SUPPORTING INFORMATION

# DESIGN AND SYNTHESIS OF NON-PEPTIDE INHIBITORS OF HEPATOCYTE GROWTH FACTOR ACTIVATION

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## PROTEASE PANEL

All analogs were tested for their inhibitory activity in a FRET protease panel consisting of the target enzymes: matriptase, hepsin, HGFA; the prototype: trypsin, and the anti-targets: thrombin, fXa. The recombinant human enzymes are commercially available (R&D Systems). The substrate is a custom peptide based on the pro-HGF substrate sequence (New England Peptide): $H_2N$(EEdansyl)GKQLRVVNGG (KDabcyl)-$NH_2$. $IC_{50}$s are determined at 10 concentrations in duplicate. HAI-1 (R&D Systems) is a positive control. The $K_m$ for each enzyme in the panel were determined (matriptase: $K_m$ = 32.6 μM; hepsin: $K_m$ = 42.9 μM; HGFA: $K_m$ = 109.5 μM; trypsin: $K_m$ = 7.6 μM; thrombin: $K_m$ = 15.6; factor $K_m$ = 14.7 μM) and using the substrate concentration (10 μM) the $IC_{50}$ were converted to their respective $K_i$ by the Chang-Prussoff method. To be considered a 'triplex inhibitor' a compound must have the $K_i$ for matriptase, hepsin and HGFA < 1 μM and the $K_i$ within 3-fold.

**Materials**

- Assay Buffer: 50 mM Tris, 20 mM NaCl, 0.01% (v/v) Tween® 20, pH 8.0
- Recombinant Human Matriptase/ST14 Catalytic Domain (rhMatriptase) (R&D Systems, Catalog # 3946-SE)
- Substrate: $H_2N$-(EEdans)GKQLRVVNGG(KDabcyl)-amide (Custom peptide from New England Peptide), 10 mM stock in DMSO
- Fluorescence micro-cuvets
- Aminco-Bowman Series 2 Luminescence Spectrometer

**Matriptase Activity Assay Protocol**

1. Dilute rhMatriptase to 0.2 μg/mL in Assay Buffer.

2. Dilute Substrate to 20 µM in Assay Buffer.
3. Load 150 µL of 0.2 µg/mL rhMatriptase into a microtube, add inhibitor or DMSO, and start the reaction by adding 150 µL of 20 µM substrate. Immediately transfer the assay solution to a cuvet and place it in the fluorometer.
4. Read at excitation and emission wavelengths of 340 nm and 490 nm, respectively, in kinetic mode for 5 minutes at room temperature. Increase in fluorescence is recorded as RFU/sec.

**Final assay conditions in assay buffer**

- 0.1 µg/ml rhMatriptase
- 10 µM Substrate
- 2% DMSO

Inhibitor concentration–response curves were analyzed using SigmaPlot by fitting data to the four-parameter logistic equation (sigmoidal dose–response curve with variable slope) from which $IC_{50}$ values were calculated.

**Proteases**

All 6 of the enzymes used in the assays of the Protease Panel were from R&D Systems:

- Recombinant Human Matriptase (Catalog Number: 3946-SE)
- Recombinant Human Hepsin (Catalog Number: 4776-SE)
- Recombinant Human HGF Activator (Catalog Number: 1514-SE)
- Recombinant Human Active Trypsin (Catalog Number: 3714-SE)
- Recombinant Human Coagulation Factor II/Thrombin (Catalog No.: 1473-SE)

- Recombinant Human Coagulation Factor Xa (Catalog Number: 1063-SE)

**Adaptation of Matriptase Protocol to other proteases in the panel**

All 6 of the proteases were assayed in essentially the same way as described in the **Matriptase Activity Assay Protocol** with the differences indicated below:

- **Hepsin Assay Protocol:** The Hepsin assay was exactly the same as the Matriptase assay. Prior to the assay the Hepsin (4776-SE) had to be activated by incubating in activation buffer (0.05M Tris, 10mM $CaCl_2$, 0.15M NaCl, 0.05% Brij-35, pH 8.0) at 37°C for 24 hours.
- **HGF Activator [HGFA] Assay:** The HGFA assay was the same as the Matriptase assay except that the concentration of the HGFA was 1 µg/ml.
- **Trypsin Assay:** The Trypsin assay was the same as the Matriptase assay except that the concentration of the Trypsin was 0.01 µg/ml and the assay buffer was 0.05M Tris, 10mM $CaCl_2$, 0.15M NaCl, 0.05% Brij-35, pH 8.0.
- **Thrombin Assay:** The Thrombin assay was the same as the Matriptase assay except that the concentration of the Thrombin was 0.08 µg/ml and the assay buffer was 0.05M Tris, 10mM $CaCl_2$, 0.15M NaCl, 0.05% Brij-35, pH 8.0.
- **Factor Xa Assay:** The Factor Xa assay was the same as the Matriptase assay except that the concentration of the Factor Xa was 0.4 µg/ml and the assay buffer was 0.05M Tris, 10mM $CaCl_2$, 0.15M NaCl, 0.05% Brij-35, pH 8.0.

## IN VITRO ADME ASSAY PANEL

**LogD.** Log D is an important lipophilicity parameter indicative of drug-likeness; since this is an issue with this series we have added it to the ADME panel. LogD was evaluated at pH 7.4 using a 'shake-flask' method adapted to a 96-well plate format. Optimal values are in the range of 1.5-4 units.

**Solubility.** Good kinetic solubility (>50 μM) is important for absorption & accurate dose response. Solubility was estimated at pH 7.4 using a μSol Explorer (PION, Billerica, MA) with an average of two determinations.

**Metabolic Stability.** The potential for a high metabolic clearance compound or first pass metabolism is estimated using a liver microsome assay. The compounds were incubated in human and mouse liver microsomes with or without the co-factor NADPH. The disappearance of the parent molecule was measured by liquid chromatography/mass spectroscopy (LC/MS) detection. The $t_{1/2}$ for each compound was determined; when necessary, metabolites can be identified by liquid chromatography/tandem mass spectrometry (LC/MS/MS).

**Supporting Information Table 1.** In vitro ADME assay panel results for **6**, **8a**-**8f**.

| | **Solubility (μM) pH 7.4** | **LogD pH 7.4** | **Human Microsomes $t_{1/2}$ (min)** | **Mouse Microsomes $t_{1/2}$ (min)** |
|---|---|---|---|---|
| **6** | 91.6 | -0.49 | 161.8 | 295.9 |
| **8a** | 73.4 | -0.68 | 31.7 | 40.2 |
| **8b** | 57.2 | 0.3 | 32.1 | 66.3 |
| **8c** | 75.5 | 0.18 | 300 | 300 |
| **8d** | 64 | -0.7 | 115.7 | 186.3 |
| **8e** | 83.7 | 0.16 | 39.1 | 204.4 |
| **8f** | 47.4 | 1.6 | 5.6 | 16.3 |

## MOUSE IN VIVO PHARMACOKINETIC (PK) STUDY

In vivo PK evaluation. In vivo PK assays were conducted in mice dosed by either a 1 mg/kg i.v. bolus or 5 mg/kg IP injection or oral gavage in 100% saline solution. Blood samples were collected by cardiac puncture at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 12 hrs. Plasma concentrations over time were determined by LC/MS/MS from which the standard PK parameters of clearance, volume of distribution, half-life ($t_{1/2}$) and fraction absorbed (%F) were derived using WinNolin software (Pharsight Inc.).

**Supporting Information Table 2**. Pharmacokinetic Parameters Calculated from Mean Plasma Concentrations of **6**
Following IV or PO Administration to Mice

IV Dose (1 mg/kg, vehicle: 100% saline)

| t½[a] (hr) | Tmax[b] (hr) | Cmax[c] (ng/mL) | $C_0$[d] (ng/mL) | AUClast[e] (hr·ng/mL) | $AUC_{INF}$[f] (hr·ng/mL) | Cl[g] (mL/hr/kg) | MRTlast[h] (hr) | $MRT_{INF}$[i] (hr) | Vss[j] (mL/kg) |
|---|---|---|---|---|---|---|---|---|---|
| 5.8 | 0.083 | 1099 | 2265 | 414 | 438 | 2283 | 0.9 | 2.0 | 4489 |

PO Dose (5 mg/kg, vehicle 100% saline)

| t½[a] (hr) | Tmax[b] (hr) | Cmax[c] (ng/mL) | AUClast[d] (hr·ng/mL) | $AUC_{INF}$[f] (hr·ng/mL) | MRTlast[h] (hr) | $MRT_{INF}$[i] (hr) | F[k] (%) |
|---|---|---|---|---|---|---|---|
| NC | 0.5 | 4.45 | 3.31 | NC | 0.9 | NC | 0.2 |

NC: Not calculated due to an insufficient number of data points beyond Tmax.

[a]Apparent half-life of the terminal phase of elimination from plasma
[b]Time maximum mean concentration was observed in plasma
[c]Maximum mean concentration observed in plasma
[d]Mean concentration in plasma at 0 hr; extrapolated value
[e]Area under the mean plasma concentration versus time curve calculated from 0 to the last time point SRI-31215 was quantifiable in plasma
[f]Area under the mean plasma concentration versus time curve calculated from 0 to infinity
[g]Total body clearance
[h]Mean residence time calculated from 0 to the last time point **6** was quantifiable in plasma
[i]Mean residence time calculated from 0 to infinity
[j]Apparent volume of distribution at steady state
[k]Oral bioavailability; $F = [\text{Mean } AUClast_{(po)} \times \text{Dose(iv)}] \div [\text{Mean } AUClast_{(iv)} \times \text{Dose(po)}] \times 100$

**Supporting Information Table 3.** IV Plasma Concentrations of **6**

| Animal ID | Nominal Timepoint (hr) | Route of Administration | Dose (mg/kg) | Plasma Concentration (ng/mL) |
|---|---|---|---|---|
| 1M 1 | 0.0083 | IV | 1 | 586 |
| 1M 2 | 0.0083 | IV | 1 | 2140 |
| 1M 3 | 0.0083 | IV | 1 | 570 |
| 1M 4 | 0.25 | IV | 1 | 157 |
| 1M 5 | 0.25 | IV | 1 | 341 |
| 1M 6 | 0.25 | IV | 1 | 271 |
| 1M 7 | 0.5 | IV | 1 | 77.3 |
| 1M 8 | 0.5 | IV | 1 | 272 |
| 1M 9 | 0.5 | IV | 1 | 106 |
| 1M 1 | 1 | IV | 1 | 91.9 |
| 1M 2 | 1 | IV | 1 | 135 |
| 1M 3 | 1 | IV | 1 | 19.7 |
| 1M 10 | 2 | IV | 1 | 11.90 |
| 1M 11 | 2 | IV | 1 | n/a |
| 1M 12 | 2 | IV | 1 | 6.66 |
| 1M 4 | 4 | IV | 1 | 6.45 |
| 1M 5 | 4 | IV | 1 | 5.43 |
| 1M 6 | 4 | IV | 1 | 5.97 |
| 1M 7 | 8 | IV | 1 | 2.62 |
| 1M 8 | 8 | IV | 1 | 3.38 |
| 1M 9 | 8 | IV | 1 | 3.09 |
| 1M 10 | 12 | IV | 1 | 1.71 |
| 1M 11 | 12 | IV | 1 | 3.62 |
| 1M 12 | 12 | IV | 1 | 3.16 |

Plasma LLOQ = 1.00 ng/mL
Plasma ULOQ = 1,000 ng/ml

**Supporting Information Table 4.** PO Plasma Concentrations of **6**

| Animal ID | Nominal Timepoint (hr) | Route of Administration | Dose (mg/kg) | Plasma Concentration (ng/mL) |
|---|---|---|---|---|
| 2M 16 | 0.25 | PO | 5 | 3.79 |
| 2M 17 | 0.25 | PO | 5 | 1.49 |
| 2M 18 | 0.25 | PO | 5 | No Peak |
| 2M 19 | 0.5 | PO | 5 | 2.16 |
| 2M 20 | 0.5 | PO | 5 | 2.60 |
| 2M 21 | 0.5 | PO | 5 | 8.58 |
| 2M 13 | 1 | PO | 5 | 1.86 |
| 2M 14 | 1 | PO | 5 | 1.37 |
| 2M 15 | 1 | PO | 5 | BQL |
| 2M 22 | 2 | PO | 5 | 3.54 |
| 2M 23 | 2 | PO | 5 | No Peak |
| 2M 24 | 2 | PO | 5 | No Peak |
| 2M 16 | 4 | PO | 5 | No Peak |
| 2M 17 | 4 | PO | 5 | No Peak |
| 2M 18 | 4 | PO | 5 | No Peak |
| 2M 19 | 8 | PO | 5 | No Peak |
| 2M 20 | 8 | PO | 5 | No Peak |
| 2M 21 | 8 | PO | 5 | No Peak |
| 2M 22 | 12 | PO | 5 | No Peak |
| 2M 23 | 12 | PO | 5 | No Peak |
| 2M 24 | 12 | PO | 5 | No Peak |

BQL = below quantitation limit

Plasma LLOQ = 1.00 ng/mL
Plasma ULOQ = 1,000 ng/mL

## CELL SCATTER ASSAY

DU145 cells were cultured in 6-well tissue culture plates at a density of $1x10^3$ cells per well. After colonies were formed (6-8 days), cells were serum-starved overnight and were treated with conditioned media from 18Co fibroblasts in the presence or absence (for the controls) of **6**, **8a**, **8b** or **8d** at 10, 5 and 1 μM concentrations for 24 hours. Cells were washed with PBS and colonies were fixed and stained with 0.5% crystal violet solution in 6% glutaraldehyde.

## CRYSTAL STRUCTURE DETERMINATION AND REFINEMENT

**Methods**: Bovine pancreatic trypsin was purchased from Sigma. Trypsin was resuspended in a buffer containing 10 mM calcium chloride and 25 mM HEPES, pH 7.0 at a concentration of 60 mg/ml. Crystals were obtained via the hanging drop vapor diffusion method using a 2 ul of trypsin mixed with 2 ul of well solution containing 200 mM ammonium sulfate, 30% Polyethylene glycol 8000, 100 mM HEPES, pH 7.0. Crystals formed in 3-5 days and reached maximum dimensions 10-14 days, with often both hexagonal and orthorhombic crystals obtained in the same drop. After the crystals had formed, less than 0.1 mg of compounds was resuspended in a solution containing the mother liquor and added to the drop containing the crystals. After the crystals were soaked for 24-48 hours, crystal were harvested and cryoprotected by removing the surrounding mother liquor in an 1:1 paraffin:silicon oil, and flash frozen in liquid nitrogen for analysis.

