## ONLINE SUPPORTING INFORMATION

# Fragment-Based Discovery of Selective Type I Inhibitors of Maternal Embryonic Leucine Zipper Kinase

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**Contents**

## 2. Kinase Inhibitory Assays

### 2.1 MELK Inhibition Assay performed at Astex ($IC_{50}$ determination)

Inhibition of MELK kinase activity was measured using a radioactive filter binding assay. Briefly, each assay well contained 1.25 nM MELK (human, residues 1 - 340) 10 µM ATP, 6.7 uCi/mL $\gamma^{33}$P-ATP (Specific activity 3000 Ci/mmol-Perkin Elmer), 3 µM biotinylated ZIP-tide peptide (Biotin-KKLNRTLSFAEPG; Bachem) in 30 µL reaction buffer (25 mM Tris pH 7.5, 10 mM $MgCl_2$, 1 mM DTT, 1 mM EGTA, 0.1% Triton X100). Kinase reactions were performed for 25 minutes at room temperature before stopping with 40 µL 2% orthophosphoric acid. Unbound radioactivity was removed by filtering the reaction through a MAPH filter plate (Millipore). The trapped $^{33}$P labelled peptide was then washed twice with 200 µL 0.5% orthophosphoric acid, 20 µL Microscint-20 added per well and the amount of radioactivity determined by scintillation counting using a Topcount (Packard) . To calculate compound $IC_{50}$, semi-log serial dilutions were used to produce 8-point dose-response curves in duplicate. $IC_{50}$ values were then derived using the four parameter logistic fit method in GraphPad Prism 5.0.

N-(4-Methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)-4-[(4-methylpiperazin-1-yl)methyl]benzamide was used as the assay standard. Using the above protocol, an $IC_{50}$ value (geometric mean) of 11 µM was obtained ($pIC_{50}$ = 4.924 ± 0.287, N = 38). All $IC_{50}$ and % inhibition data on compounds **1** – **7** (see **Table 2.1**) represent the geometric mean of 2 independent duplicate experiments, except where otherwise stated.

### 2.2 MELK Inhibition Assay Performed at Millipore ($IC_{50}$ determination)

MELK protein was incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 250 µM KKLNRTLSFAEPG, 10 mM magnesium acetate and [$\gamma$-$^{33}$PATP] (specific activity approx. 500 cpm/pmol). After incubation for 40 minutes at room temperature, the reaction was stopped by the addition of 3% phosphoric acid solution. 10 µL of the reaction was then spotted onto a P30 filtermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting. To calculate compound $IC_{50}$, semi-log serial dilutions were used to produce 8-point dose-response curves in duplicate. $IC_{50}$ values were then derived using the four parameter logistic fit method in GraphPad Prism 5.0.

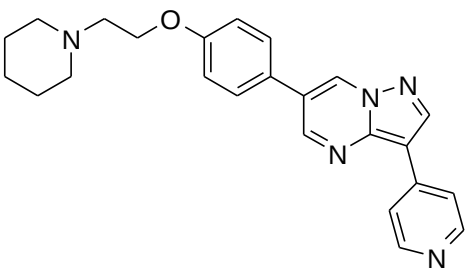
**Table 2.1: MELK Inhibition Data for Compounds 1 - 7**

| Compound | $IC_{50}$ µM | Geometric Mean $IC_{50}$ µM | % Inhibition |
|---|---|---|---|
| **1** | 160 | (N = 1) | 29% at 50 µM |
| | | | 91% at 500 µM |
| **2** | 2.1 | 2.1 | 74% at 10 µM |
| | 2.1 | | |
| **3** | 4.6 | 4.2 | |
| | 3.8 | | |
| **4** | 1.1 | 1.0 | |
| | 0.99 | | |
| **5** | 31 | (N = 1) | |
| | | | |
| **6** | 0.38 | 0.41 | 68% at 1 µM |
| | 0.44 | | |
| **7** | 0.041 | 0.037 | 67% at 0.1 µM |
| | 0.033 | | |

