average purity of the two samples was quoted as the final compound purity. The purity of caffeine control samples (98.3%) was consistent with its specification (98.5-101.0%). The SD between three NMR tubes was less than 3% and the SD between two samples was 0.2%.

6.4 qNMR analysis for CCT369260

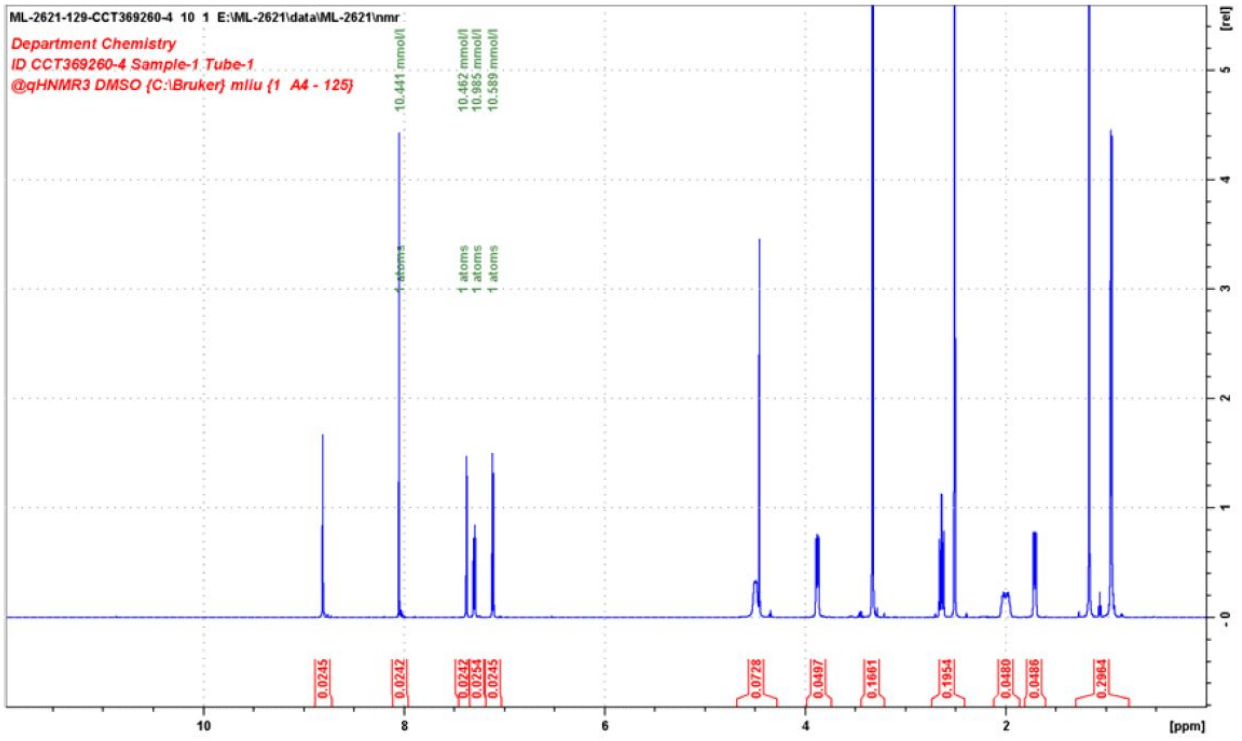
Purity analysis for caffeine control

Compound	Batch	Sample	tube	Weight (mg)	MW	Volume (uL)	Calc Conc. (mM)	qNMR Conc. (mM)	Purity (%)	Average	SD	Average	SD
Caffeine	SLBT7668	1	1	2.152	194.14	500	22.170	22.359	100.9%				
Caffeine	SLBT7668	1	2	2.152	194.14	500	22.170	21.891	98.7%	98.1%	2.6%		
Caffeine	SLBT7668	1	3	2.152	194.14	500	22.170	20.977	94.6%			**98.3 %**	**0.2 %**
Caffeine	SLBT7668	2	1	2.704	194.14	500	27.856	27.47	98.6%				
Caffeine	SLBT7668	2	2	2.704	194.14	500	27.856	27.373	98.3%	98.5%	0.2%		
Caffeine	SLBT7668	2	3	2.704	194.14	500	27.856	27.489	98.7%				