Data from single crystals were collected with a Rigaku MicroMax 007 HF rotating copper anode system and a Saturn 944 HG CCD detector. Data were integrated and scaled using HKL3000. Initial models were obtained by molecular replacement using Phaser from the PHENIX package with the search models derived from PDB codes 3UNR for the $P2_12_12_1$ data set and 4ABG

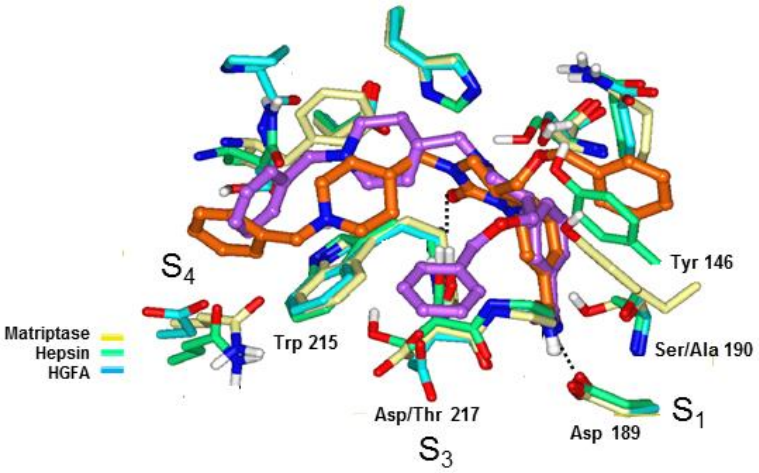
for the $P3_121$ data set. The compound model was built in Coot and the protein structure was refined using PHENIX. The atomic coordinates and structure factors have been deposited in the PDB. Data collection and refinement statistics are summarized in Supporting Information Table 2.

**Supporting Information Table 5**: Data collection and refinement statistics

| Data collection | Compound **6** | Compound **8f** |
|---|---|---|
| Space group | $P2_12_12_1$ | $P3_121$ |
| Cell dimensions: a, b, c (Å) α, β, γ (°) | 54.5 58.4 66.6 90 90 90 | 54.5 54.5 107.8 90 90 120 |
| Resolution (Å) | 50-1.20 (1.22-1.20) | 50-1.09 (1.11-1.09) |
| $R_{merge}$ | 0.108 (0.573) | 0.085 (0.655) |
| I/σ I | 17.8 (1.5) | 24.6 (1.4) |
| Completeness (%) | 91.0 (76.4) | 94.0 (90.6) |
| Redundancy | 8.3 (3.4) | 8.5 (2.0) |
| Refinement | | |
| $R_{work}$/ $R_{free}$ | 0.147/0.173 | 0.159/0.178 |
| Average *B* factor | 20.6 | 25.7 |
| RMSD bond length (Å) | 0.009 | 0.006 |
| RMSD bond angles (°) | 1.352 | 1.134 |
| Ramachandran analysis: favored/allowed (%) | 97.9/2.1 | 98.6/1.4 |

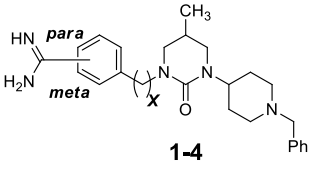
Values in parenthesis are for the highest resolution shell

**Supporting Information Figure 1.** Co-crystal structure of compound **8f** with trypsin (purple) and modeled in matriptase (orange).

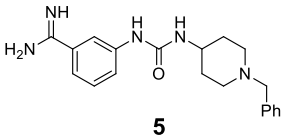



To confirm that the 5-substituent is directed toward the S3 subsite, we obtained a co-crystal structure of **8f** with bovine pancreatic trypsin (SI Figure 1). In general, the trypsin structure of **8f** confirms that the 5-substituent is directed toward the S3 subsite. In general, the trypsin structure of **8f** is very similar to **6** with the exception that the N-benzylpiperidine group is rotated slightly and displaced upward but still engaged with the indole of Trp 215 in the S4 subsite. This shift is due to the accommodation of the 5-methyl(benzyloxy)- group in S3 . There is clear density for the benzyloxy group over the Ser 217 residue of trypsin; this group sits in a small aliphatic groove between main chain N of Gly 218 and the carbonyl of Gly 216. However, in a model derived by minimization of the trypsin binding pose in matriptase (SI Figure 1), the 5-methyl(benzyloxy)- group 'flips' and adopts a conformation that forms a prominent aryl face-to-face lipophilic interaction with Tyr 146; this interaction is possible in hepsin (residue 146 = Tyr) but not in HGFA, thrombin or factor Xa (residue 146= Glu).

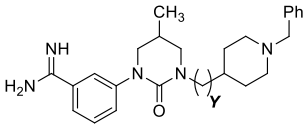
**Supporting Information Table 6.** Comprehensive SAR data with statistical analysis. (Ki, μM)*



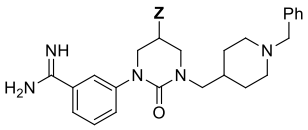

**1-4**




**5**




**6,7**




**8 a-l**

| | | | Matriptase | Hepsin | HGFA | Trypsin | Thrombin | Factor Xa |
|---|---|---|---|---|---|---|---|---|
| 1 | meta | X = 0 | 0.83 ± 0.0094 | 3.4 ± 0.0910 | 9.8 ± 0.7279 | 0.78 ± 0.0975 | 35.9 ± 0.9169 | 0.24 ± 0.0094 |
| 2 | para | X = 0 | 48.9 ± 3.3206 | 61.2 ± 5.7750 | 46.5 ± 1.8176 | 35.4 ± 9.8963 | >100 | 25.5 ± 3.7467 |
| 3 | meta | X = 1 | 13.5 ± 1.0440 | 15.1 ± 0.9856 | >100 | 4.8 ± 0.0671 | >100 | 49.4 ± 3.3277 |
| 4 | para | X = 1 | 15.4 ± 0.4430 | 20.8 ± 1.0212 | 39.6 ± 1.1796 | 4.8 ± 0.2907 | 82.2 ± 6.2229 | 22.8 ± 3.6023 |
| 5 | | | 40.5 ± 0.2732 | 53.1 ± 3.3181 | 54.6 ± 3.5470 | 5.3 ± 0.5600 | >100 | 5.4 ± 0.2732 |
| 6 | Y = 1 (SRI 31215) | | 0.53 ± 0.0267 | 0.54 ± 0.0248 | 0.48 ± 0.0111 | 0.43 ± 0.0248 | 11.6 ± 0.5173 | 0.38 ± 0.0080 |
| 7 | Y = 2 | | 0.51 ± 0.0252 | 2.12 ± 0.0252 | 1.11 ± 0.0230 | 2.77 ± 0.1861 | >100 | 0.40 ± 0.0292 |
| 8a | Z = HO- | | 0.28 ± 0.0195 | 0.21 ± 0.0142 | 0.88 ± 0.0539 | 0.39 ± 0.0175 | 21.9 ± 1.3220 | 0.85 ± 0.0760 |
| 8b | Z = $HOCH_2$- | | 0.58 ± 0.0163 | 0.69 ± 0.0188 | 0.23 ± 0.0037 | 0.15 ± 0.0081 | 7.4 ± 0.5403 | 0.25 ± 0.0051 |
| 8c | Z = $H_3CO$- | | 0.81 ±0.0197 | 0.76 ± 0.0373 | 2.49 ± 0.0721 | 0.76 ± 0.0663 | >100 | 1.23 ± 0.1417 |
| 8d | Z = $H_3COCH_2$- | | 0.85 ± 0.0231 | 0.92 ± 0.0116 | 0.16 ± 0.0064 | 0.21 ± 0.0229 | 8.1 ± 0.6569 | 0.20 ± 0.0100 |
| 8e | Z = $PhCH_2O$- | | 1.54 ± 0.0593 | 0.76 ± 0.0111 | 1.92 ± 0.1923 | 0.55 ± 0.0448 | 29.7 ± 0.8797 | 0.92 ± 0.0523 |
| 8f | Z = $PhCH_2OCH_2$- | | 0.85 ± 0.0647 | 0.56 ± 0.0320 | 0.43 ± 0.0099 | 0.10 ± 0.0046 | 2.13 ± 0.0888 | 0.18 ± 0.0057 |
| 8g | Z = $PhC(O)NHCH_2$- | | 0.47 ± 0.0106 | 1.1 ± 0.0476 | 1.1 ± 0.0401 | 0.33 ± 0.0244 | >100 | 0.75 ± 0.0488 |
| 8h | Z = $PhCH_2C(O)NH$- | | 1.28 ± 0.0456 | 1.12 ± 0.0839 | 0.38 ± 0.0198 | 0.5 ± 0.0355 | 42.3 ± 0.9817 | 0.74 ± 0.0129 |
| 8i | Z = $PhCH_2C(O)NHCH_2$- | | 1.51 ± 0.0528 | 0.31 ± 0.0130 | 1.08 ± 0.0530 | 0.59 ± 0.0382 | >100 | 1.55 ± 0.1663 |
| 8j | Z = $H_3CSO_2NH$- | | 0.33 ± 0.0147 | 0.72 ± 0.0288 | 0.35 ± 0.0097 | 0.42 ± 0.0409 | >100 | 0.51 ± 0.0365 |
| 8k | Z = $PhSO_2NH$- | | 0.52 ± 0.0560 | 0.67 ± 0.0134 | 0.52 ± 0.0111 | 0.34 ± 0.0438 | 17 ± 2.2597 | 0.61 ± 0.0497 |
| 8l | Z = $PhCH_2SO_2NH$- | | 0.35 ± 0.0049 | 0.53 ± 0.0102 | 0.44 ± 0.0093 | 0.31 ± 0.0071 | 13.4 ± 2.6138 | 0.66 ± 0.0713 |
| 8m | Z = $PhCH_2CH_2NHCH_2$- | | 1.34 ± 0.0365 | 1.86 ± 0.1067 | 2.18 ± 0.0431 | 1.57 ± 0.1206 | >100 | 1.2 ± 0.0746 |
| 8n | Z = $Et_2NCH_2$- | | 3.25 ± 0.3325 | 4.86 ± 0.2340 | 2.39 ± 0.1446 | 2.80 ± 0.2618 | >100 | 1.20 ± 0.0952 |
| 8o | Z = $CH_3C(O)NH$- | | 1.68 ± 0.0464 | 2.16 ± 0.1617 | 1.44 ± 0.0659 | 0.53 ± 0.0621 | 38 ± 8.1386 | 0.58 ± 0.0283 |
| 8p | Z = $H_3CC(O)NHCH_2$- | | 0.72 ± 0.0173 | 1.91 ± 0.1143 | 1.26 ± 0.0277 | 0.53 ± 0.0566 | >100 | 0.72 ± 0.0661 |
| 8q | Z = $PhC(O)NH$- | | 0.65 ± 0.0120 | 1.82 ± 0.1282 | 0.88 ± 0.0131 | 0.33 ± 0.0410 | 19.9 ± 2.3957 | 1.4 ± 0.0663 |
| 8r | Z = $PhCH_2CH_2C(O)NH$- | | 0.76 ± 0.0337 | 1.13 ± 0.1072 | 1.22 ± 0.1187 | 0.57 ± 0.0338 | 34 ± 0.7357 | 1.09 ± 0.0332 |
| 8s | Z = $PhCH_2CH2C(O)NHCH_2$- | | 1.32 ± 0.1200 | 2.11 ± 0.0940 | 1.38 ± 0.0501 | 0.60 ± 0.0191 | >100 | 1.56 ± 0.0168 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 8t | Z = $H_3CSO_2NHCH_2$- | 0.78 ± 0.0344 | 1.97 ± 0.0691 | 1.06 ± 0.0293 | 0.39 ± 0.0362 | 19.9 ± 2.6602 | 0.29 ± 0.0386 |
| 8u | Z = $PhSO_2NHCH_2$- | 0.78 ± 0.0702 | 0.89 ± 0.0406 | 1.04 ± 0.0492 | 0.60 ± 0.0241 | 44.1 ± 0.9372 | 0.24 ± 0.0314 |
| 8v | Z = $PhCH_2SO_2NHCH_2$- | 0.92 ± 0.0207 | 0.99 ± 0.0574 | 1.40 ± 0.0453 | 0.57 ± 0.0180 | >100 | 0.53 ± 0.0237 |
| | HAI-1 | 0.0032 ±0.0002 | 0.0038 ± 0.0002 | 0.0034 ± 0.0001 | 0.0008 ±0.0001 | >1.0 | >1.0 |

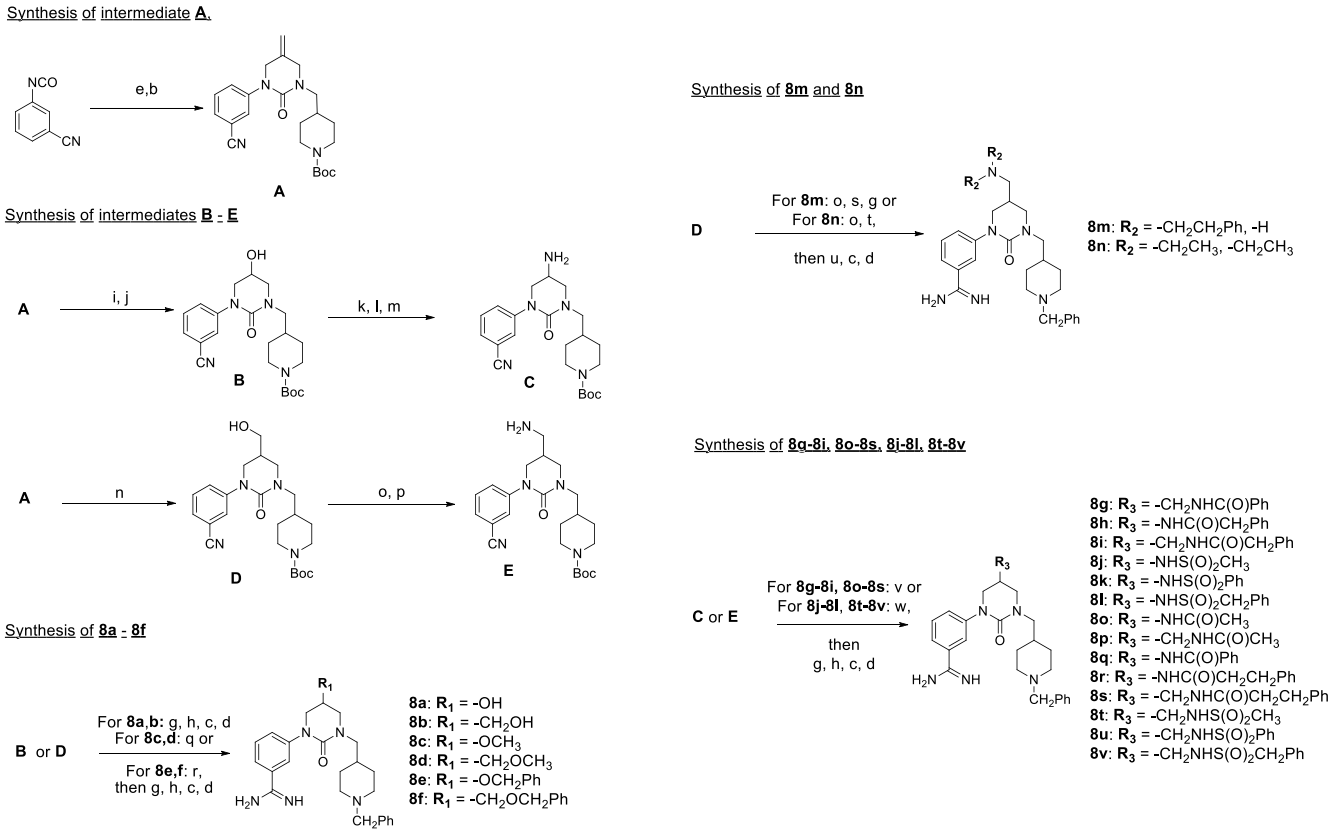
*All compounds have an *N*=1 with the exception of **6** [SRI 31215] with an *N*=2. $IC_{50}$ experiments for each compound with each protease were determined in duplicate with 2 fold serial dilutions of each compound over a concentration range of 100-0.050 uM (up to 12 concentrations). Inhibitor concentration–response curves were analyzed using SigmaPlot by fitting data to the four-parameter logistic equation (sigmoidal dose–response curve with variable slope) from which $IC_{50}$ values were calculated. SigmaPlot calculates the standard error for the $IC_{50}$ calculation and that is the SE that is shown in the table. The $K_i$ were estimated from the $IC_{50}$ using the Chang-Prussoff relationship. See 'Protease Panel' on page 3.