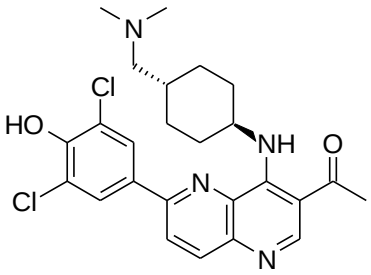
**2.3 Kinase Cross Screening Profile Performed at Millipore (% Inhibition and $IC_{50}$).** The experiments were conducted by Merck Millipore under their KinaseProfile™ service. Kinase assays were performed according to the published protocol (http://www.millipore.com/techpublications/tech1/pf3036) using peptide or protein substrates in the presence of 10 µM ATP in a filter-binding radioactive ATP transferase assay. Inhibition was first determined at 1 µM concentration of the inhibitor. A total of 235 kinases were screened; when $\geq$ 50% inhibition was observed at 1 µM, $IC_{50}$ values were determined in a five-point dose-response curve. Definition of abbreviations used in Table 2: Flt3, Fms-like tyrosine kinase 3; CAMKIIδ, calcium/calmodulin-dependent protein kinase IIδ; Mnk2, mitogen-activated protein kinase–interacting kinase 2; CAMKIIγ, calcium/calmodulin-dependent protein kinase IIγ; MLCK, myosin light-chain kinase; AMPKα1, AMP-activated protein kinase α1; AMPKα2, AMP-activated protein kinase α2. Kinase cross screening data on Dorsomorphin and OTSSP167 (below) are published by Millipore and are available via the following links:

http://www.kinase-screen.mrc.ac.uk/screening-compounds/341053

http://www.kinase-screen.mrc.ac.uk/screening-compounds/594372



**Dorsomorphin**



**OTSSP167**

### 3. Ba/F3 Cell Line Assays

**Ba/F3-Flt3 proliferation assay in the absence or presence of IL3.** Compounds dissolved in DMSO were sprayed into costar 3712 384-well plates (100 nL/well). A suspension of Ba/F3-Flt3 cells (courtesy of Prof. Jan Cools, University of Leuven, Belgium) was added (20,000 cell/well), followed by the addition of 10 ng/mL IL3. The cells were incubated for 24 h at 37 $^{o}$C and 5% $CO_2$. Alamar Blue solution was added, and after 4 h incubation at 37 $^{o}$C, the fluorescent intensity was measured on a Fluorescence plate reader (540 nm excitation and 590 nm emission). The control experiment in the absence of IL3 was performed in the same way. To calculate compound $IC_{50}$, semi-log serial dilutions were used to produce 8-point dose-response curves in duplicate. A best-fit curve was fitted by a minimum sum of squares method to the plot of %Control vs. compound concentration. From this an $IC_{50}$ value (inhibitory concentration causing 50 % cytotoxicity) was calculated.

**Ba/F3-FGFR1 proliferation assay in the presence or absence of IL3.** Assay performed as described for Ba/F3-Flt3. Ba/F3-FGFR1 cells were made available by Prof. Jan Cools, University of Leuven, Belgium.

**Ba/F3-FGFR3 proliferation assay in the presence or absence of IL3.** Assay performed as described for Ba/F3-Flt3.

**Ba/F3-KDR proliferation assay in the presence or absence of IL3.** Assay performed as described for Ba/F3-Flt3. Ba/F3-KDR cells were made available by Prof. Jan Cools, University of Leuven, Belgium.

### 4. Fragment Screen Details

Screening of fragments by $T_m$ was carried out using a Stratagene mx3005p apparatus with MELK (MELK His6-TEV-1-337) at 0.5 µM protein concentration in the presence of Hepes (50 mM), NaCl (100 uM), $MgCl_2$ (1 mM), DTT (5 mM) and DMSO (1%) at pH 7.5 over a 25 – 75 $^{o}$C temperature range. The NMR fragment screen was carried out using a Bruker AV500

spectrometer equipped with a TXI-cryoprobe. Fragments which bound to MELK were detected using a ligand-detected NMR method, WaterLOGSY. Screening samples contained 7 µM MELK (MELK 1-337, N213T, V214A, M215A, Y218V, K219A) and cocktails of 4 fragments at a concentration of 500 µM each, in TRIS buffer (20 mM TRIS pH7.5, 150 mM NaCl, 5 mM DTT).