Purity analysis for CCT369260

Compound	Batch	Sample	tube	Weight (mg)	MW	Volume (uL)	Calc Conc. (mM)	qNMR Conc. (mM)	Purity (%)	Average	SD	**Average**	**SD**
CCT369260	4	1	1	2.76	508.99	500	10.845	10.619	97.9%	97.1%	0.6%	**96.9%**	**0.1%**
CCT369260	4	1	2	2.76	508.99	500	10.845	10.478	96.6%				
CCT369260	4	1	3	2.76	508.99	500	10.845	10.481	96.6%				
CCT369260	4	2	1	2.164	508.99	500	8.503	8.249	97.0%	96.8%	1.0%		
CCT369260	4	2	2	2.164	508.99	500	8.503	8.329	98.0%				
CCT369260	4	2	3	2.164	508.99	500	8.503	8.118	95.5%				

Example ^{1}H NMR spectrum used in CCT369260 qNMR analysis



7. Supplementary data tables

Supplementary Table S1: summary statistics and individual replicate values

Compound number	BCL6 FP IC_{50} Geometric Mean (μM)	BCL6 FP IC_{50} individual replicates (μM)	BCL6 FP pIC_{50} mean	BCL6 FP pIC_{50} SD	BCL6 FP pIC_{50} SEM	n
2	120	110, 105, 165, 97, 127, 127	3.92	0.08	0.03	6
5	207	208, 207	3.68	N/A	N/A	2
3	70	53, 57, 114	4.15	0.18	0.11	3
4	86	86	4.07	N/A	N/A	1
6 (CCT365386)	10.5	14, 13, 6.8, 13, 9.3, 8.6	4.98	0.13	0.05	6
BCOR peptide control	12.2	9.7, 9.3, 8.4, 11, 16, 17, 15, 15, 13, 16, 11, 13, 12, 12, 11	4.91	0.09	0.024	15

Supplementary Table S2: summary statistics and individual replicate values, including TR-FRET data for compounds 2-5.

Compound number	BCL6 TR-FRET IC_{50} Geometric Mean (μM)	BCL6 TR-FRET IC_{50} individual replicates (μM)	BCL6 TR-FRET pIC_{50} mean	BCL6 TR-FRET pIC_{50} SD	BCL6 TR-FRET pIC_{50} SEM	LE	LLE	n
1 (CCT369260)	0.52	0.42, 0.46, 0.24, 0.74, 0.67, 0.19, 0.98, 0.73, 0.31, 0.55, 1.54	6.28	0.27	0.08	0.25	1.9	11
2	69	57*, 64*, 82*, 75*	4.16	0.07	0.03	0.34	1.3	4
3	49	41*, 46*, 32*, 98*	4.31	0.21	0.09	0.22	0.23	4
4	100	100*	4.00	-	-	0.27	2.1	1
5	61	61*	4.22	-	-	0.42	2.2	1
6 (CCT365386)	3.42	1.7, 2.23, 9.38, 3.32, 3.23*, 4.22*	5.47	0.26	0.10	0.35	3.2	6
11a	5.42	5.56, 5.37, 5.33	5.27	0.01	0.01	0.28	2.6	3
11b	3.40	3.15, 3.57, 3.48	5.47	0.03	0.02	0.28	2.3	3
11c	4.56	5.11, 4.27, 4.34	5.34	0.04	0.03	0.29	2.7	3
11d	4.55	5.86, 4.74, 3.39	5.34	0.12	0.07	0.29	2.7	3
11e	6.55	5.56, 8.01, 6.32	5.18	0.08	0.05	0.29	1.6	3