## SYNTHESIS OF INTERMEDIATES A-E AND COMPOUNDS 8a-v

The intermediates **A**-**E** and compounds **8a-v** were prepared by the methods outlined in Supporting Information Scheme 1. Intermediate A was obtained by reaction of *meta*-benzonitrile isocyanate with tert-butyl 4-(aminomethyl)piperidine-1-carboxylate. This gave a urea intermediate that is cyclized with 3-chloro-2-(chloromethyl)prop-1-ene to give **A**, a versatile synthetic intermediate that we exploit to prepare the 5-tetrahydropyrimidin-2(1H)-one analogs **8a-v** to probe the $S_3$ subsite. Ozonolysis of intermediate **A** followed by reduction of the ozonide with dimethylsulfide to the ketone, then further reduction of the ketone with sodium borohydride gave a 5-hydoxy-tetrahydropyrimidin-2(1H)-one, intermediate **B**. Intermediate **B** was transformed to the 5-amino-tetrahydropyrimidin-2(1H)-one **C** by a three step tosylation, azide displacement and reduction sequence. Hydroboration and oxidation of intermediate **A** gave access to a 5-(hydroxymethyl)-tetrahydropyrimidin-2(1H)-one **D**; intermediate **D** was oxidized using the Dess-Martin periodinane procedure to give the aldehyde in quantitative yield. The primary amine **E** was derived from this aldehyde by reductive amination using sodium cyanoborohydride with a mixture of ammonium hydroxide and ammonium acetate in 60% yield. For analogs **8a-f**, intermediates **B**

or **D** were treated with trifluoroacetic acid to remove the N-t-butoxycarbonyl protecting group, then N-benzylation with benzylbromide and triethylamine. These derivatives of **B** and **D** underwent amidine formation using the two-step procedure described above to give the hydroxy analogs **8a** and **8b**, respectively. The ether analogs **8c** – **f** were obtained by O-methylation or O-benzylation of **B** and **D** before N-t-butoxycarbonyl deprotection of the piperidine. Subsequent progression of the ethers derived from **B** and **D** through the four step N-benzylation and amidine formation protocol gave **8c** – **f** in good yield. To obtain **8m** the aldehyde derived from **D** was reacted with 2-phenylethan-1-amine in the presence of sodium cyanoborohydride to give a secondary amine that was protected as the p-nitrobenzylcarbamate for clean N-benzylation of the piperidine nitrogen. Following N-benzylation and during amidine formation, the p-nitrobenzylcarbamate group was removed by the Raney nickel hydrogenolysis step and gave **8m**. The tertiary amine **8n** was obtained from same aldehyde without the need for p-nitrobenzylcarbamate protection. Compounds **8g**– **8v** were derived from intermediates **C** and **E**. N-acylation followed by removal of the N-t-butoxycarbonyl group and N-benzylation then two-step amidine formation gave amides **8g-8i, 8o-8s**; by an analogous sequence, after N-sulfonylation of **C** and **E**, there was obtained the sulfonamides **8i-l, u** and **v**.

**Supporting Information Scheme 1**. Synthesis of intermediates **A**-**E** and compounds **8a-v**[a]




[a]Reagents and conditions: (a) 1-benzylpiperidin-4-amine, DMF, r.t. (b) 3-chloro-2-(chloromethyl)prop-1-ene, NaH, THF (c) hydroxylamine hydrochloride, $Et_3N$, EtOH (d) Raney Nickel, $H_2$, 60 p.s.i, r.t. (e) tert-butyl 4-(aminomethyl)piperidine-1-carboxylate, DMF, r.t. (f) for 7 (y = 2): tert-butyl 4-(2-aminoethyl)piperidine-1-carboxylate, DMF, r.t. (g) TFA (h) benzylbromide, $Et_3N$, DMF, r.t. (i) $O_3$, MeOH, -78 $^oC$, then $(CH_3)_2S$ (j) $NaBH_4$, MeOH (k) tosyl chloride, $Et_3N$, DCM, r.t. (l) $NaN_3$, DMF, 60 $^oC$ (m) $H_2$ at 1 atm., 10% Pd-C, MeOH (n) 9-BBN, THF then $NaBO_3$ (o) Dess-Martin periodinane, TFA, DCM (p) $NH_4OH$, $NH_4OAc$, $NaCNBH_3$, EtOH, reflux (q) for 8c or 8d: $CH_3I$, NaH, THF (r) for 8e or 8f: benzylbromide, NaH, THF (s) for 14: 2-phenylethanamine, $NaBH(OAc)_3$, DCM then 4-nitrobenzyl carbonochloridate, $Et_3N$, DCM

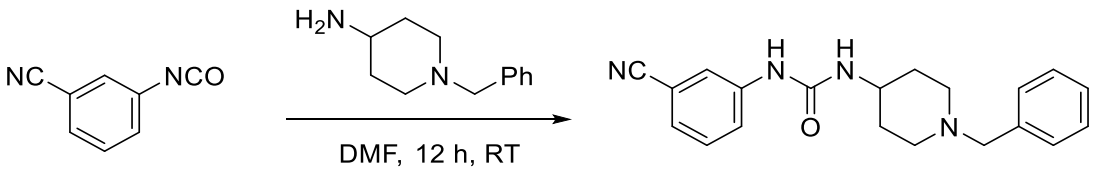
(t) for 15: diethylamine, $NaBH(OAc)_3$, DCM (u) benzaldehyde, $NaBH(OAc)_3$, DCM (v) for 8g-8i, 8o-8s: **R**C(O)Cl, $Et_3N$, N,N-dimethylpyridin-4-amine, DCM (w) for 8j-8l, 8t-8v: **R**$S(O)_2Cl$ $Et_3N$, N,N-dimethylpyridin-4-amine, DCM.

## CHEMISTRY EXPERIMENTAL

**General methods**: All reactions were carried out in oven- or flame-dried glassware under argon atmosphere using standard gas-tight syringes, cannulae, and septa. Stirring was achieved with oven dried magnetic bars. All the reactions were done in anhydrous solvents (DMF, THF, $CHCl_3$, $CH_2Cl_2$) purchased from Sigma-Aldrich. All commercially purchased reagents were used without purification. Flash column chromatography was performed with Redisep $R_f$® normal-phase silica flash columns 230-400 mesh, on Teledyne-ISCO *CombiFlash* $R_f$® purification system. Thin layer chromatography was performed on Analtech silica gel GF 0.25 mm plates and EMD Millipore silica gel 60 $F_{254}$ plates. Deuterated solvents were purchased from Cambridge Isotope Laboratories and Sigma-Aldrich. $^1$H NMR spectra were recorded on a Varian Unity 400 nmr spectrometer operating at 400 MHz and calibrated to the solvent peak. The chemical formula for target compounds were determined from the $(M+H)^+$ by high resolution mass spectroscopy using an Agilent 6210 Electrospray Time of Flight Spectrometer. The purity of the final compounds were assayed by HPLC/MS method using an Agilent 6210 Electrospray Time of Flight Spectrometer coupled with an Agilent 1100 Series LC using a Kinetex phenyl-hexyl (2.6µm, 50x4.6 mm) column and an isocratic mobile phase consisting of water/ methanol/ formic acid (95/ 4.9/ 0.1; v/ v/ v).

3-(3-(1-benzylpiperidin-4-yl)-5-methyl-2-oxotetrahydropyrimidin-1(2H)-yl)benzimidamide (**1**)

**STEP 1 – Urea Synthesis**:



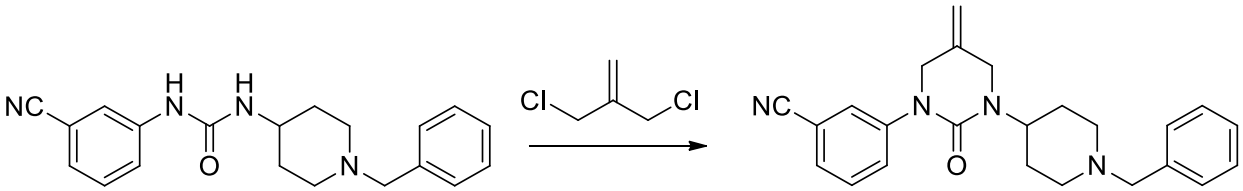

A solution of 3-cyanophenylisocyanate (2g, 13.88 mmol) and 4-amino-1-benzylpiperidine (3 ml, 15.26 mmol) in dry DMF (10 ml) was stirred at ambient temperature for 3h. The reaction was

complete by TLC (hexane: EtOAc 4:1). This mixture was diluted with water (120 ml) then extracted with EtOAc (3x50 ml); the extracts were combined, washed with water (4x50 ml), brine (50 ml), dried and evaporated to give the crude product. This material was purified by silica gel chromatography on an ISCO CombiFlash $R_f$® using a gradient of $CHCl_3$/MeOH to obtain 4.2 g of the desired product as a white color solid in 91% yield.

**HRMS**: for $C_{20}H_{22}N_4O$ calculated $(M+H)^+$ = 335.18664 m/z, found $(M+H)^+$ =335.18700 m/z.

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ: 8.93 (s, 1H), 7.65 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 8.8 Hz, 2H), 7.35 – 7.22 (m, 5H), 6.40 (d, *J* = 7.6 Hz, 1H), 3.53 – 3.46 (m, 1H), 3.45 (s, 2H), 2.70 (d, *J* = 11.0 Hz, 2H), 2.07 (t, *J* = 11.0 Hz, 2H), 1.79 (dd, *J* = 13.0, 3.9 Hz, 2H), 1.46-1.35 ppm (ddd, *J* = 23.5, 10.7, 3.6 Hz, 2H).

**<u>STEP 2 – Cyclic Urea Synthesis</u>**:

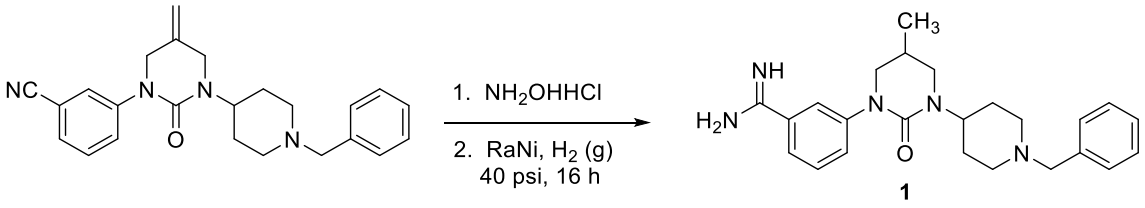



To a cooled solution (0°C) of (1-benzylpiperidin-4-yl)-3-(3-cyanophenyl)urea (4.8 g, 14.35 mmol) in tetrahydrofuran (100 ml) and 3-chloro-2-(chloromethyl)prop-1-ene (4.15 ml, 35.9 mmol) was added sodium hydride (1.722 g, 43.1 mmol). The reaction mixture was allowed to come to room temperature and then was heated at 75°C for 5 hrs. After reaction completion as monitored by LCMS and TLC, added water to quench the reaction, solvent removed and the residue partitioned between sat $NH_4Cl$ solution and EtOAc (2x75ml). The combined extracts were dried over $Na_2SO_4$, filtered and evaporated. The crude product was purified by column chromatography on silica gel on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ to obtain 4.5 g of the desired product as a white solid in 82% yield.

**HRMS**: $C_{24}H_{26}N_4O$ calculated $(M+H)^+$ = 387.21794 m/z, found $(M+H)^+$ = 387.21776 m/z.

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ: 7.72 (ddd, *J* = 2.2, 1.5, 0.5 Hz, 1H), 7.61 (ddd, *J* = 7.9, 2.3, 1.4 Hz, 1H), 7.54 (dt, *J* = 7.6, 1.5 Hz, 1H), 7.49 (dd, J = 13.7, 7.7 Hz, 1H), 7.35 – 7.28 (m, 4H), 7.27 – 7.22 (m, 1H), 5.15 (dq, *J* = 26.0, 1.2 Hz, 2H), 4.26 (s, 2H), 4.08 (tt, *J* = 12.1, 4.0 Hz, 1H), 3.90 (t, *J* = 1.3 Hz, 2H), 3.46 (s, 2H), 2.92 – 2.84 (m, 2H), 1.99 (td, *J* = 11.8, 2.3 Hz, 2H), 1.76 (ddd, *J* = 24.5,12.2, 3.8 Hz, 2H), 1.59 – 1.50 ppm (m, 2H).

**STEPS 3 and 4**:




**Oxime**: A mixture of 3-(3-(1-benzylpiperidin-4-yl)-5-methylene-2-oxotetrahydropyrimidin-1(2H)-yl)benzonitrile (321 mg, 0.83 mmol), hydroxylamine hydrochloride (116 mg, 1.66 mmol) and diisopropyl ethylamine (0.28 ml, 1.66 mmol) in EtOH (15 ml) were heated at reflux for 4 h followed by overnight reaction at roomtmperature. After reaction completion as monitored by LCMS and TLC analysis, the solvent was removed and residue purified by column chromatography on silica gel on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$, to obtain the desired product as a gummy solid (194 mg, 56% yield).

**HRMS**: for $C_{24}H_{29}N_5O_2$ $(M+2H)^{+2}$ = 210.62447 m/z; calculated $(M+H)^+$ = 420.23940 m/z, found $(M+H)^+$ = 420.23871 m/z.

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ: 7.54 (t, J = 1.9 Hz, 1H)7.44 (dt, *J* = 7.3, 1.7 Hz, 2H), 7.40-7.28 (m, 5H), 7.26 (dt, *J* = 8.0, 1.8 Hz, 1H), 5.56 (broad s, 2H), 5.12 (dq, *J* = 13.9, 1.4 Hz, 2H), 4.22 (s, 2H), 4.20-4.10 (m, 1H), 3.91 (s, 2H), 3.08-2.90 (m, 4H), 2.09-1.90 (m, 2H), 1.67 (d, *J* = 11.9 Hz, 2H), 1.35-1.29 ppm (m, 2H).