## 5. MELK Crystallography and PDB Accession Codes

For the work described, two soakable crystal forms of MELK were developed that were suitable for high throughput structure determination of protein-ligand complexes. A number of directed protein side chain residue substitutions were introduced aimed at disrupting crystal contact points, without affecting kinase function. This gave the $P3_121$ form as described below. Additional mutations to residues on the activation loop were made in order to knock out phosphorylation sites, thus giving the *P*1 form as described below. The structure of MELK (wild-type, residues 1 - 342) was solved by molecular replacement using MARK2 (Panneerselvam et. al., *Structure* **2006** *14* 173 - 183) as a search model, then manual rebuilding in Coot and refinement using Refmac5. This structure was then in turn used as a search model for solution, by molecular replacement, of the MELK constructs described below, with subsequent manual rebuilding (Coot) and refinement (Refmac5). Compound **1** (X-ray validated hit from the fragment screen) is available from Aurora Building Blocks (Catalog no. A03.727.194).

### *P*$3_1$21 Form

A human MELK construct was generated consisting of a thrombin cleavable N-terminal His tag linked to residues 1 – 337 (kinase + UBA domains) with the following mutations: N213T, V214A, M215A, Y218V, K219A. The protein was concentrated to ~10 mg/mL in 20 mM Tris.HCl pH 7.5, 150 mM NaCl, 5 mM DTT and N-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)-4-[(4-methylpiperazin-1-yl)methyl]benzamide was added to 1 mM immediately prior to crystallisation. Protein crystals were obtained using the hanging vapor diffusion method following the addition of an equal volume of 2-5% (w/v) PEG3350, 0.4-0.9 M LiCl, 0.1 M MES pH 6.5 and incubation at 20 ˚C. Crystals appeared overnight and continued to grow for several days. Crystals were soaked and cryo-protected in 10% (w/v) PEG3350, 1 M LiCl, 0.1 M MES pH 6.5, 23% mesoerythritol and flash frozen in liquid nitrogen prior to data collection (synchrotron, ESRF). This construct gave trigonal crystals (**Figure S1**) with a single protomer in the asymmetric unit with a molecule of N-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)-4-[(4-methylpiperazin-1-yl)methyl]benzamide bound. The bound ligand could be displaced by soaking with an appropriate test compound as described above. The MELK-ligand complex structures for the following compounds

were solved using the *P*$3_1$21 crystal form (with corresponding PDB codes given): **1**, 4umr; **3**, 4umq.

### *P*1 Form

An alternative construct was generated similar to that described above, with additional mutations to remove two phosphorylation sites on the activation loop, i.e. a thrombin cleavable N-terminal His tag linked to residues 1 – 337 (kinase + UBA domains) with the following mutations: T167A, S171A, N213T, V214A, M215A, Y218V, K219A. The thrombin cleaved non-phosphorylated protein was concentrated to ~10 mg/mL in 20 mM Tris.HCl pH 7.5, 150 mM NaCl, 5 mM DTT. Protein crystals were obtained using the hanging vapor diffusion method following the addition of an equal volume of 5% (w/v) PEG3350, 0.1 M HEPES pH 7.5 and incubation at 20 ˚C. Crystals appeared overnight and continued to grow for several days. Crystals were soaked with test compounds in 11% (w/v) PEG3350, 0.11 M HEPES pH 7.5 prior to cryo-protection in 11% (w/v) PEG3350, 0.11 M HEPES pH 7.5, 22% mesoerythritol and flash frozen in liquid nitrogen prior to data collection (synchrotron, ESRF). This construct gave triclinic crystals with 4 protomers in the asymmetric unit (**Figure S2**). The system gave apo crystals that were amenable to soaking with ligands as described above. The MELK-ligand complex structures for the following compounds were solved using the *P*1 crystal form (with corresponding PDB codes given): **2**, 4d2p; **4**, 4d2t, **5**, 4d2w; **7**, 4d2v.

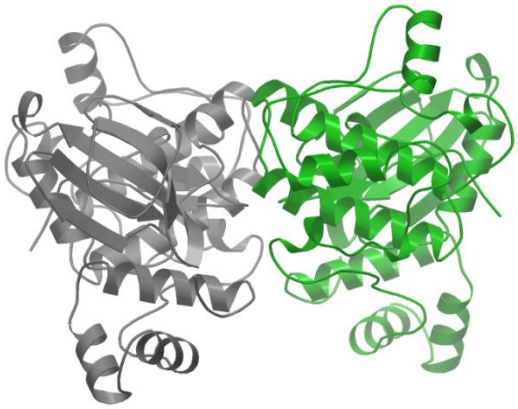


**Figure S1.** Trigonal (*P*$3_1$21) crystal form of MELK showing one copy in the asymmetric unit in green, with a symmetry copy in gray.