Compound number	BCL6 TR-FRET IC_{50} Geometric Mean (μM)	BCL6 TR-FRET IC_{50} individual replicates (μM)	BCL6 TR-FRET pIC_{50} mean	BCL6 TR-FRET pIC_{50} SD	BCL6 TR-FRET pIC_{50} SEM	LE	LLE	n
11f	1.67	1.56, 1.48, 2.03	5.78	0.07	0.04	0.31	3.6	3
11g	3.93	3.35, 3.78, 4.78	5.41	0.08	0.05	0.29	3.2	3
17a	0.86	0.91, 0.79, 0.87	6.07	0.03	0.02	0.32	3.7	3
17b	1.36	1.48, 1.45, 1.17	5.87	0.06	0.03	0.32	2.7	3
17c	0.99	0.89, 0.93, 0.82, 1.19	6.00	0.07	0.04	0.34	3.7	4
17d	2.47	1.88, 2.94, 2.74	5.61	0.10	0.06	0.29	3.3	3
17e	2.12	2.15, 2.29, 1.95	5.67	0.03	0.02	0.31	2.6	3
17f	37	41.11, 36.18, 22.65, 53.88	4.44	0.16	0.08	0.24	1.5	4
17g	0.68	0.65, 0.73, 0.67	6.17	0.03	0.01	0.32	2.9	3
18	1.86	1.88, 2.23, 1.53	5.73	0.08	0.05	0.31	2.7	3
19	1.16	1.12, 0.94, 1.49	5.94	0.10	0.06	0.30	3.1	3
20a	0.27	0.28, 0.2, 0.34	6.57	0.11	0.06	0.30	4.0	3
20b	1.04	0.98, 1.08, 0.94, 1.21	5.98	0.06	0.03	0.27	2.2	4
21	1.40	1.72, 1.02, 1.56	5.85	0.12	0.07	0.26	2.8	3

Compound number	BCL6 TR-FRET IC_{50} Geometric Mean (μM)	BCL6 TR-FRET IC_{50} individual replicates (μM)	BCL6 TR-FRET pIC_{50} mean	BCL6 TR-FRET pIC_{50} SD	BCL6 TR-FRET pIC_{50} SEM	LE	LLE	n
22	0.48	0.62, 0.36, 0.5	6.32	0.12	0.07	0.29	4.0	3
23a	0.71	0.83, 0.72, 0.59	6.15	0.07	0.04	0.29	3.2	3
23b	0.40	0.37, 0.43, 0.34, 0.4	6.40	0.03	0.02	0.29	3.2	4
23c	0.43	0.22, 0.33, 0.43, 0.56	6.37	0.12	0.07	0.29	3.3	4
23d	0.26	0.29, 0.27, 0.22	6.59	0.06	0.03	0.29	3.2	3
24a	0.64	0.81, 0.89, 0.36	6.19	0.21	0.12	0.27	2.1	3
24b	0.76	0.99, 0.65, 0.68	6.12	0.10	0.06	0.26	1.7	3
25a	0.15	0.15, 0.15, 0.17	6.81	0.04	0.02	0.29	3.5	3
25b	0.10	0.1, 0.09, 0.13, 0.08	7.01	0.10	0.05	0.28	3.3	4
25c	0.19	0.19, 0.16, 0.23	6.72	0.07	0.04	0.28	3.1	3
26a	0.79	0.76, 0.81, 0.81	6.10	0.02	0.01	0.27	2.6	3
26b	3.49	3.87, 3.35, 3.28	5.46	0.04	0.02	0.23	0.9	3
26c	0.93	0.93, 0.86, 1.02	6.03	0.04	0.02	0.26	2.6	3
27a	0.40	0.39, 0.63, 0.26	6.40	0.19	0.11	0.26	2.1	3