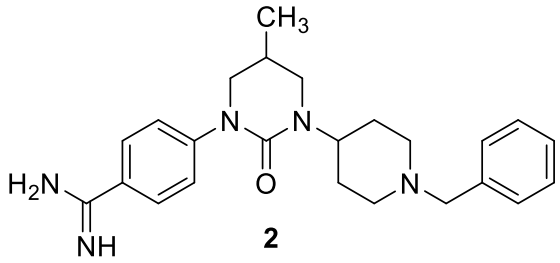
**Compound 1**: The oxime prepared above (180 mg) was dissolved in EtOH (30 ml) and shaken under an $H_2$ atmosphere (38 psi) in the presence of Raney Nickel catalyst (170 mg) for 18 hours at ambient temperature. The reaction was purged with Ar then filtered through a Celite® pad and evaporated. The crude product was purified by column chromatography on silica gel on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive; it was submitted as a trifluoroacetate salt following lyophilization.

**HRMS**: for $C_{24}H_{31}N_5O$ found $(M+2H)^{+2}$ = 203.6321 m/z; calculated $(M+H)^+$ = 406.26014 m/z, found (M+H) = 406.26004 m/z;

**PURITY**: 100%, retention time: 4.41 min.

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 9.04 (br.s, 3H), 7.75 (t, *J* = 1.9 Hz, 1H), 7.67 (dt, *J* = 7.3, 1.9 Hz, 1H), 7.61 – 7.55 (m, 2H), 7.41 – 7.34 (m, 4H), 7.34 – 7.28 (m, 1H), 4.20 (tt, *J* = 12.2, 4.1 Hz, 1H), 3.73 (ddd, *J* = 11.3, 4.3, 1.5 Hz, 1H), 3.52 (s, 2H), 3.45-3.43 (m, 2H), 3.02 – 2.91 (m, 3H), 3.30-2.20 (m, 1H), 2.10 – 1.99 (m, 2H), 1.87 – 1.72 (m, 2H), 1.61 – 1.52 (m, 2H), 1.10 (d, *J* = 6.5 Hz, 3H).

4-(3-(1-benzylpiperidin-4-yl)-5-methyl-2-oxotetrahydropyrimidin-1(2H)-yl)benzimidamide (**2**)



**2**

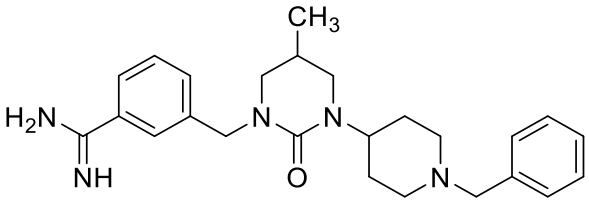
**Compound 2** was synthesized following a similar synthetic sequence as described for **compound 1** starting with 4-isocyanatobenzonitrile in place of 3-isocyanatobenzonitrile. The crude product was purified by column chromatography on silica gel on an ISCO CombiFlash $R_f^{®}$ using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, to obtain the desired product as a white solid (43 mg, 26% yield).

**HRMS**: for $C_{24}H_{31}N_5O$ found $(M+2H)^{+2}$ = 203.63569 m/z; calculated $(M+H)^{+}$ = 406.26014 m/z, found $(M+H)^{+}$ = 406.25896 m/z.

**PURITY**: 100%, retention time: 4.21 min.

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ: 8.97 (broad s, 3H), 7.75 (d, *J* = 8.8 Hz, 2H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.35-7.28 (m, 4H), 7.28-7.22 (m, 1H), 4.14 (ddt, *J* = 12.0, 7.9, 4.0 Hz, 1H), 3.67 (dd, *J* = 11.2, 4.1 Hz, 1H), 3.46 (s, 2H), 3.42 – 3.33 (m, 2H), 2.95 – 2.85 (m, 3H), 2.22-2.14 (m, 1H), 1.99 (t, *J* = 11.4 Hz, 2H), 1.73 (ddd, *J* = 11.7, 7.1, 4.2 Hz, 2H), 1.52 (d, *J* = 12.3 Hz, 2H), 1.02 ppm (d, *J* = 6.6 Hz, 3H).

4-(3-(3-(Amino(iminio)methyl)benzyl)-5-methyl-2-oxotetrahydropyrimidin-1(2H)-yl)-1-benzylpiperidin-1-ium bis trifluoroacetate (**3**)



**3**

**Compound 3** was synthesized following a similar synthetic sequence as described for **compound 1** starting with 3-(isocyanatomethyl)benzonitrile in place of 3-isocyanatobenzonitrile. The crude product was purified by column chromatography on silica gel on an ISCO CombiFlash $R_f^{®}$ using

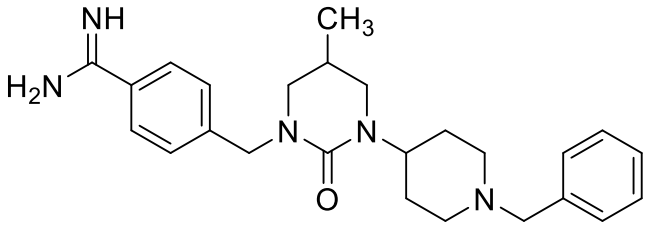
a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, to obtain the desired product as a white solid (40 mg, 75% yield).

**HRMS:** for $C_{25}H_{33}N_5O$ found $(M+2H)^{+2}$ = 210.64296 m/z; calculated $(M+H)^+$ = 420.27579 m/z, found $(M+H)^+$ = 420.27538 m/z.

**PURITY**: 100%, retention time: 4.50 min.

**$^1$H NMR** (DMSO-$d_6$, 400 MHz) δ: 9.66-9.04 (broad s, 3H), , 7.68-7.62 (m, 1H), 7.60 (s, 1H), 7.58-7.50 (m, 2H), , 7.34-7.22 (m, 5H), 4.5 (d, *J*= 3.5 Hz, 2H), 4.19-4.11 (dddd, *J* = 16.0, 8.0, 4.0, 4.0 Hz, 1H), , 3.45 (s, 2H), 3.23 (dd, J = 12.0, 2.8 Hz, 1H), , 3.15 (dd, *J* = 11.6, 3.2 Hz, 1H), 2.90-2.76 (m, 4H), 2.06-1.92 (m, 3H), , 1.75-1.58 (m, 2H), 1.52-1.40 (m, 2H), 0.9 ppm (d, *J* = 6.6 Hz, 3H).

4-(3-(4-(Amino(iminio)methyl)benzyl)-5-methyl-2-oxotetrahydropyrimidin-1(2H)-yl)-1-benzylpiperidin-1-ium bis trifluoroacetate (**4**)




**4**

**Compound 4** was synthesized following similar a synthetic sequence as described for **compound 1** starting with 4-(isocyanatomethyl)benzonitrile in place of 3-isocyanatobenzonitrile. The crude product was purified by column chromatography on silica gel on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, to obtain the desired product as a white solid (88 mg, 48% yield).

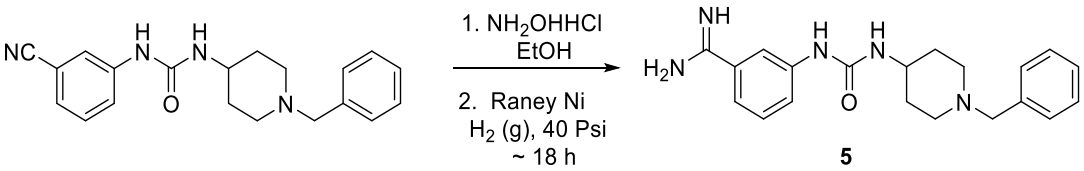
**HRMS**: for $C_{25}H_{33}N_5O$ found $(M+2H)^{+2}$ = 210.64420 m/z; calculated $(M+H)^+$ = 420.27579 m/z, found $(M+H)^+$ = 420.27532 m/z;.

**PURITY**: 100%, retention time: 4.15 min.

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ: 9.15 (broad d, *J* = 102.5 Hz, 3H), 7.77 (d, *J* = 8.4 Hz, 2H), 7.43 (d, *J* = 8.3 Hz, 2H), 7.35 – 7.28 (m, 4H), 7.27-7.22(m, 1H), 4.51 (dd, *J* = 22.1,16.1 Hz, 2H), 4.15 (ddd, *J* = 12.2, 8.1, 4.1 Hz, 1H), 3.47 (s, 2H), 3.23 (dd, J = 11.4, 4.0 Hz, 1H), 3.15 (dd, J = 11.4,

4.9 Hz, 1H), 2.90 – 2.76 (m, 4H), 2.05-1.94 (m, 3H), 1.75-1.59 (m, 2H), 1.45 (t, *J* = 12.4 Hz, 2H), 0.90 ppm (d, *J* = 6.6 Hz, 3H).

4-(3-(3-(Amino(iminio)methyl)phenyl)ureido)-1-benzylpiperidin-1-ium bis trifluoroacetate (**5**)




**Oxime**: A mixture of urea (289 mg, 0.83 mmol), hydroxylamine hydrochloride (114 mg, 1.66 mmol) and diisopropyl ethylamine (0.291 ml, 1.66 mmol) in EtOH (15 ml) were heated at reflux for 6 h followed by stirring at room temperature for 12-16 h. After the reaction was complete by TLC (5% MeOH in $CHCl_3$), the solvent was removed by evaporation *in vacuo* and the compound purified by column chromatography on silica gel with an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$. There was obtained 220 mg of the desired product as a white solid in 69% yield.

**HRMS**: for $C_{20}H_{25}N_5O_2$ $(M+2H)^{+2}$ = 184.60860 m/z; calculated $(M+H)^+$ = 368.20810 m/z, found $(M+H)^+$ = 368.20784 m/z.

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ: 9.55 (s, 1H), 8.47 (s, 1H), 7.61 (t, *J* = 4 Hz, 1H), 7.45 – 7.41 (m, 1H), 7.40-7.25 (d, *J* = 24.1 Hz, 5H), 7.20 (t, *J* = 7.8 Hz, 1H), 7.16 (dt, *J* = 7.7, 1.5 Hz, 1H), 6.25 (broad s, 1H), 5.68 (s, 2H), 3.65-3.49 (m, 3H), 2.95-2.66 (m, 2H), 2.42-2.04 (m, 2H), 1.90-1.79 (m, 2H), 1.53-1.37 ppm (m, 2H).

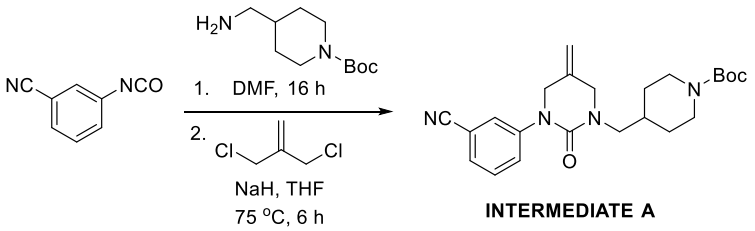
**Compound 5**: The purified oxime from the above reaction (190 mg, 0.517 mmol) was dissolved in EtOH (10 mL) and placed in a shaker under an $H_2$ atmosphere (38 psi) in the presence of Raney Nickel catalyst (183 mg, 3.01 mmol, wet weight) for 18 h at ambient temperature. After reaction completion, the crude material was purged with Ar gas then filtered through a Celite® pad washing with MeOH. The solvent was evaporated followed by purification by column chromatography on silica with an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive provided 45 mg of the desired product as a white solid in 24% yield. This product was treated with trifluoroacetic acid in water then lyophilized to give the final product as the bis-trifluoroacetate salt.

**HRMS**: for $C_{20}H_{25}N_5O$ found $(M+2H)^{+2}$ = 176.61176 m/z; calculated $(M+H)^+$ = 352.21319 m/z, found $(M+H)^+$ = 352.21321 m/z.

**PURITY**: 100%, retention time 4.12 min.

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ: 9.26 (broad s, 1H), 8.95 (s, 2H), 7.89 (t, *J* = 2.0 Hz, 1H), 7.59 (ddd, *J* = 8.2, 2.2, 1.0 Hz, 1H), 7.44 (t, *J* = 8.0 Hz, 1H), 7.35 – 7.27 (m, 4H), 7.27 – 7.22 (m, 2H), 6.48 (d, *J* = 7.6 Hz, 1H), 3.54-3.47 (m, 1H), 3.46 (s, 2H), 2.71 (d, *J* = 11.7 Hz, 2H), 2.08 (t, *J* = 11.0 Hz, 2H), 1.84 – 1.76 (m, 2H), 1.47 – 1.35 ppm (m, 2H).

tert-butyl 4-((3-(3-cyanophenyl)-5-methylene-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (**INTERMEDIATE A**)




**INTERMEDIATE A**

**Urea Synthesis**: A solution of 3-isocyanatobenzonitrile (6 g, 41.6 mmol) and tert-butyl 4-(aminomethyl)piperidine-1-carboxylate (10.57 ml, 50.0 mmol) in DMF (30 ml) was stirred at room temperature overnight. The reaction solution was poured in water and a white gummy solid separated out. This material was extracted with $CHCl_3$ (2 x 100 ml), then the combined organic extracts were washed with brine, dried ($MgSO_4$) and evaporated. The resulting crude material was purified by column chromatography on silica gel with an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ to obtain 15 g of the desired product as a white solid in quantitative yield.

**HRMS**: for $C_{19}H_{26}N_4O_3$ calculated $(M+Na)^+$ = 381.18971 m/z, found $(M+Na)^+$ = 381.18941 m/z.

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.71 (ddd, *J* = 8.3, 2.3, 1.1 Hz, 1H), 7.66 (s, 1H), 7.64 – 7.61 (m, 1H), 7.34 (dd, *J* = 8.3, 7.7 Hz, 1H), 7.24 (dt, *J* = 7.7, 1.3 Hz, 1H), 5.56 (t, *J* = 6.1 Hz, 1H), 4.17 – 4.06 (m, 2H), 3.35-3.02 (m, 2H), 2.80-2.61 (m, 2H), 1.78 – 1.70 (m, 1H), 1.68 (s, 2H), 1.46 (s, 9H), 1.15 (ddd, *J* = 24.7, 13.1, 4.1 Hz, 2H).

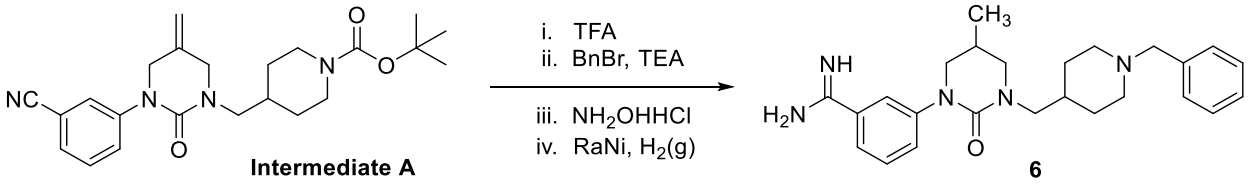
**Cyclic Urea Synthesis**: To tert-butyl 4-((3-(3-cyanophenyl)ureido)methyl)piperidine-1-carboxylate (15 g, 41.8 mmol) in THF (250 ml) was added 3-chloro-2-(chloromethyl)prop-1-ene (7.75 ml, 67.0 mmol) followed by the addition of sodium hydride (60% suspension in mineral oil, 4.35 g, 109 mmol) at ambient temperature. After 10 min., the reaction was heated at 75 °C for 5 h

after which the reaction was complete by TLC (1:1 hexane: EtOAc); then water (1 ml) was added to quench the reaction and the solvent removed by distillation *in vacuo*. The residue was partitioned between saturated $NH_4Cl$ solution and EtOAc (75 ml); then aqueous layer was extracted with EtOAc (75 ml) a second time. The combined EtOAc extracts were dried over $Na_2SO_4$, filtered and evaporated. This material was purified by column chromatography on silica gel on an ISCO CombiFlash $R_f$® using a gradient of EtOAc in hexane; to yield 5.5 g (13.4 mmol, 32%) of the desired.