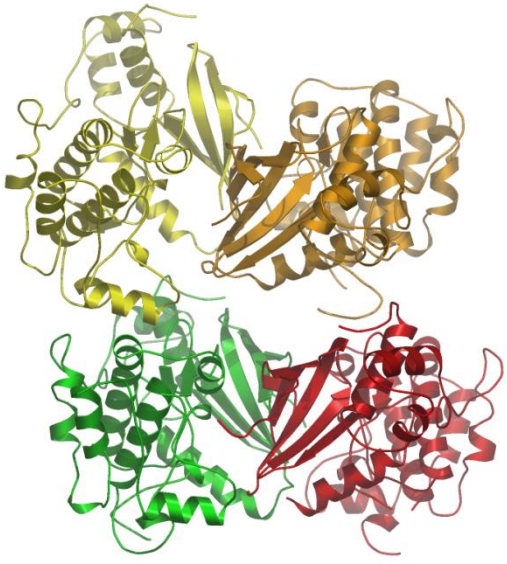


**Figure S2.** Triclinic (*P*1) crystal form of MELK showing the individually colored 4 protomers in the asymmetric unit.

## 6. Experimental Details for the Synthetic Procedures and Characterization Data of Compounds 2 - 7.

**Chemistry**. All solvents employed were commercially available anhydrous grade, and reagents were used as received unless otherwise noted. Hereafter, petrol denotes the petroleum ether fraction boiling at 40 – 60 $^{o}$C. Flash column chromatography was performed on a Biotage SP1 system (32–63 µm particle size, KP-Sil, 60 Å pore size). NMR spectra were recorded on a Bruker AV400 (Avance 400 MHz) spectrometer. Analytical LC–MS was conducted using an Agilent 1200 series with Mass Spec Detector coupled with an Agilent 6140 single quadrupole mass detector and an Agilent 1200 MWD SLUV detector. LC retention times, molecular ion (m/z) and LC purity (by UV) were based on the method below. Purity of compounds **2 – 7** (as measured by peak area ratio) was >95%.

### LC Method (BASIC)

Eluent A: 95:5 10 mM $NH_4HCO_3$+$NH_4OH$:$CH_3CN$ (pH = 9.2)
Eluent B: $CH_3CN$
Gradient: 5-95% eluent B over 1.1 minutes
Flow: 0.9 ml/min
Column: Waters Acquity UPLC BEH C18; 1.7µ; 2.1x50 mm
Column T: 50°C

### Synthesis of Compound 2

**tert-Butyl 7-(4-bromobenzamido)-1,2,3,4-tetrahydroisoquinoline-2-carboxylate.** To a stirred solution of tert-butyl 7-amino-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (0.12 g, 0.5 mmol) and diisopropylethylamine (0.075 g, 0.6 mmol) in $CH_2Cl_2$ (5 mL) at 20 $^{o}$C was added 4-bromobenzoyl chloride. Stirring was carried out for 4 h, then the mixture was partitioned between aqueous NaOH (0.5 M, 20 mL) and $CH_2Cl_2$ (2 x 15 mL). The combined organic extracts were dried ($Na_2SO_4$) and evaporated in vacuo. Chromatography of the residue ($SiO_2$, 0 - 40% EtOAc in petrol) gave the title compound (0.18 g, 86%). $^{1}$H NMR (400 MHz, $CDCl_3$): 7.85-7.72 (3H, m), 7.65 (2H, d), 7.59-7.45 (1H, m), 7.42-7.31 (1H, m), 7.15 (1H, d), 4.61 (2H, s), 3.67 (2H, t), 2.84 (2H, t), 1.52 (9H, s).

**4-[(3-Hydroxy-5-methoxyphenyl)amino]-N-(1,2,3,4-tetrahydroisoquinolin-7-yl)benzamide (2).** A mixture of tert-butyl 7-(4-bromobenzamido)-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (0.18 g, 0.42 mmol), 3-amino-5-methoxyphenol (0.07 g, 0.50 mmol), potassium phosphate (0.125 g, 0.59 mmol), tris(dibenzylideneacetone)dipalladium (0) (0.019 g, 0.021 mmol) and