Compound number	BCL6 TR-FRET IC_{50} Geometric Mean (μM)	BCL6 TR-FRET IC_{50} individual replicates (μM)	BCL6 TR-FRET pIC_{50} mean	BCL6 TR-FRET pIC_{50} SD	BCL6 TR-FRET pIC_{50} SEM	LE	LLE	n
27b	0.31	0.33, 0.27, 0.34	6.51	0.05	0.03	0.28	2.8	3
27c	0.33	0.33, 0.37, 0.29	6.49	0.05	0.03	0.27	2.4	3
27d	0.13	0.12, 0.11, 0.17	6.88	0.09	0.05	0.29	4.0	3
27e	0.09	0.05, 0.09, 0.1, 0.11	7.07	0.13	0.07	0.28	4.4	4
27f	0.20	0.21, 0.2, 0.2	6.69	0.01	0.01	0.26	3.6	3
27g	0.39	0.36, 0.5, 0.34	6.40	0.09	0.05	0.27	3.1	3
27h	0.96	0.77, 0.98, 1.19	6.02	0.10	0.06	0.24	1.6	3
28a	0.71	1.01, 0.47, 0.75, 0.71	6.15	0.13	0.07	0.25	2.1	4
28b	0.23	0.27, 0.24, 0.19	6.63	0.07	0.04	0.27	2.4	3

***** *for early examples, indicated with an asterisk, TR-FRET assay was run using 10 nM BCL6 instead of 1 nM. LE calculated using 1.4*(pIC50)/HAC. LLE calculated using AlogP values.*

Supplementary Table S3: Crystallographic data collection and refinement statistics.

Protein construct	BCL6 5-129	BCL6 5-129	BCL6 5-129
Ligand	**4**	**5**	**6 (CCT365386)**
PDB code	6TOF	6TOG	6TOH
Crystal			
Space group	P 6_1 2 2	P 6_1 2 2	P 6_1 2 2
Unit cell dimensions (a/b/c in Å)	66.22/66.22/151.27	67.33/67.33/154.40	67.79/67.79/165.98
Unit cell angles (α/β/γ in °)	90/90/120	90/90/120	90/90/120
Data collection and processing			
Beamline	DLS I03	DLS I03	ESRF ID30A-1
Wavelength (Å)	0.9763	0.9763	0.9660
Integration program	DIALS	XDS	XDS
Reduction program	AIMLESS	AIMLESS	AIMLESS
Resolution range	37.87 – 1.67	29.15 – 1.69	47.93 – 1.58
Number of unique reflections [a]	23741 (1190)	24088 (1197)	31907 (3048)
Completeness [a]	100 (100)	100 (100)	100 (100)
Redundancy [a]	14.9 (13.7)	36.7 (35.2)	18.8 (19.4)
R_{merge} (%) [a]	16.5 (421.2)	15.6 (355.9)	7.3 (211.2)
I/σ(I) [a]	8.2 (0.5)	16.9 (1.4)	21.0 (1.7)
$CC_{1/2}$ [a, b]	0.994 (0.454)	0.999 (0.434)	0.999 (0.677)
Refinement			
Program	BUSTER	BUSTER	BUSTER
R_{work} (%)	18.25	23.13	17.28
R_{free} (%)	21.56	25.01	19.72
Number of residues	130	130	130
Number of water molecules	119	72	171
Average B-factor (Å^2)	35.92	41.52	36.29
Ramachandran favoured (%)	99.20	98.40	96
Ramachandran outliers (%)	0	0	0
RMSD bonds (Å)	0.014	0.014	0.014
RMSD angles (°)	1.627	1.714	1.510

[a] Values in parentheses are for the highest resolution shell.

[b] Half-dataset correlation coefficient, see: Karplus, P. A.; Diederichs, K. Linking crystallographic model and data quality. *Science* **2012**, *336*, 1030−1033.