**HRMS** for $C_{23}H_{30}N_4O_3$ calculated $(M+Na)^+$ = 433.22101 m/z, found $(M+Na)^+$ = 433.22068 m/z.

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ: 7.58-7.56 (m, 1H), 7.56-7.51 (m, 1H), 7.43 – 7.40 (m, 2H), 5.13 (d, *J* = 6.0 Hz, 2H), 4.25 (s, 2H), 3.99 (s, 2H), 3.48 (broad s, 2H), 3.41 – 3.12 (m, 2H), 2.69 (t, *J* = 12.7 Hz, 2H), 1.90 (ttt, *J* = 11.1, 7.3, 3.7 Hz, 1H), 1.67 (d, *J* = 12.8 Hz, 2H), 1.45 (s, 9H), 1.18 ppm (ddd, *J* = 24.7, 12.4, 4.5 Hz, 2H).

<u>Preparation of 4-((3-(3-(amino(iminio)methyl)phenyl)-5-methyl-2-oxotetrahydro-pyrimidin-1(2H)-yl)methyl)-1-benzylpiperidin-1-ium trifluoroacetate bis trifluoroacetate (**6**)</u>




**Boc Deprotection**: To a solution of tert-butyl 4-((3-(3-cyanophenyl)-5-methylene-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (5.5g, 13.40 mmol) in dichloromethane (75 ml) was added 2,2,2-trifluoroacetic acid (10.32 ml, 134 mmol). The solution turned orange-red. TLC (1:1 hexane: EtOAc) after 2 h showed that the starting material was consumed. The reaction was evaporated, 70 ml of EtOH added and then evaporated again to remove the last trace of acid. This material was dried *in vacuo* for 2 h then triturated with 10 mL of $CH_2Cl_2$ (2x) to give purified material. A second crop was obtained by combining the supernatant solutions and evaporating. Trituration of this material gave a second crop of product. The product was obtained in quantitative yield as the trifluoroacetic acid salt

**LRMS**: $(M+H)^+$ = 311 m/z.

**N-Benzylation**: To a solution of 3-(5-methylene-2-oxo-3-(piperidin-4-ylmethyl)tetrahydropyrimidin-1(2H)-yl)benzonitrile (260mg, 0.838 mmol) and triethylamine (0.257 ml, 1.843 mmol) in DMF (4 ml) was added (bromomethyl)benzene (0.109 ml, 0.921 mmol), and the solution was stirred at room temperature for 4 hrs. When the reaction was complete by LC/MS, it was poured into water and extracted with EtOAc (2x 40ml). The combined extracts were washed with brine, dried ($Na_2SO_4$) then evaporated. This crude product was separated purified by column chromatography on silica gel on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ in 77% yield (260 mg, 0.649 mmol).

**HRMS**: for $C_{25}H_{28}N_4O$ calculated $(M+H)^+$ = 401.23359 m/z, found $(M+H)^+$ = 401.23282 m/z.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.66 – 7.64 (m, 1H), 7.60 – 7.55 (m, 1H), 7.53 – 7.49 (m, 2H), 7.33 – 7.30 (m, 4H), 7.30 – 7.23 (m, 1H), 5.17 (dq, *J* = 5.1, 1.2 Hz, 2H), 4.31 (s, 2H), 4.04 (s,2H), 3.53 (s, 2H), 3.29 (d, *J* = 7.1 Hz, 2H), 2.92 (broad d, *J* = 11.6 Hz, 2H), 2.02 (dd, *J* = 11.8, 2.4 Hz, 2H), 1.77 (dd, *J* = 10.9, 3.4 Hz, 1H), 1.70 (broad d, *J* = 13.2 Hz, 2H), 1.32 (ddd, *J* = 24.4, 12.2, 3.8 Hz, 2H).

**Oxime**: A mixture of 3-(3-((1-benzylpiperidin-4-yl)methyl)-5-methylene-2-oxotetrahydropyrimidin-1(2H)-yl)benzonitrile (260 mg, 0.649 mmol), hydroxylamine HCl (90 mg, 1.298 mmol) and N-ethyl-N-isopropylpropan-2-amine (0.226 ml, 1.298 mmol) in EtOH (10 ml) was refluxed for 5 h followed by stirring overnight at ambient temperature. The solvent was evaporated and the residue was purified by column chromatography on silica gel with an ISCO CombiFlash Rf®, using gradient of MeOH in $CHCl_3$, provided 233 mg of the desired product as a white solid in 83% yield.

**HRMS**: for $C_{25}H_{31}N_5O_2$ calculated $(M+H)^+$ = 434.25505 m/z, found $(M+H)^+$ = 434.25527 m/z.

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 7.52-7.50 (m, 1H), 7.50 – 7.46 (m, 1H), 7.45 – 7.35 (m, 6H), 7.33-7.29 (m, 1H), 5.17 (d, *J* = 6.7 Hz, 2H), 4.30 (s, 2H), 4.05 (s, 2H), 3.94 (broad s, 2H), 3.33 (broad s, 2H), 3.26-3.18 (m, 2H), 2.64-2.49 (m, 2H), 1.99 – 1.88 (m, 1H), 1.84 (d, *J* = 14.3 Hz, 2H), 1.49-1.40 (m, 2H).

**Compound 6**: The oxime prepared above (233 mg, 0.535 mmol) was dissolved in EtOH (30 ml) and shaken under an $H_2$ atmosphere (40 psi) in a Parr apparatus in the presence of Raney Nickel catalyst (250 mg, 4.23 mmol, wet weight) for 18 h at ambient temperature. The reaction was purged with Ar then filtered through a Celite® pad and evaporated. The crude product was purified by column chromatography on silica gel with an ISCO CombiFlash $R_f$® using a gradient of MeOH with 2% $NH_4OH$ in $CHCl_3$ to provide 68 mg of the desired product as a white solid in 30% yield;

this material was taken up in a dilute solution of trifluoroacetic acid and lyophilized to give the bis trifluoroacetate salt.

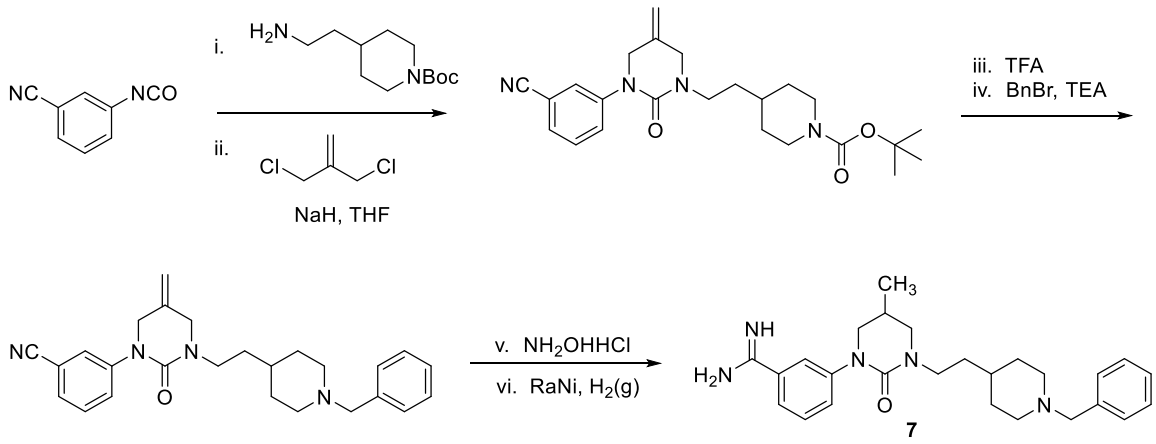
**HRMS**: for $C_{25}H_{33}N_5O$, calculated $(M+2H)^{+2}$ = 210.64153 m/z; found $(M+2H)^{+2}$ = 210.64215 m/z; $(M+H-CH_2C_6H_5)^+$ = 330.22801 m/z; calculated $(M+H)^+$ = 420.27579 m/z; found $(M+H)^+$ = 420.27472.

**PURITY**: 100%, retention time: 4.30 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.66 (dt, *J* = 1.7, 1.0 Hz, 1H), 7.64 – 7.59 (m, 1H), 7.59 – 7.56 (m, 2H), 7.36 – 7.28 (m, 5H), 3.71 (ddd, *J* = 11.4, 4.3, 1.7 Hz, 1H), 3.66 (s, 2H), 3.49 (dd, *J* = 11.4, 9.3 Hz, 1H), 3.44 (ddd, *J* = 12.1, 4.7, 1.7 Hz, 1H), 3.30 – 3.28 (m, 2H), 3.18 (dd, *J* = 11.9, 9.3 Hz, 1H), 3.02 (d, *J* = 11.8 Hz, 2H), 2.41-2.32 (m, 1H), 2.20 (t, *J* = 11.7 Hz, 2H), 1.81 (tt, *J* = 7.1, 3.8 Hz, 1H), 1.74 (d, *J* = 13.7 Hz, 2H), 1.45 – 1.31 (m, 2H), 1.10 (d, *J* = 6.6 Hz, 3H).

**$^{13}$C NMR** (101 MHz, Methanol-$d_4$) δ 168.1, 156.8, 146.1, 132.8, 132.4, 132.3, 131.2, 131.1, 131.0, 130.4, 130.3, 129.9, 127.2, 125.7, 62.2, 56.7, 55.4, 54.1, 54.00, 50.3, 34.1, 28.7 (2C), 28.7, 16.4.

<u>Amino(3-(3-(2-(1-benzylpiperidin-4-yl)ethyl)-5-methyl-2-oxotetrahydropyrimidin-1(2H)-yl)phenyl)methaniminium bis trifluoroacetate (**7**)</u>




**Urea Synthesis**: 3-Isocyanatobenzonitrile ( 2.6 g, 18.04 mmol ) was dissolved in anhydrous DMF (15 ml) and cooled to at 0 °C, then tert-butyl 4-(2-aminoethyl)piperidine-1-carboxylate ( 5.0 g, 21.90 mmol, 5.0 ml ) was added. This mixture was stirred and allowed to thaw to ambient temperature over 12 h. After this time product formation was confirmed by TLC (1:1 hexane: EtOAc) and LRMS, then the reaction mixture was poured into water (120 ml) resulting in the formation of a white precipitate. The aqueous layer then extracted with $CHCl_3$ (3 X 100 ml) and

the combined organic extracts were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The resulting crude product was purified over silica gel using MeOH in $CHCl_3$ as a gradient to obtain 3.5 g of the desired product as a white solid in 52% yield.

**HRMS**: for $C_{20}H_{28}N_4O_3$ calculated (M+Na) = 395.20536 m/z, found $(M+Na)^+$ = 395.20523 m/z.

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.68 (s, 1H), 7.63 (d, *J* = 8.3 Hz, 1H), 7.36 (t, *J* = 7.9 Hz, 1H), 7.29 (d, *J* = 1.5 Hz, 1H), 6.87 (s, 1H), 4.90 (t, *J* = 5.6 Hz, 1H), 4.08 (d, *J* = 12.6 Hz, 2H), 3.35-3.26 (m, 2H), 2.69 (t, *J* = 12.9 Hz, 2H), 1.69 (d, *J* = 13.0 Hz, 2H), 1.58 (s, 3H), 1.46 (s, 9H), 1.20-1.07 (m, 2H).

**Cyclic Urea Synthesis**: *tert*-Butyl 4-(2-(3-(3-cyanophenyl)ureido)ethyl)piperidine-1-carboxylate (3.5g, 9.4 mmol) was dissolved in anhydrous THF ( 80 ml ), then the solution was cooled to 0$^o$ C (ice bath) and 3-chloro-2-(chloromethyl)prop-1-ene (1.88g, 15.04 mmol, 1.741 ml ) was added. Sodium hydride (60% suspension in mineral oil, 0.977g, 24.43 mmol) was added portion-wise to the solution while cooling was maintained. Once sodium hydride addition was complete and the gas evolution subsided, the cooling bath was removed and the resulting mixture was stirred with heating at 75 °C for 5 h. After the reaction was complete, as indicated by TLC (1:1 hexane: EtOAc), this mixture was cooled to 0 °C and quenched by dropwise addition of a small amount of saturated aqueous $NH_4Cl$ solution. The quenched reaction was evaporated and partitioned between EtOAc (100 ml) and water (100 ml). The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to obtain a solid. Further purification by silica gel chromatography using a gradient of EtOAc in hexane (ISCO CombiFlash $R_f$®) provided 1.88 g of the desired product as an off-white solid in 48% yield.

**HRMS:** for $C_{24}H_{32}N_4O_3$ calculated (M+Na) = 447.23666 m/z, found $(M+Na)^+$ = 447.23695 m/z

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.57 – 7.51 (m, 2H), 7.43-7.39 (m,2H), 5.13 (d, J = 10.9 Hz, 2H), 4.23 (s, 2H), 4.06 (broad s, 2H), 3.97 (s, 2H), 3.43 (t, *J* = 7.5 Hz, 2H), 2.68 (t, *J* = 12.8 Hz, 2H), 1.70 (d, *J* = 13.3 Hz, 2H), 1.60 – 1.52 (m, 3H), 1.45 (s., 9H), 1.14 (ddd, *J* = 24.5, 12.4, 4.3 Hz, 2H).

**Boc-deprotection:** *tert*-Butyl 4-(2-(3-(3-cyanophenyl)-5-methylene-2-oxotetrahydropyrimidin-1(2H)-yl)ethyl)piperidine-1-carboxylate (1.88g, 4.43 mmol) was dissolved in anhydrous $CH_2Cl_2$ (25 ml) and then treated with 2,2,2-trifluoroacetic acid (5.05g, 44.3 mmol, 3.41ml). After the reaction was complete as indicated by TLC (1:1 hexane: EtOAc) the solvent was removed and the

crude product taken up into $CHCl_3$ and dried ($Na_2SO_4$). The $CHCl_3$ was evaporated and this material was taken up in EtOH and evaporated (2 x) to remove residual trifluoroacetic acid. The resulting amorphous solid was carried through to the next step.