XPhos (0.020 g, 0.042 mmol) in 1,2-dimethoxyethane (5 mL) was stirred at 110 $^{o}$C (block temperature) for 18 h. The mixture was cooled then partitioned between water (30 mL) and $CH_2Cl_2$ (3 x 10 mL). The combined organic extracts were dried ($Na_2SO_4$) and evaporated in vacuo. Chromatography of the residue ($SiO_2$, 0 - 50% EtOAc in petrol) gave tert-butyl 7-{4-[(3-hydroxy-5-methoxyphenyl)amino]benzamido}-1,2,3,4-tetrahydroiso-quinoline-2-carboxylate (0.166 g), which was stirred with trifluoroacetic acid (2 mL) and $CH_2Cl_2$ (5 mL) for 18 h. The resulting solution was evaporated in vacuo and the residue partitioned between aqueous HCl (0.2 M, 50 mL) and EtOAc (2 x 30 mL). The aqueous phase was basified (pH 8) with saturated aqueous $NaHCO_3$ and the resulting solid collected by filtration. The filter cake was washed with water and dried in vacuo to give the title compound (0.057 g, 35% over 2 steps). $^{1}$H NMR (400 MHz, DMSO-D6): 9.81 (1H, s), 9.36 (1H, s), 8.50 (1H, s), 7.85 (2H, d), 7.46 (2H, s), 7.10 (2H, d), 7.01 (1H, d), 6.23 (1H, s), 6.17 (1H, s), 5.95 (1H, s), 3.83 (2H, s), 3.68 (3H, s), 2.94 (2H, t), 2.65 (2H, s). Analytical LCMS, *m/z* 390 $[M+H]^+$, $t_R$ = 1.13 min.

**Synthesis of Compound 3**

**4-Bromo-2-fluoro-N-phenylbenzamide (14).** A mixture of 4-bromo-2-fluorobenzoyl chloride (4.75 g, 20 mmol), aniline (2.05 g, 22 mmol) and triethylamine (2.53 g, 25 mmol) in $CH_2Cl_2$ (100 mL) was stirred at 20 $^{o}$C for 3 h. The resulting solution was washed with aqueous HCl (2 M, 100 mL) then evaporated in vacuo. Chromatography of the residue ($SiO_2$, 10 - 40% EtOAc in petrol) gave the title compound (1.92 g, 30%) as a colorless solid. $^{1}$H NMR (400 MHz, DMSO-D6): 10.47 (1H, s), 7.76 (1H, dd), 7.70 (2H, d), 7.64 (1H, t), 7.57 (1H, dd), 7.36 (2H, t), 7.13 (1H, t).

**tert-Butyl N-{3-[5-bromo-2-(phenylcarbamoyl)phenoxy]propyl}carbamate (15).** A mixture of 4-bromo-2-fluoro-N-phenylbenzamide (1.87 g, 6.36 mmol), N-Boc-hydroxypropylamine (1.23 g, 7.0 mmol), cesium carbonate (4.56 g, 14 mmol) and N, N-dimethylformamide was stirred at 60 °C for 18 h, then evaporated in vacuo. The residue was partitioned between water (100 mL) and $CH_2Cl_2$ (100 mL) and the organic phase was dried ($Na_2SO_4$) and evaporated in vacuo to give the title compound as a yellow oil. $^{1}$H NMR (400 MHz, DMSO-D6): 10.08 (1H, s), 7.71 (2H, d), 7.58 (1H, d), 7.38 (1H, s), 7.34 (2H, t), 7.27 (1H, dd), 7.10 (1H, t), 6.95 (1H, t), 4.13 (2H, t), 3.17-3.00 (2H, m), 1.93-1.79 (2H, m), 1.36 (9H, s).

**2-(3-Aminopropoxy)-4-[(3-hydroxy-5-methoxyphenyl)amino]-N-phenylbenzamide hydrochloride (3•HCl).** A mixture of tert-butyl N-{3-[5-bromo-2-(phenylcarbamoyl)phenoxy]propyl}carbamate **15** (0.225 g, 0.50 mmol), 3-methoxy-5-aminophenol (0.084 g, 0.60 mmol), tris(dibenzylideneacetone)dipalladium (0) (0.023 g, 0.025 mmol), XPhos (0.024 g, 0.05 mmol), potassium phosphate (0.212 g, 1.0 mmol) and 1,2-