Protein construct	BCL6 5-129	BCL6 5-129	Flag-TEV-BCL6
Ligand	**11a**	**11f**	**17a**
PDB code	6TOO	6TOI	6TOJ
Crystal			
Space group	P 6_1 2 2	P 6_1 2 2	P 6_1 2 2
Unit cell dimensions (a/b/c in Å)	67.78/67.78/152.29	67.66/67.66/166.64	67.00/67.00/166.73
Unit cell angles (α/β/γ in °)	90/90/120	90/90/120	90/90/120
Data collection and processing			
Beamline	ESRF ID30A-1	ESRF ID30A-1	DLS I04-1
Wavelength (Å)	0.9660	0.9660	0.9159
Integration program	XDS	XDS	XDS
Reduction program	AIMLESS	AIMLESS	AIMLESS
Resolution range	47.85 – 1.53	55.28 – 1.58	47.62 – 1.85
Number of unique reflections [a]	34884 (1686)	31728 (1556)	19812 (1185)
Completeness [a]	100 (100)	99.9 (99.5)	100 (100)
Redundancy [a]	18.0 (17.9)	12.4 (11.9)	24.8 (25.5)
R_{merge} (%) [a]	13.3 (298.4)	9.1 (76.9)	13.8 (344.9)
I/σ(I) [a]	16.6 (1.6)	16.9 (3.3)	13.2 (1.3)
$CC_{1/2}$ [a, b]	0.998 (0.685)	0.999 (0.352)	0.999 (0.570)
Refinement			
Program	BUSTER	BUSTER	BUSTER
R_{work} (%)	17.58	16.33	18.54
R_{free} (%)	19.45	18.38	20.48
Number of residues	131	131	131
Number of water molecules	186	156	107
Average B-factor (Å^2)	28.55	29.54	44.08
Ramachandran favoured (%)	98.41	98.41	96.88
Ramachandran outliers (%)	0	0	0
RMSD bonds (Å)	0.015	0.014	0.014
RMSD angles (°)	1.536	1.623	1.566

[a] Values in parentheses are for the highest resolution shell.

[b] Half-dataset correlation coefficient, see: Karplus, P. A.; Diederichs, K. Linking crystallographic model and data quality. *Science* **2012**, *336*, 1030−1033.

Protein construct	BCL6 5-129	BCL6 5-129	Flag-TEV-BCL6
Ligand	**23d**	**25b**	**25a**
PDB code	6TOK	6TON	6TOL
Crystal			
Space group	P 6_1 2 2	P 6_1 2 2	P 6_1 2 2
Unit cell dimensions (a/b/c in Å)	68.59/68.59/168.73	67.50/67.50/170.05	67.14/67.14/167.45
Unit cell angles (α/β/γ in °)	90/90/120	90/90/120	90/90/120
Data collection and processing			
Beamline	DLS I04-1	ESRF ID30A-1	DLS I04-1
Wavelength (Å)	0.9282	0.9660	0.9159
Integration program	DIALS	DIALS	XDS
Reduction program	AIMLESS	AIMLESS	AIMLESS
Resolution range	59.40 – 1.43	48.17 – 2.36	47.76 – 1.64
Number of unique reflections [a]	44308 (2200)	10122 (1011)	28338 (1345)
Completeness [a]	100 (100)	99.8 (98.8)	100 (100)
Redundancy [a]	12.4 (10.7)	14.5 (15.5)	23.6 (19.9)
R_{merge} (%) [a]	6.1 (308.7)	29.4 (320.8)	6.9 (309.0)
I/σ(I) [a]	14.6 (0.7)	7.6 (1.4)	23.2 (1.0)
$CC_{1/2}$ [a, b]	0.999 (0.343)	0.996 (0.411)	1.000 (0.544)
Refinement			
Program	BUSTER	BUSTER	BUSTER
R_{work} (%)	18.24	19.47	18.13
R_{free} (%)	18.72	23.22	20.47
Number of residues	131	131	131
Number of water molecules	178	86	161
Average B-factor (Å^2)	36.52	46.66	41.11
Ramachandran favoured (%)	98.37	96.83	98.45
Ramachandran outliers (%)	0	0	0
RMSD bonds (Å)	0.016	0.015	0.014
RMSD angles (°)	1.679	1.746	1.638

[a] Values in parentheses are for the highest resolution shell.