**HRMS**: for $C_{19}H_{24}N_4O$ calculated $(M+H)^+ = 325.20229$ m/z; found $(M+H)^+ = 325.20243$ m/z

**N-Benzylation:** To a stirred solution of 3-(5-methylene-2-oxo-3-(2-(piperidin-4-yl)ethyl)tetrahydropyrimidin-1(2H)-yl)benzonitrile (1.01g, 3.1 mmol ) and triethylamine (0.95 ml, 0.69g, 6.82 mmol) in $CH_2Cl_2$ (20 ml) was added benzylbromide (0.405 ml, 0.583g, 3.41 mmol) at ambient temperature. The combined reaction mixture was stirred for 30 min after which TLC (10% MeOH in $CHCl_3$) confirmed that the starting material was consumed and a product formed. The solvent was evaporated and the product isolated by silica gel chromatography using a gradient of MeOH in $CHCl_3$. The product containing fractions were combined and evaporated to give 1.2 g of the product in 92% yield after the Boc-deprotection and *N*-benzylation steps.

**Oxime formation:** An ethanol solution (15 ml) of 3-(3-(2-(1-benzylpiperidin-4-yl)ethyl)-5-methylene-2-oxotetrahydropyrimidin-1(2H)-yl)benzonitrile (320 mg, 0.772 mmol), hydroxylamine hydrochloride (107 mg, 1.544 mmol) and Hunig's base (0.240 ml, 1.544 mmol) was stirred for 6 hr at ambient temperature then stirred for 16 h at 85 °C. After the reaction was complete by TLC (10% MeOH in $CHCl_3$), the solvent was removed and the product isolated by silica gel chromatography with an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ This provided 160 mg of the desired product in 46% yield.

**HRMS**: for $C_{26}H_{33}N_5O_2$ found $(M+2H)^{+2} = 224.63962$ m/z; calculated $(M+H)^+ = 448.27070$ m/z, found $(M+H)^+ = 448.27053$.

**Compound 7:** The oxime (158 mg, 0.353 mmol) prepared above was dissolved in EtOH (10 mL) and placed in a Parr apparatus and shaken under an $H_2$ atmosphere (38 psi) in the presence of Raney Nickel catalyst (124 mg, 2.11 mmol, wet weight) for 18 h at ambient temperature. After the reaction was complete by TLC (18% MeOH with 2% $NH_4OH$ in $CHCl_3$) the reaction mixture was purged with argon then filtered through a Celite® pad. The solvent was evaporated the crude product was purified by column chromatography on silica gel with an ISCO CombiFlash $R_f$®, using a gradient of MeOH with 2% $NH_4OH$ in $CHCl_3$. This provided 31 mg of the desired product

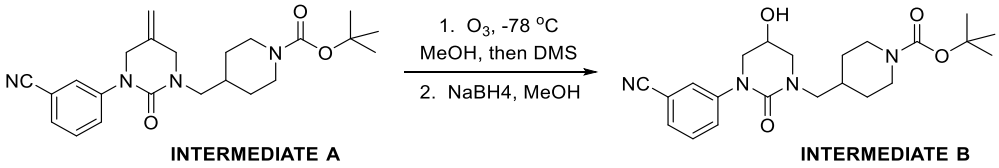
as a white solid in 20% yield. This product was taken up in dilute trifluoroacetic acid solution and lyophilized to give the bis trifluoroacetate salt.

**HRMS**: for $C_{20}H_{25}N_5O$ found $(M+2H)^{+2}$ = 217.64972 m/z; calculated $(M+H)^+$ = 434.29144 m/z, found $(M+H)^+$ = 434.29109 m/z.

**PURITY**: 100%, retention time: 4.71 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ: 7.67 (q, *J* = 1.2 Hz, 1H), 7.64 – 7.56 (m, 3H), 7.34 – 7.25 (m, 5H), 3.71 (ddd, *J* = 11.5, 4.3, 1.7 Hz, 1H), 3.56 (s, 2H), 3.52 – 3.34 (m, 5H), 3.15 (dd, *J* = 11.8, 9.1 Hz, 1H), 2.93 (d, *J* = 11.5 Hz, 2H), 2.08 (s, 2H), 1.77 (d, *J* = 10.0 Hz, 2H), 1.58 – 1.50 (m, 2H), 1.32 (s, 3H), 1.11 ppm (d, *J* = 6.6 Hz, 3H).

tert-butyl 4-((3-(3-cyanophenyl)-5-hydroxy-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (**INTERMEDIATE B**)




**Ozonolysis**: tert-Butyl 4-((3-(3-cyanophenyl)-5-methylene-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (440 mg, 1.072 mmol) was dissolved in MeOH (7 ml) and bubbled with $O_3$ gas at -78 °C for 4 h. Once the starting material was consumed the reaction was purged with argon then was quenched with dimethylsulfide (0235 ml, 3.22 mmol). The quenched mixture was stirred at room temperature for an additional 1 h. After 1 h, the solvent was evaporated and the residue was dissolved in $CH_2Cl_2$ and washed with water. The layers were separated and the aqueous layer was extracted with $CH_2Cl_2$ (3x25 ml). The combined organic layers were dried over $Na_2SO_4$ and concentrated under reduced pressure to isolate the product as a viscous oil. Further purification by silica gel chromatography with an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ provided the desired product (250 mg, 57%) as a white solid.

**HRMS**: for $C_{22}H_{28}N_4O_4$ calculated $(M+Na)^+$ = 435.02228 m/z; found $(M+H)^+$ = 435.19962 m/z.

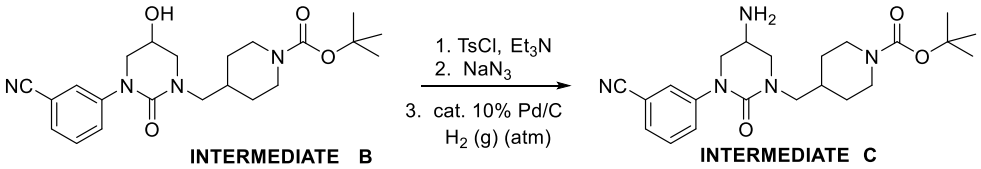
**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.50 (dd, *J* = 2.4, 0.8 Hz, 1H), 7.48 – 7.44 (m, 3H), 4.24 (s, 2H), 4.09 (d, *J* = 18.9 Hz, 2H), 4.01 (d, *J* = 0.6 Hz, 2H), 3.32 (s, 2H), 2.70 (t, *J* = 12.7 Hz, 2H), 1.84 (dtq, *J* = 11.2, 7.4, 3.6 Hz, 1H), 1.65 (d, *J* = 14.3 Hz, 3H), 1.45 (d, *J* = 0.6 Hz, 9H), 1.30 – 1.12 (m, 3H).

**Reduction to intermediate B**: To a stirring solution of tert-butyl 4-((3-(3-cyanophenyl)-2,5-dioxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (240 mg, 0.582 mmol) in MeOH (5 mL), sodium borohydride (44.0 mg, 1.164 mmol) was added in portions at room temperature; this reaction was stirred for 6 h. After the reaction was complete, the mixture was quenched with saturated $NH_4Cl$ and the solvent was removed under reduced pressure. The residue was taken up in $CH_2Cl_2$, water was added and the layers were separated. The aqueous layer was extracted with $CH_2Cl_2$ (3x25 ml) and the combined organic layers were dried over $Na_2SO_4$ then concentrated under reduced pressure. Purification of the residue by silica gel chromatography with an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ provided the desired product (206 mg, 85%) as a white solid.

**HRMS**: for $C_{22}H_{30}N_4O_4$ calculated $(M+Na)^+$ = 437.21593 m/z; found $(M+Na)^+$ = 437.21479 m/z.

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.62 – 7.59 (m, 1H), 7.58 – 7.54 (m, 1H), 7.43 – 7.40 (m, 2H), 4.29 (br.s, 1H), 4.09 (d, *J* = 13.1 Hz, 2H), 3.88 (dd, J = 11.8, 3.0 Hz, 1H), 3.64 (ddt, *J* = 12.1, 7.5, 2.8 Hz, 2H), 3.37 (ddd, *J* = 12.4, 4.2, 2.1 Hz, 1H), 3.24 (br.s, 2H), 2.83 – 2.62 (m, 3H), 1.90 (ttt, *J* = 11.0, 7.3, 3.7 Hz, 1H), 1.44 (s, 9H), 1.29 – 1.10 (m, 3H).

<u>tert-butyl 4-((5-amino-3-(3-cyanophenyl)-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (**INTERMEDIATE C**)</u>




**Tosylation**: To tert-butyl 4-((3-(3-cyanophenyl)-5-hydroxy-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (1.93 g, 4.66 mmol) in $CH_2Cl_2$ (23.28 ml), TEA (1.947 ml, 13.97 mmol) followed by DMAP (0.057 g, 0.466 mmol) were added at 0°C. This mixture was stirred for 20 min, then p-toluenesulfonyl chloride (0.976 g, 5.12 mmol) was added at 0 °C and this mixture was stirred for another 30 min. The reaction was left for overnight and stirred at room temperature. After 20 h the solvent was removed by evaporation and the crude product was applied directly onto silica gel and purified by column chromatography with an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$. After purification the desired product (2.21 g, 83% yield) was obtained as a yellow color viscous oil.

**HRMS**: for $C_{29}H_{36}N_4O_6S$ calculated $(M+Na)^+$ = 591.22478 m/z; found $(M+Na)^+$ = 591.22453 m/z.

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.74 (d, *J* = 8.3 Hz, 2H), 7.46 – 7.37 (m, 4H), 7.31 (d, *J* = 8.1 Hz, 2H), 5.07 (dt, *J* = 5.9, 2.9 Hz, 1H), 4.06 (s, 2H), 3.90 (dd, *J* = 13.1, 2.5 Hz, 1H), 3.75 – 3.65 (m, 2H), 3.51 (dt, *J* = 13.5, 2.9 Hz, 1H), 3.16 (s, 2H), 2.64 (t, *J* = 12.7 Hz, 2H), 2.43 (s, 3H), 1.85-1.75 (m, 1H), 1.58 (s, 2H), 1.43 (s, 9H), 1.11 (dq, *J* = 11.5, 6.3, 5.4 Hz, 2H).

**Azidation**: To tert-butyl 4-((3-(3-cyanophenyl)-2-oxo-5-(tosyloxy)tetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (2.21 g, 3.89 mmol) in DMF (19.43 ml), sodium azide (3.03 g, 46.68 mmol) was added and the mixture was heated overnight at 60 °C. After this time the reaction was cooled to room temperature and the solvent was condensed under reduced pressure. The resulting material was taken up in $CH_2Cl_2$ (150 ml) and extracted with water (5x), then sat. $NaHCO_3$ and brine. The organic layer was dried over $Na_2SO_4$ and evaporated. Further purification of the crude by silica gel chromatography with an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ provided the desired product (1.53 g, 90% yield) as a colorless viscous oil.

**HRMS**: $C_{22}H_{29}N_7O_3$ calculated $(M+Na)^+$ = 462.22241 m/z, found $(M+Na)^+$ = 462.22264 m/z. **$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.59 (tq, *J* = 2.1, 1.1 Hz, 1H), 7.55 (dtd, *J* = 6.4, 2.5, 0.8 Hz, 1H), 7.47 – 7.42 (m, 2H), 4.13 – 4.07 (m, 2H), 4.01 – 3.94 (m, 1H), 3.70 (dddd, *J* = 9.3, 4.8, 2.4, 1.3 Hz, 2H), 3.44 (ddd, *J* = 12.6, 4.4, 2.1 Hz, 1H), 3.31 (s, 2H), 2.70 (t, *J* = 12.7 Hz, 2H), 1.88 (ddp, *J* = 11.3, 7.5, 3.7 Hz, 1H), 1.67 (h, *J* = 3.6 Hz, 3H), 1.44 (d, *J* = 0.8 Hz, 9H), 1.19 (tt, *J* = 13.5, 8.3 Hz, 2H).

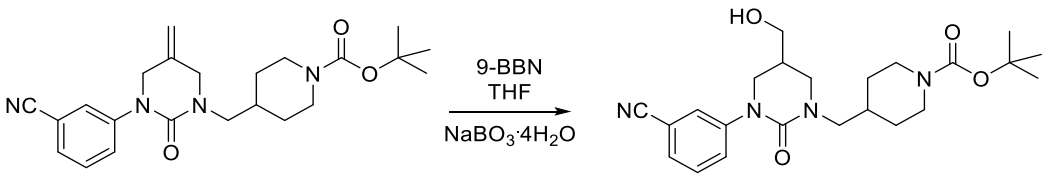
**Reduction of azide to intermediate C**: To tert-butyl 4-((5-azido-3-(3-cyanophenyl)-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (550 mg, 1.251 mmol) in MeOH (2503 µl), 10% Pd-C (133 mg, 0.125 mmol) was added. This suspension was stirred under $H_2$ (gas) at atmospheric pressure for 1 h. After the reaction was complete, the flask was flushed with Ar gas, then was diluted with methanol and filtered through a Celite pad to remove the catalyst. Purification by silica gel chromatography with an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ provided the desired product (517 mg) as a solid in quantitative yields

**HRMS**: $C_{22}H_{31}N_5O_3$ calculated $(M+K)^+$ = 452.20585 m/z, found $(M+K)^+$ = 252.20590 m/z.

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.60 (dt, *J* = 2.2, 0.9 Hz, 1H), 7.58 – 7.53 (m, 1H), 7.41 (dt, *J* = 4.2, 0.7 Hz, 2H), 4.10 (s, 2H), 3.87 – 3.77 (m, 1H), 3.60 – 3.53 (m, 1H), 3.52 – 3.45 (m,

2H), 3.21 (ddd, *J* = 11.5, 5.4, 1.9 Hz, 2H), 2.69 (t, *J* = 12.8 Hz, 2H), 1.90 (ttt, *J* = 11.1, 7.3, 3.7 Hz, 1H), 1.70-1.62 (m, 2H), 1.44 (s, 9H), 1.24 – 1.11 (m, 3H).

tert-butyl 4-((3-(3-cyanophenyl)-5-(hydroxymethyl)-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate ( **INTERMEDIATE D**)



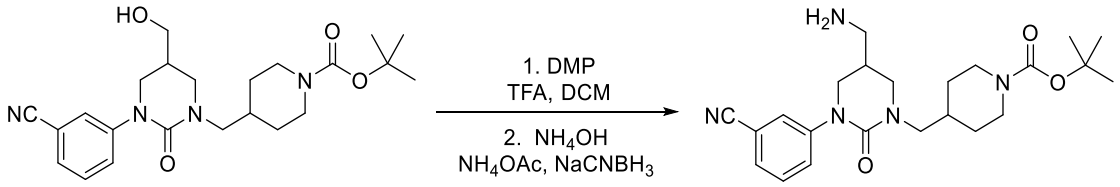

**INTERMEDIATE A** **INTERMEDIATE D**

To a solution of tert-butyl 4-((3-(3-cyanophenyl)-5-methylene-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (250mg, 0.609 mmol) in THF (1 ml) was added 9-BBN (1.462 ml, 0.731 mmol) slowly and the mixture was stirred at room temp for 6 hrs, followed by the addition of a suspension of sodium perborate (276mg) in water (3ml). The combined reaction mixture was then stirred overnight. Thereafter, the crude reaction mixture was filtered, washed solid with diethyl ether, extracted the aqueous layer with more ether, combined ether extracts dried and evaporated to give crude material which was purified by silica gel chromatography with an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$. After purification 120 mg of the desired product was obtained as a white solid in 46% yield.