dimethoxyethane (8 mL) was heated at reflux under nitrogen for 2 h. The mixture was cooled and evaporated in vacuo, then the residue was partitioned between aqueous HCl (1 M, 30 mL) and $CH_2Cl_2$ (50 mL). The organic phase was dried ($Na_2SO_4$) and evaporated in vacuo. Chromatography of the residue ($SiO_2$, 30 - 70% EtOAc in petrol) gave tert-butyl N-(3-{5-[(3-hydroxy-5-methoxyphenyl)amino]-2-(phenylcarbamoyl)phenoxy}-propyl)carbamate as a pale yellow foam. This material was dissolved in EtOAc and treated with HCl in dioxane (4 M, 3 mL) and the resulting solution stirred at 20 °C for 18 h, then evaporated in vacuo to give the title compound (0.18 g, 88% over 2 steps). $^1$H NMR (DMSO-D6) 9.90 (1H, s), 9.44 (1H, br s), 8.59 (1H, br s), 7.96 (3H, br s), 7.72 (2H, d), 7.66 (1H, d), 7.34 (2H, t), 7.08 (1H, t), 6.78 (1H, d), 6.73 (1H, dd), 6.29 (1H, t), 6.19 (1H, t), 5.97 (1H, t), 4.18 (2H, t), 3.69 (3H, s), 3.02 (2H, m), 2.15 (2H, m). Analytical LCMS, *m/z* 408 $[M+H]^+$, $t_R$ = 1.05 min.

**Synthesis of Compound 4**

**2-(3-Aminopropoxy)-N-phenyl-4-(1H-pyrazol-4-yl)benzamide hydrochloride (4•HCl).** A mixture of tert-butyl N-{3-[5-bromo-2-(phenylcarbamoyl)phenoxy]propyl}carbamate **15** (0.169 g, 0.38 mmol), 4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-pyrazole-1-carboxylic acid tert-butyl ester (0.124 g, 0.42 mmol), tetrakis(triphenylphosphinepalladium (0) (0.044 g, 0.038 mmol), sodium carbonate (0.398 g, 3.8 mmol), 1,2-dimethoxyethane (5 mL) and water (5 mL) was stirred at 80 °C for 2 h under nitrogen. The mixture was cooled, then partitioned between aqueous HCl (1 M, 30 mL) and EtOAc (30 mL). The organic phase was dried ($Na_2SO_4$) and evaporated in vacuo to give tert-butyl N-{3-[2-(phenylcarbamoyl)-5-(1H-pyrazol-4-yl)phenoxy]propyl}carbamate as a colorless solid. This material was dissolved in EtOAc then treated with HCl in dioxane (4 M, 3 mL) and the resulting solution stirred at 20 °C for 18 h, then evaporated in vacuo. The resulting solid was triturated with EtOAc to give the title compound (0.06 g, 47%). $^1$H NMR (400 MHz, DMSO-D6): 10.13 (1H, s), 8.24 (2H, s), 8.08-7.91 (3H, m), 7.75 (2H, d), 7.65 (1H, d), 7.45-7.30 (4H, m), 7.10 (1H, t), 4.32 (2H, t), 3.07-2.95 (2H, m), 2.18-2.06 (2H, m). Analytical LCMS, *m/z* 337 $[M+H]^+$, $t_R$ = 1.11 min.

**Synthesis of Compound 5**

**7-Amino-1,2,4,5-tetrahydro-benzo[d]azepine-3-carboxylic acid tert-butyl ester (10).** Sulfuric acid (1 M, 30 mL) was added dropwise to a stirred solution of 2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine **8** (2.50 g, 17.0 mmol) in 1,4-dioxane (30 mL) and the solvent was removed *in vacuo* to afford a sticky paste. A small volume of isopropanol (*ca*. 5 mL) was added to induce