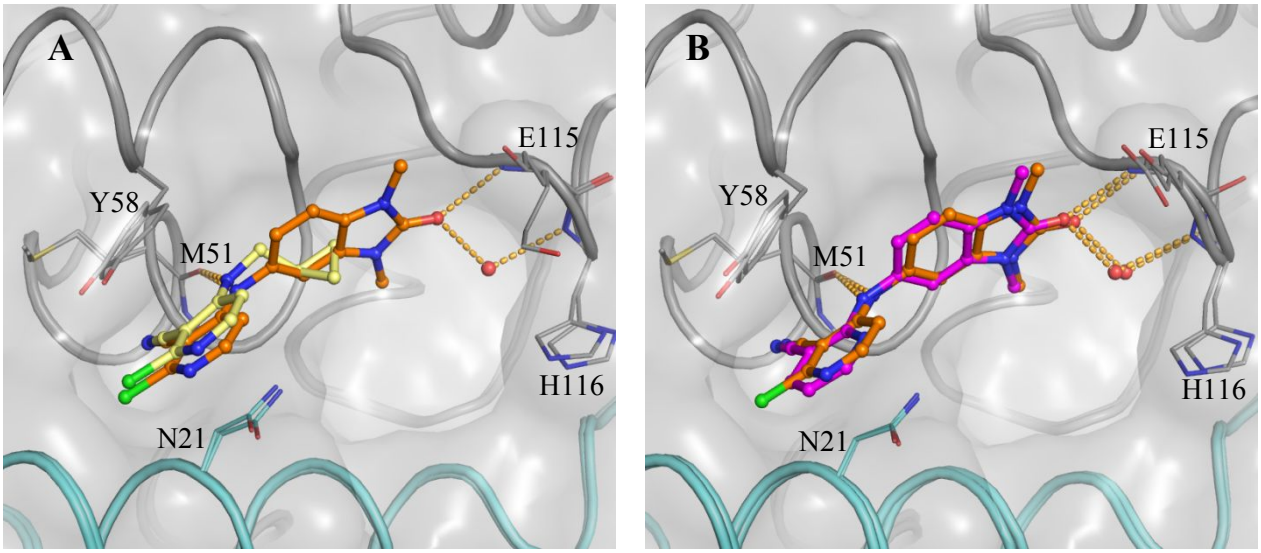
[b] Half-dataset correlation coefficient, see: Karplus, P. A.; Diederichs, K. Linking crystallographic model and data quality. *Science* **2012**, *336*, 1030−1033.

Protein construct	Flag-TEV-BCL6
Ligand	**1 (CCT369260)**
PDB code	6TOM
Crystal	
Space group	P 6_1 2 2
Unit cell dimensions (a/b/c in Å)	67.28/67.28/166.38
Unit cell angles (α/β/γ in °)	90/90/120
Data collection and processing	
Beamline	DLS I04-1
Wavelength (Å)	0.9159
Integration program	DIALS
Reduction program	AIMLESS
Resolution range	47.72 – 1.90
Number of unique reflections [a]	18447 (1159)
Completeness [a]	100 (100)
Redundancy [a]	12.0 (12.7)
R_{merge} (%) [a]	9.6 (196.2)
I/σ(I) [a]	11.4 (0.9)
$CC_{1/2}$ [a, b]	0.998 (0.558)
Refinement	
Program	BUSTER
R_{work} (%)	19.69
R_{free} (%)	21.81
Number of residues	131
Number of water molecules	95
Average B-factor (Å^2)	49.90
Ramachandran favoured (%)	97.67
Ramachandran outliers (%)	0
RMSD bonds (Å)	0.015
RMSD angles (°)	1.611

[a] Values in parentheses are for the highest resolution shell.