**HRMS**: $C_{23}H_{32}N_4O_4$ Calculated $(M+Na)^+$ = 451.23158 m/z, found $(M+Na)^+$ = 451.23187 m/z.

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 7.73 (dt, *J* = 2.2, 1.0 Hz, 1H), 7.62 (ddd, *J* = 7.9, 2.3, 1.5 Hz, 1H), 7.55 – 7.52 (m, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 4.79 (t, *J* = 5.2 Hz, 1H), 3.92 (d, *J* = 13.1 Hz, 2H), 3.74 – 3.67 (m, 1H), 3.60 – 3.52 (m, 1H), 3.46 (t, *J* = 5.8 Hz, 2H), 3.42 – 3.36 (m, 1H), 3.25 – 3.18 (m, 1H), 3.17 (d, *J* = 5.2 Hz, 1H), 3.12 (dd, *J* = 13.5, 7.0 Hz, 1H), 2.76-2.63 (m, 2H), 2.30-2.21 (m, 1H), 1.89-1.79 (1H), 1.64-1.53 (m, 2H), 1.39 (s, 9H), 1.00 (tq, *J* = 12.3, 7.4, 6.2 Hz, 2H).

tert-butyl 4-((5-(aminomethyl)-3-(3-cyanophenyl)-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (**INTERMEDIATE E**)




**INTERMEDIATE D** **INTERMEDIATE E**

**Oxidation**: To a stirred solution of tert-butyl 4-((3-(3-cyanophenyl)-5-(hydroxymethyl)-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (277 mg, 0.646 mmol) in $CH_2Cl_2$ (3.5 ml), Dess-Martin Periodinane (548 mg, 1.293 mmol) followed by TFA (149 µl, 1.939 mmol) were added at ambient temperature and stirred for 90 min. Subsequently, the reaction was quenched with 10% of $Na_2S_2O_3$ (5 ml) followed by sat. $NaHCO_3$ solution (5 ml) and stirred vigorously for 30 min. Afterwards the reaction mixture was transferred to a separatory funnel and the separated aqueous layer washed thrice with $CH_2Cl_2$ (10 ml). The combined organic layers were dried over MgSO4 and concentrated under reduced pressure. After purification by silica gel chromatography with an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ there was obtained the desired product as a gummy solid in quantitative yields (275 mg).

**HRMS**: for hemiacetal $C_{24}H_{34}N_4O_5$: Calculated $(M+Na)^+$ = 481.24214 m/z, found $(M+Na)^+$ = 481.24163 m/z

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 9.79 (s, 1H), 7.60 – 7.58 (m, 1H), 7.54 (dtd, *J* = 7.0, 2.3, 0.8 Hz, 1H), 7.47 – 7.43 (m, 2H), 4.18 – 3.95 (m, 4H), 3.80 (ddd, *J* = 12.1, 4.4, 1.4 Hz, 1H), 3.72 – 3.66 (m, 1H), 3.55 – 3.08 (m, 2H), 3.04 – 2.95 (m, 1H), 2.70 (t, *J* = 12.7 Hz, 2H), 1.91 (qdt, *J* = 10.6, 7.1, 3.9 Hz, 2H), 1.65 (t, *J* = 12.4 Hz, 2H), 1.59 – 1.54 (m, 1H), 1.45 (s, 9H), 1.29 – 1.11 (m, 2H).

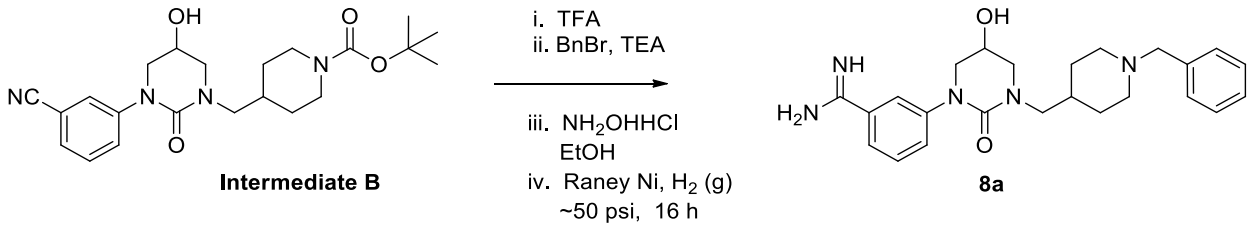
**Reductive Amination to intermediate E**: To a stirred solution of the above aldehyde (121 mg, 0.284 mmol) in saturated $NH_4OAc$ in EtOH (5 ml), was added sodium cynaoborohydride (53.5 mg, 0.851 mmol) and ammonia (2.2 mL, 102 mmol) (30% aq. solution, $NH_4OH$ solution) at room temperature. This reaction mixture was refluxed for 15 h. The reaction was then cooled to room temperature, the solvent was evaporated and the resulting heterogeneous residue was separated between $H_2O$ and $CH_2Cl_2$. The aqueous layer washed twice with $CH_2Cl_2$ (2 X 10 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to obtain an off white solid. Further purification by silica gel chromatography with an

ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ yielded 62 mg (51%) of the desired product as a colorless oil.

**HRMS**: $C_{23}H_{33}N_5O_3$ calculated $(M+Na)^+$ = 450.24756 m/z, found $(M+Na)^+$ = 450.24684 m/z.

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.60 (dq, *J* = 1.9, 0.9 Hz, 1H), 7.59 – 7.54 (m, 1H), 7.41 – 7.38 (m, 2H), 4.10 (d, *J* = 11.9 Hz, 2H), 3.77 (ddd, *J* = 11.4, 4.3, 1.3 Hz, 1H), 3.56 (dd, *J* = 11.3, 8.6 Hz, 1H), 3.51 – 3.44 (m, 1H), 3.24 (dd, *J* = 11.7, 8.5 Hz, 2H), 2.83 (dd, *J* = 6.9, 1.2 Hz, 2H), 2.69 (t, *J* = 12.8 Hz, 2H), 2.30 – 2.18 (m, 1H), 1.91 (ddp, *J* = 11.4, 7.6, 3.7 Hz, 1H), 1.65 (dq, *J* = 12.8, 3.7, 2.9 Hz, 2H), 1.44 (d, *J* = 0.7 Hz, 9H), 1.37 – 1.23 (m, 2H), 1.24 – 1.11 (m, 3H).

3-(3-((1-benzylpiperidin-4-yl)methyl)-5-hydroxy-2-oxotetrahydropyrimidin-1(2H)-yl)benzimidamide (**8a**)




**Boc Deprotection**: To a stirring solution of tert-butyl 4-((3-(3-cyanophenyl)-5-hydroxy-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (188 mg, 0.454 mmol) in $CH_2Cl_2$ (3628 µl), TFA (907 µl) was added at room temperature; this mixture was stirred for 30 min. After the reaction was complete, the crude was evaporated with chloroform then ethanol; this process was repeated three times to remove residual TFA. There was obtained the desired product of suitable purity to be used directly in the next step.

**LRMS**: $(M+H)^+$ = 315.2 m/z.

**N-Benzylation**: To a solution of 3-(5-hydroxy-2-oxo-3-(piperidin-4-ylmethyl)tetrahydro pyrimidin-1(2H)-yl)benzonitrile (143 mg, 0.455 mmol) in acetonitrile (5 ml), benzylbromide (0.060 ml, 0.500 mmol) and triethylamine (0.190 ml, 1.365 mmol) were added; this mixture was stirred for 30 min. After the reaction was complete, the solvent was evaporated and the product isolated by silica gel chromatography with an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ to obtain the desired material as a solid in quantitative yield.

**HRMS**: for $C_{24}H_{28}N_4O_2$ calculated $(M+H)^+$ = 405.22850 m/z; found $(M+H)^+$ = 405.22848 m/z.
**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.66 – 7.64 (m, 1H), 7.60 (s, 2H), 7.57-7.52 (m, 1H), 7.44 – 7.37 (m, 5H), 4.31 (s, 2H), 4.17 (s, 2H), 3.83 (d, *J* = 12.3 Hz, 1H), 3.69 (d, *J* = 12.3 Hz, 1H), 3.59 (d, *J* = 12.4 Hz, 1H), 3.55 – 3.40 (m, 3H), 2.98 (d, *J* = 12.6 Hz, 1H), 2.86 (s, 2H), 2.00 (s, 3H), 1.89-1.75 (m, 3H)

**N-hydroxybenzimidamide:** To a reaction flask purged with argon was added 3-(3-((1-benzylpiperidin-4-yl)methyl)-5-hydroxy-2-oxotetrahydropyrimidin-1(2H)-yl)benzonitrile (200 mg, 0.494 mmol) followed by Ethanol (4944 µl), hydroxylamine hydrochloride (68.7 mg, 0.989 mmol) and Hunig'sBase (173 µl, 0.989 mmol) at RT. The combined solution was stirred for 6 h at 85 °C followed by stirring the reaction overnight at room temperature. After reaction completion as monitored by TLC and LCMS analysis, the solvent was evaporated and the material purified by silica gel chromatography with an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$, provided the desired product as a white solid. (90 mg, 42% yield).

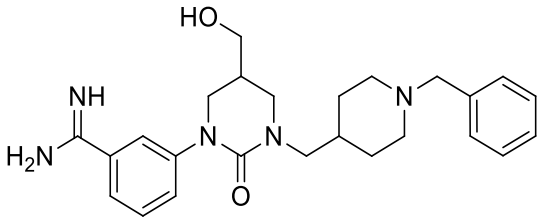
**HRMS**: for $C_{24}H_{31}N_5O_3$ calculated $(M+H)^+$ = 438.24997; found $(M+H)^+$ = 438.24982 m/z;
**$^1$H NMR** (400 MHz, Methanol-d4) δ 7.65 - 7.56 (m, 3H), 7.52-7.46 (m, 6H), 4.30 (s, 2H), 4.28 (t, J = 3.4 Hz, 1H), 3.97 (dd, J = 12.2, 2.7 Hz, 1H), 3.68 (dd, J = 12.4, 3.2 Hz, 1H), 3.62 (dt, J = 11.8, 6.2 Hz, 1H), 3.52 (br.d, J = 12.5 Hz, 2H), 3.40 - 3.32 (m, 3H), 2.99 (t, J = 12.9 Hz, 2H), 2.02 (t, J = 10.9 Hz, 3H), 1.49 (q, J = 13.3, 12.8 Hz, 2H).

**Compound 8a**: The purified N-hydroxybenzimidamide (81 mg, 0.185 mmol), from the previous step, was dissolved in ethanol (5 ml), to which Raney Ni (110 mg, wet weight, washed with ethanol) was added. The combined mixture was placed on a Parr apparatus and shaken for 15-18 h under a hydrogen atmosphere at 50 psi. After the reaction was complete, the reaction mixture was purged with Ar gas and filtered through Celite pad washing with methanol. The solvent was evaporated and the product isolated by silica gel column chromatography on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, to obtain the desired product as a white solid (13 mg, 17% yield).
**HRMS**: for $C_{24}H_{31}N_5O_2$ calculated $(M+H)^+$ = 422.25505; found $(M+H)^+$ = 422.25498 m/z;
**Purity**: 100%, retention time 2.01 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.71 (td, *J* = 1.9, 1.0 Hz, 1H), 7.65 – 7.61 (m, 1H), 7.60 – 7.57 (m, 2H), 7.36 – 7.33 (m, 4H), 7.32 – 7.28 (m, 1H), 4.26 (dt, *J* = 6.5, 3.2 Hz, 1H), 3.98 – 3.93 (m, 1H), 3.67 (ddd, *J* = 12.4, 3.4, 0.9 Hz, 1H), 3.61 (dd, *J* = 4.2, 2.2 Hz, 1H), 3.39 – 3.32 (m, 2H), 3.26 (dd, *J* = 13.8, 6.9 Hz, 1H), 3.04 – 2.97 (m, 2H), 2.19 (dd, *J* = 12.0, 2.5 Hz, 2H), 2.16 – 2.14 (m, 1H), 1.82 – 1.74 (m, 3H), 1.43-1.32 (m, 3H).

**$^{13}$C NMR** (101 MHz, Methanol-$d_4$) δ 171.9, 156.8, 145.7, 137.3, 135.8, 131.1(2C), 130.8, 130.0, 129.4(2C), 128.8, 126.8, 125.9, 63.9, 62.4, 56.0, 54.7, 54.4, 54.2, 48.8, 35.5, 30.2, 30.1.

3-(3-((1-benzylpiperidin-4-yl)methyl)-5-(hydroxymethyl)-2-oxotetrahydropyrimidin-1(2H)-yl)benzimidamide (**8b**)




**8b**

**Compound 8b** was synthesized from **intermediate D** following a similar synthetic sequence as described for **Compound 8a**. After purification by silica gel column chromatography on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, the product was obtained as a white solid in 13% yield (14 mg).

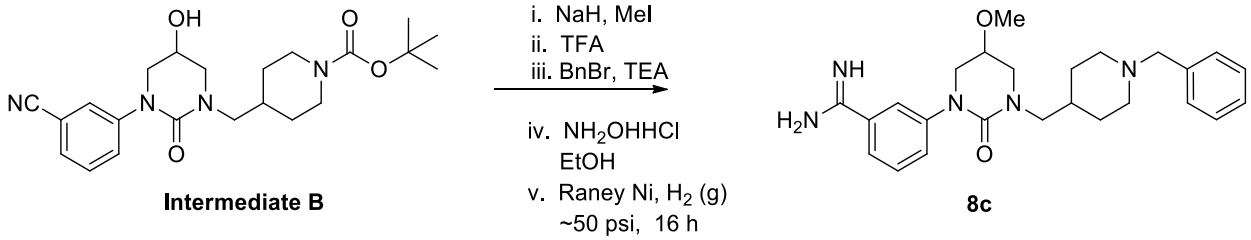
**HRMS**: $C_{25}H_{33}N_5O_2$: Calculated $(M+H)^+$ = 436.27070 m/z, found $(M+H)^+$ = 436.27050 m/z.

**Purity**: 96%, retention time 3.80 min and 4.80 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.64 – 7.62 (m, 1H), 7.58 – 7.55 (m, 1H), 7.55 – 7.51 (m, 2H), 7.34 – 7.30 (m, 4H), 7.30 – 7.23 (m, 1H), 3.80 (dd, *J* = 11.6, 4.2 Hz,1H), 3.67 – 3.59 (m, 3H), 3.55-3.49 (m, 3H), 3.30 – 3.26 (m, 3H), 2.92 (br.d, *J* = 11.6 Hz, 2H), 2.43-2.33 (m, 1H), 2.08 – 1.98 (m, 2H), 1.70 (d, *J* = 13.5 Hz, 3H), 1.33 (q, *J* = 10.8, 10.4 Hz, 2H).