solidification followed by EtOAc (50 mL) and the resulting mixture was stirred at room temperature for 1 h. The solids were collected by filtration, and the filter cake was washed with EtOAc (25 mL) and petrol (50 mL) and sucked dry to afford 2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine sulphate (2.62 g, 63%) as an off-white solid. $^1$H NMR (DMSO-D6) 8.80 (2H, br s), 7.21 (4H, m), 3.19 (4H, br m), 3.07 (4H, br m). 2,3,4,5-Tetrahydro-1*H*-benzo[*d*]azepine sulphate (2.62 g, 10.69 mmol) was added at 0 °C in small portions over 15 minutes to a stirred mixture of concentrated sulfuric and nitric acids (1:1; 15 mL) and the resulting mixture was stirred at 0 °C for 2 h. The mixture was poured onto ice and allowed to warm to room temperature. The mixture was basified by the careful portionwise addition of sodium hydrogen carbonate (50.0 g) and extracted with EtOAc (3 × 300 mL). The combined organic extracts were dried ($Na_2SO_4$) and evaporated in vacuo to afford 7-nitro-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine **9** (1.60 g, 78%) as a dark oil which was used without further purification. $^1$H NMR (DMSO-D6) 7.99 (1H, d), 7.95 (1H, dd), 7.39 (1H, d), 2.97 (4H, m), 2.79 (4H, m). MS, *m/z* 193 $[M+H]^+$. Di-*tert*-butyl dicarbonate (2.00 g, 9.16 mmol) was added to a stirred solution of 7-nitro-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine **9** (1.60 g, 8.33 mmol) in $CH_2Cl_2$ (60 mL) and the mixture was stirred at room temperature for 2 h. The solvent was removed *in vacuo* and the residue subjected to column chromatography on silica. Elution with 10-40% EtOAc in petrol afforded 7-nitro-1,2,4,5-tetrahydro-benzo[*d*]azepine-3-carboxylic acid *tert*-butyl ester (2.40 g, 98%) as a pale yellow oil which solidified upon standing. $^1$H NMR (400 MHz, $CDCl_3$): 8.07-7.97 (2H, m), 7.37-7.29 (1H, m), 3.61 (4H, d), 3.10-2.95 (4H, m), 1.51 (9H, s). MS, *m/z* 237 $[M+H-{}^tBu]^+$. A mixture of 10% palladium on carbon (0.3 g) and 7-nitro-1,2,4,5-tetrahydro-benzo[*d*]azepine-3-carboxylic acid *tert*-butyl ester (2.40 g, 8.22 mmol) in methanol (60 mL) was stirred and held at room temperature under an atmosphere of hydrogen for 16 h. The mixture was filtered, the catalyst rinsed with methanol (20 mL) and the combined filtrates were evaporated *in vacuo* to afford the title compound (2.06 g, 96%) as a dark oil which was used without further purification. $^1$H NMR (DMSO-D6) 6.77 (1H, d), 6.36 (1H, d), 6.30 (1H, dd), 4.81 (2H, br s), 3.39 (4H, m), 2.65 (4H, m), 1.41 (9H, s). MS, *m/z* 207 $[M+H-{}^tBu]^+$.

**4-Bromo-N-(2,3,4,5-tetrahydro-1H-3-benzazepin-7-yl)benzamide hydrochloride (5•HCl).** A mixture of 7-amino-1,2,4,5-tetrahydro-benzo[d]azepine-3-carboxylic acid tert-butyl ester **10** (0.131 g, 0.50 mmol), triethylamine (0.1 mL, 0.72 mmol) and 4-bromobenzoyl chloride (0.133 g, 0.50 mmol) in $CH_2Cl_2$ (8 mL) was stirred at 20 °C for 3 h. The mixture was washed with saturated aqueous $NaHCO_3$ (10 mL) and aqueous HCl (10 mL), dried ($Na_2SO_4$) and evaporated in vacuo to give tert-butyl 7-(4-bromobenzamido)-2,3,4,5-tetrahydro-1H-3-benzazepine-3-carboxylate (**12**, R = H), used without further purification. $^1$H NMR (DMSO-D6) 10.22 (1H, s), 7.92 (2H, d), 7.74 (2H, d), 7.53 (2H, m), 7.13 (1H, d), 3.46 (4H, br m), 2.82 (4H, br m), 1.42 (9H, m). This material was dissolved in EtOAc then treated with HCl in dioxane (4 M, 3 mL), stirred

at 20 °C for 18 h, then evaporated in vacuo to give the title compound (0.175 g, 98%. $^1$H NMR (DMSO-D6) 10.32 (1H, s), 9.21 (2H, br s), 7.93 (2H, d), 7.77 (2H, m), 7.65 (1H, d), 7.56 (1H, dd), 7.19 (1H, d), 3.69 (4H, br m), 3.08 (4H, m). Analytical LCMS, *m/z* 345 $[M+H]^+$, $t_R$ = 1.20 min.