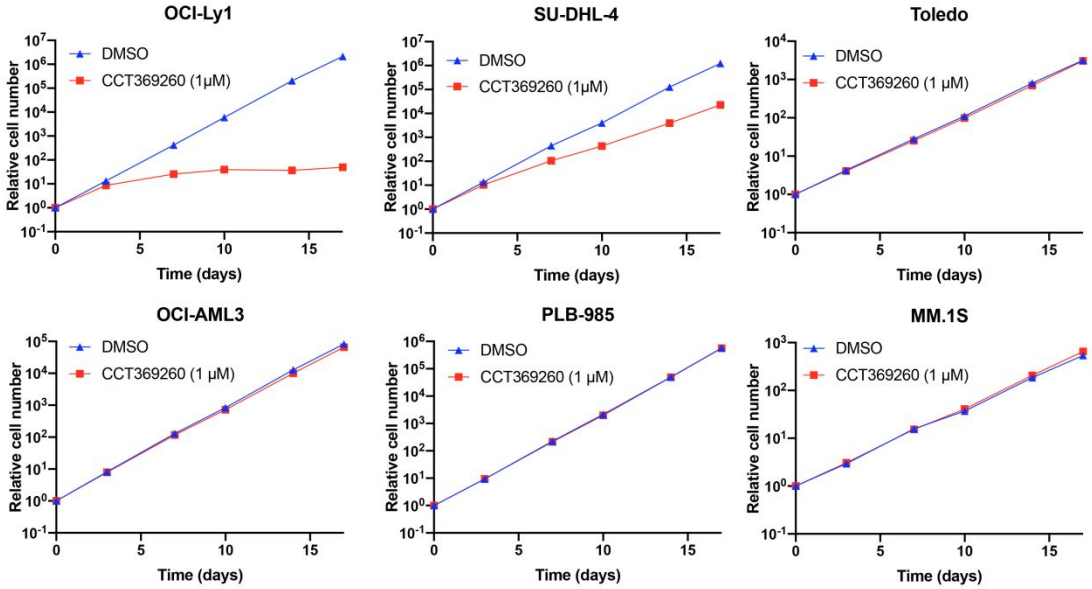
[b] Half-dataset correlation coefficient, see: Karplus, P. A.; Diederichs, K. Linking crystallographic model and data quality. *Science* **2012**, *336*, 1030−1033.

8. Supplementary figures



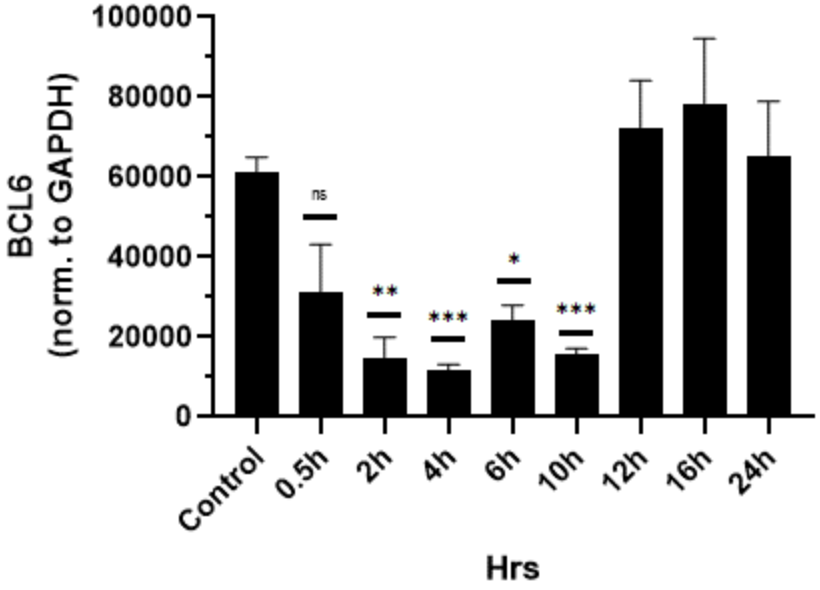
Supplementary Figure S1:

Overlays of X-ray structure of compound ***6*** *(PDB code 6TOH) with that of* ***5*** *(A, PDB code 6TOG) and* ***4*** *(B, PDB code 6TOF) to show consistent binding mode.*