**Synthesis of Compound 6**

**4-(1H-Pyrazol-4-yl)-N-(2,3,4,5-tetrahydro-1H-3-benzazepin-7-yl)benzamide hydrochloride (6•HCl).** A mixture of tert-butyl 7-(4-bromobenzamido)-2,3,4,5-tetrahydro-1H-3-benzazepine-3-carboxylate (**12**, R = H) (0.130 g 0.29 mmol), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (0.062 g, 0.33 mmol), tetrakis(triphenylphosphinepalladium (0) (0.040 g, 0.029 mmol), sodium carbonate (0.31 g, 2.9 mmol), water (5 mL) and dimethoxyethane (5 mL) was stirred at 80 °C for 18 h. The mixture was cooled then partitioned between aqueous HCl (1 M, 30 mL) and EtOAc (30 mL) and the organic phase was dried ($Na_2SO_4$) and evaporated in vacuo. Chromatography of the residue ($SiO_2$, 0 - 100% EtOAc in petrol) gave tert-butyl 7-[4-(1H-pyrazol-4-yl)benzamido]-2,3,4,5-tetrahydro-1H-3-benzazepine-3-carboxylate as a colorless solid, which was dissolved in EtOAc and treated with HCl in dioxane (4 M, 3 mL) and the resulting solution stirred at 20 °C for 18 h, then evaporated in vacuo. The resulting solid was triturated with EtOAc to give the title compound (0.012 g, 12%). $^1$H NMR (DMSO-D6) 10.18 (1H, s), 9.21 (2H, br s), 8.20 (2H, s), 7.97 (2H, d), 7.77 (2H, d), 7.67 (1H, d), 7.60 (1H, dd), 7.19 (1H, d), 3.18 (4H, br s), 3.08 (4H, br s). Analytical LCMS, *m/z* 333 $[M+H]^+$, $t_R$ = 1.00 min.

**Synthesis of Compound 7**

**2-Methoxy-4-(1H-pyrazol-4-yl)-N-(2,3,4,5-tetrahydro-1H-3-benzazepin-7-yl)benzamide hydrochloride (7•HCl).** A mixture of 7-amino-1,2,4,5-tetrahydro-benzo[d]azepine-3-carboxylic acid tert-butyl ester **10** (0.215 g, 0.82 mmol), 4-bromo-2-methoxybenzoic acid (0.198 g, 0.86 mmol), ({[3-(dimethylamino)propyl]imino}methylidene)(ethyl)amine hydrochloride (0.192 g, 1.0 mmol), triethylamine (0.2 mL, 1.44 mmol) and 1-hydroxybenzotriazole (0.135 g, 1.0 mmol) in $CH_2Cl_2$ (10 mL) was stirred at 20 °C for 18 h. Resulting mixture was washed with saturated aqueous $NaHCO_3$ and aqueous HCl (1 M, 20 mL) and the organic phase was dried ($Na_2SO_4$) and evaporated in vacuo to give tert-butyl 7-(4-bromo-2-methoxybenzamido)-2,3,4,5-tetrahydro-1H-3-benzazepine-3-carboxylate (**12**, R = OMe) (0.39 g, used without further purification). MS, *m/z* 476 $[M+H]^+$. A mixture of this material, 4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-pyrazole-1-carboxylic acid tert-butyl ester (0.24 g, 0.82 mmol),

tetrakis(triphenylphosphinepalladium (0) (0.11 g, 0.082 mmol), sodium carbonate (0.87 g, 8.2 mmol), water (6 mL) and dimethoxyethane (6 mL) was stirred at 80 °C for 18 h. The mixture was cooled then partitioned between aqueous HCl (1 M, 30 mL) and EtOAc (30 mL) and the organic phase was dried ($Na_2SO_4$) and evaporated in vacuo. Chromatography of the residue ($SiO_2$, 30 - 100% EtOAc in petrol) gave tert-butyl 7-[2-methoxy-4-(1H-pyrazol-4-yl)benzamido]-2,3,4,5-tetrahydro-1H-3-benzazepine-3-carboxylate. This material was dissolved in EtOAc and treated with HCl in dioxane (4 M, 3 mL) and the resulting solution stirred at 20 °C for 18 h, then evaporated in vacuo. The resulting solid was triturated with EtOAc to give the title compound (0.17 g, 57% over 3 steps) as an off-white solid. $^1$H NMR (DMSO-D6) 10.01 (1H, s), 9.34 (2H, br s), 8.24 (2H, s), 7.70 (1H, d), 7.61 (1H, s), 7.55 (1H, dd), 7.40 (1H, s), 7.34 (1H, dd), 7.17 (1H, d), 4.01 (3H, m), 3.18 (4H, br m), 3.08 (4H, br m). Analytical LCMS, *m/z* 363 $[M+H]^+$, $t_R$ = 1.06 min.