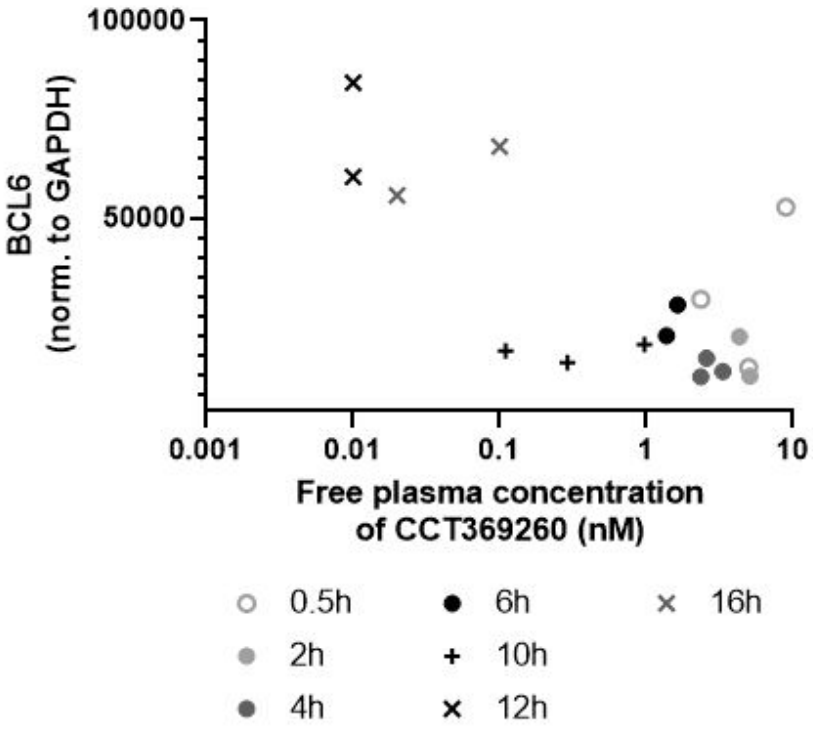
Supplementary Figure S2:

17-day cell proliferation curves for BCL6-dependent (OCI-Ly1, SU-DHL-4) and BCL6-independent (Toledo, OCI-AML3, PLB-985, MM.1S) cell lines. Cells were treated with 1 μM CCT369260 or DMSO vehicle control for 17 days and relative cell number was calculated.



Supplementary Figure S3:

PD effect of compound ***1*** *(CCT369260) on tumour BCL6 levels following a single 15 mg/kg po dose. Experimental details are described in Supplementary Experimental section 5 above, and in the legend for Figure 7. BCL6 levels in tumour were quantified using capillary electrophoresis and normalised to a GAPDH loading control. The significance (* p≤0.05, ** p≤0.01, *** p≤0.001) values for changes in BCL6 levels in treated samples (n=3 at each time point except 2, 6, 12h when n=2) are described at each time point relative to the vehicle control (n=21, 0 – 24h), and were analysed using a one-way ANOVA (p < 0.0001) and Dunnett's multiple comparisons test. All experiments were carried out according to the UK guidelines for animal experimentation. All data were analysed and plotted using GraphPad Prism.*



Supplementary Figure S4:

PK/PD relationship between free plasma concentration of compound ***1*** *(CCT369260) and tumour BCL6 levels following a single 15 mg/kg po dose. Experimental details are described in Supplementary Experimental section 5 above, and in the legend for Figure 7. BCL6 levels in tumour were quantified using capillary electrophoresis and normalised to a GAPDH loading control. At 0.5 h (open circles), free concentration is high but incomplete suppression of BCL6 levels is observed, consistent with a short time-lag in PD effect onset due to compound distribution to tumour and/or time required for degradation of target. At 10h (+ shape), free concentration has dropped below the expected level required for degradation, but low levels of BCL6 are still observed, consistent with a sustained PD effect due to the time required for resynthesis of BCL6 following degradation.*

9. References for supplementary information

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