**$^{13}$C NMR** (101 MHz, Methanol-$d_4$) δ 167.8, 157.2, 145.7, 138.4, 135.8, 130.8, 130.7, 130.5, 130.0, 129.2, 128.4, 126.6, 125.9, 125.4, 64.3, 62.3, 54.9, 54.3 (2C), 51.8, 50.1, 36.4, 35.9, 30.6 (2C).

3-(3-((1-benzylpiperidin-4-yl)methyl)-5-methoxy-2-oxotetrahydropyrimidin-1(2H)-yl)benzimidamide (**8c**)




**O-Methylation:** To a solution of tert-butyl 4-((3-(3-cyanophenyl)-5-hydroxy-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (500 mg, 1.206 mmol) in THF (8 ml), sodium hydride (73 mg, 1.809, 60% in mineral oil) was added in portions at room temperature; this mixture was stirred for 15 min. After 15 min, iodomethane (0.150 ml, 2.413 mmol) was added and the reaction was stirred for an additional 15 min at room temperature followed by heating at reflux for 2 h. After the reaction was complete, as monitored by TLC and LCMS, it was cooled to room temperature and quenched with aq. $NH_4Cl$, extracted with $CH_2Cl_2$ (3 X 25 ml), dried over $Na_2SO_4$ and concentrated under reduced pressure. Further purification by silica gel column chromatography with an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$, provided the desired product (323 mg, 65% yield) as a yellow oil.

**HRMS**: for $C_{23}H_{32}N_4O_4$ calculated $(M+Na)^+$ = 451.23158 m/z; found $(M+Na)^+$ = 451.23128 m/z.

**$^1$H NMR** (400 MHz, Chloroform-d) δ 7.59 (dp, J = 2.1, 0.7 Hz, 1H), 7.58 - 7.54 (m, 1H), 7.42 - 7.39 (m, 2H), 4.17 - 4.02 (m, 2H), 3.88 - 3.76 (m, 2H), 3.71 (ddd, J = 11.7, 4.7, 1.9 Hz, 1H), 3.61 - 3.53 (m, 1H), 3.44 (dd, J = 4.4, 1.9 Hz, 1H), 3.42 - 3.39 (m, 3H), 3.25 - 3.19 (m, 1H), 2.69 (t, J = 12.7 Hz, 2H), 1.89 (ddp, J = 11.3, 7.5, 3.8 Hz, 1H), 1.67 (s, 2H), 1.44 (d, J = 0.5 Hz, 9H), 1.28 - 1.10 (m, 3H).

**Boc Deprotection**: To a stirred solution of tert-butyl 4-((3-(3-cyanophenyl)-5-methoxy-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (303 mg, 0.707 mmol) in $CH_2Cl_2$ (7071 µl) was added TFA (545 µl, 7.07 mmol); this mixture was stirred for 30 min at room temperature. After the reaction was complete, the crude was triturated with chloroform followed by ethanol. The trituration repeated three times with each solvent. After solvent evaporation an

orange colored viscous oil was obtained. The crude material was carried through to the next step without any purification.

**LRMS**: $(M+H)^+$ = 329.2 m/z

**N-Benzylation**: To a solution of 3-(5-methoxy-2-oxo-3-(piperidin-4-ylmethyl)tetrahydropyrimidin-1(2H)-yl)benzonitrile (232 mg, 0.706 mmol) in $CH_2Cl_2$ (7 mL), (bromomethyl)benzene (0.092 mL, 0.777 mmol) and TEA (0.295 mL, 2.119 mmol) were added at room temperature then stirred for 30 min. After reaction completion, the solvent was removed by evaporation to obtain the crude material as an orange colored viscous oil. Further purification by silica gel column chromatography with an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$, provided the desired product as a solid in quantitative yields (350 mg).

**HRMS**: for $C_{25}H_{30}N_4O_2$ calculated $(M+H)^+$ = 419.24415 m/z; found $(M+H)^+$ = 419.24424 m/z.

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.62 – 7.57 (m, 1H), 7.56 – 7.47 (m, 3H), 7.44 – 7.36 (m, 5H), 4.15 (d, *J* = 18.2 Hz, 2H), 3.83 (dd, *J* = 11.9, 2.6 Hz, 1H), 3.74 (p, *J* = 3.4 Hz, 1H), 3.71 – 3.64 (m, 1H), 3.61 (dd, *J* = 12.4, 3.5 Hz, 1H), 3.48 (d, *J* = 11.9 Hz, 2H), 3.43 – 3.36 (m, 2H), 3.35 (d, *J* = 0.9 Hz, 2H), 3.27 (dd, *J* = 13.9, 6.1 Hz, 1H), 2.69 (s, 2H), 1.98 (q, *J* = 7.9, 6.9 Hz, 3H), 1.84 (d, *J* = 13.4 Hz, 1H).

**N-hydroxybenzimidamide:** To 3-(3-((1-benzylpiperidin-4-yl)methyl)-5-methoxy-2-oxotetra hydropyrimidin-1(2H)-yl)benzonitrile (340 mg, 0.812 mmol) in ethanol (16.200 mL), hydroxylamine hydrochloride (113 mg, 1.625 mmol) followed by Hunig's Base (0.284 mL, 1.625 mmol) were added at room temperature. The resulting orange colored reaction mixture was stirred for 6 h at 85 °C followed by overnight stirring at room temperature. After the reaction was complete, the solvent was evaporated and the product isolated by silica gel column chromatography with an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$, to obtain the desired product (189 mg, 52% yield) as a yellow color solid.

**HRMS**: for $C_{25}H_{33}N_5O_3$ calculated $(M+H)^+$ = 452.26562 m/z; found $(M+H)^+$ = 452.26590 m/z.

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 9.60 (s, 1H), 7.55 – 7.45 (m, 2H), 7.45 – 7.32 (m, 5H), 7.32 – 7.20 (m, 2H), , 5.79 (s, 2H), 4.35-3.94 (m, 2H), 3.88 – 3.77 (m, 2H), 3.67 – 3.60 (m, 1H), 3.55 (dd, *J* = 12.4, 3.4 Hz, 1H), 3.37-3.22 (m, 4H), 3.30 (s, 3H), 3.24 – 3.11 (m, 3H), 1.75 (s, 3H), 1.53 – 1.28 (m, 2H).

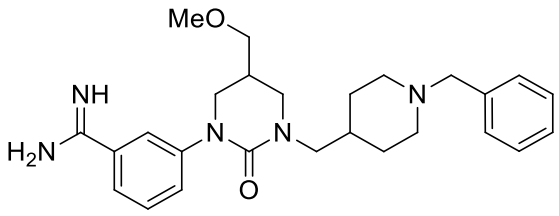
**Compound 8c:** To the oxime, 3-(3-((1-benzylpiperidin-4-yl)methyl)-5-methoxy-2-oxotetra hydropyrimidin-1(2H)-yl)-N-hydroxybenzimidamide (121 mg, 0.268 mmol) in ethanol (6 ml), Raney nickel (157 mg, 2.68 mmol,) was added and the suspension shaken overnight on a Parr apparatus under $H_2$ atmosphere at 50 psi. After reaction completion, the crude mixture was purged with argon gas then filtered through a Celite pad with copious washing with methanol. Further purification of the product by silica gel column chromatography on an Isco CombiFlash $R_f^®$ using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, provided the desired product (44 mg, 38% yield) as a light yellow solid.

**HRMS**: for $C_{25}H_{33}N_5O_2$ calculated $(M+2H)^{+2}$ = 218.63899 m/z; found $(M+2H)^{+2}$ = 218.63928 m/z.

**Purity**: 100%, retention time 0.92 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.67 (dt, *J* = 1.9, 1.1 Hz, 1H), 7.64 – 7.57 (m, 3H), 7.42 – 7.31 (m, 5H), 3.96 (dd, *J* = 12.4, 2.6 Hz, 1H), 3.89 – 3.85 (m, 1H), 3.83 (s, 2H), 3.78 (ddd, *J* = 12.4, 3.5, 2.5 Hz, 1H), 3.66 (dd, *J* = 12.9, 3.2 Hz, 1H), 3.50 (dt, *J* = 12.9, 2.9 Hz, 1H), 3.42 (s, 3H), 3.14 (dd, *J* = 11.5, 3.2 Hz, 2H), 2.42 (tt, *J* = 12.1, 3.0 Hz, 2H), 1.96 – 1.79 (m, 4H), 1.49 – 1.35 (m, 2H), 1.29 (br. S, 1H).

3-(3-((1-benzylpiperidin-4-yl)methyl)-5-(methoxymethyl)-2-oxotetrahydropyrimidin-1(2H)-yl)benzimidamide (**8d**)




**8d**

**Compound 8d** was synthesized from **intermediate D** following similar synthetic sequence as described for **Compound 8c**. After purification by silica gel column chromatography on an ISCO CombiFlash $R_f^®$ using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, the desired product was obtained as a white solid in 39% yield (60 mg).

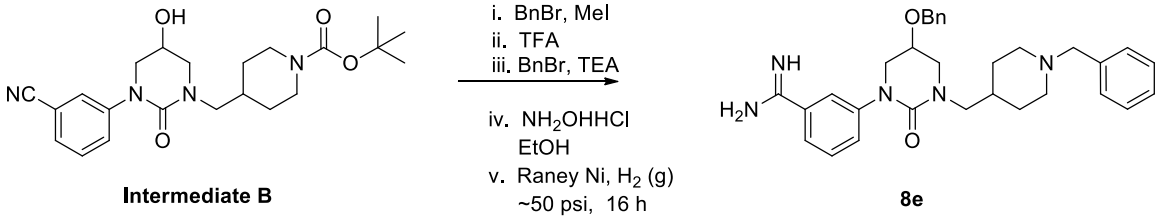
**HRMS**: $C_{26}H_{35}N_5O_2$: Calculated $(M+H)^+$ = 450.28635 m/z, found $(M+H)^+$ = 450.28638 m/z.

**Purity**: 100%, retention time 4.40 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.68 (dq, *J* = 1.8, 1.3 Hz, 1H), 7.65 – 7.59 (m, 3H), 7.45 (td, *J* = 7.4, 6.4, 3.2 Hz, 5H), 4.11 (br. s, 2H), 3.82 (ddd, *J* = 11.6, 4.3, 1.3 Hz, 1H), 3.66 (dd, *J* = 11.6, 8.0 Hz, 1H), 3.53 (ddd, *J* = 11.9, 4.8, 1.3 Hz, 2H), 3.50 (dd, *J* = 6.7, 1.5 Hz, 2H), 3.40-3.33 (m, 7H), 2.78 (br.s, 2H), 2.61 – 2.50 (m, 1H), 2.06-1.96 (m, 1H), 1.90 (d, *J* = 14.7 Hz, 2H), 1.49 (d, *J* = 14.2 Hz, 2H).

**$^{13}$C NMR** (101 MHz, Methanol-$d_4$) δ 168.0, 156.9, 146.1, 132.3, 132.1, 132.0 (2C), 131.0 (2C), 130.5, 130.0, 129.9, 126.8, 125.8, 72.7, 62.0, 59.3 (2C), 53.8, 53.3, 51.8, 50.0, 34.0, 33.9, 28.5 (2C).

3-(5-(benzyloxy)-3-((1-benzylpiperidin-4-yl)methyl)-2-oxotetrahydropyrimidin-1(2H)-yl)benzimidamide (**8e**)




**O-Benzylation**: To a reaction flask purged with argon was added tert-butyl 4-((3-(3-cyanophenyl)-5-hydroxy-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (405 mg, 0.977 mmol) followed by THF (7 ml). To the above clear solution sodium hydride (58.6 mg, 1.466 mmol) was added in portions at RT and stirred for 15 min. After 15 min, (bromomethyl)benzene (0.232 ml, 1.954 mmol) was added dropwise over 5 min and the combined solution stirred for additional 15 min at RT followed by reflux at 65 °C for 4 h. After reaction completion as monitored by TLC and LCMS analysis, the mixture was cooled to room temperature and quenched with aq. $NH_4Cl$, extracted with $CH_2Cl_2$ (3 X 10 ml), dried over $Na_2SO_4$ and concentrated under reduced pressure. Further purification of the crude by silica gel column chromatography silica gel column chromatography with an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$, provided the desired product as a colorless viscous oil (201 mg, 41% yield).

**HRMS**: for $C_{29}H_{30}N_4O_4$ calculated $(M+Na)^+$ = 527.26288 m/z; found $(M+Na)^+$ = 527.26298 m/z.

**$^1$H NMR** (400 MHz, Chloroform-d) δ 7.56 (td, J = 1.3, 0.6 Hz, 1H), 7.55 - 7.52 (m, 1H), 7.42 - 7.39 (m, 2H), 7.37 - 7.27 (m, 5H), 4.61 (s, 2H), 4.15 - 4.02 (m, 2H), 4.02 - 3.95 (m, 1H), 3.86 -

3.81 (m, 1H), 3.72 (ddd, J = 12.0, 5.0, 1.8 Hz, 1H), 3.60 - 3.54 (m, 1H), 3.44 (ddd, J = 12.3, 4.6, 1.8 Hz, 1H), 2.65 (t, J = 10.7 Hz, 2H), 1.88 (dqd, J = 11.3, 7.5, 3.8 Hz, 1H), 1.72 - 1.62 (m, 3H), 1.44 (s, 9H), 1.23 - 1.09 (m, 3H).

**Boc Deprotection**:To a stirring solution of tert-butyl 4-((5-(benzyloxy)-3-(3-cyanophenyl)-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (180 mg, 0.357 mmol) in $CH_2Cl_2$ (3 ml), TFA (0.8 ml) was added at once and combined solution stirred for 30 min at room temperature. After reaction completion, the solvent was evaporated off by diluting with chloroform followed by ethanol. After solvent evaporations the desired product was obtained as an off white color viscous oil. The resulting crude material was directly carried through to the next step without purification.
**LRMS**: $(M+H)^+$ = 405 m/z

**N-Benzylation**: To a stirring solution of 3-(5-(benzyloxy)-2-oxo-3-(piperidin-4-ylmethyl)tetrahydropyrimidin-1(2H)-yl)benzonitrile (180 mg, 0.445 mmol) in $CH_2Cl_2$ (5 ml), (bromomethyl)benzene (58.2 µl, 0.489 mmol) followed by TEA (186 µl, 1.335 mmol) were added and the resulting yellow colored reaction mixture stirred for 30 min at room temperature. After reaction completion as monitored by TLC and LCMS analysis, solvent evaporation followed by purification via silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$, provided the desired product as a colorless viscous oil (190 mg, 86% yield).
**HRMS**: for $C_{31}H_{34}N_4O_2$ calculated $(M+H)^+$ = 495.27545 m/z; found $(M+H)^+$ = 495.27630 m/z.
**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.59-7.53 (m, 2H), 7.52 (s, 1H), 7.47-7.41 (m, 1H), 7.39-7.34 (m, 5H), 7.28 – 7.19 (m, 5H), 4.53 (s, 2H), 4.13 (s, 2H), 3.97 – 3.93 (m, 1H), 3.88 (dd, *J* = 12.3, 2.5 Hz, 1H), 3.70 – 3.55 (m, 2H), 3.43-3.35 (m, 2H), 3.34-3.21 (m, 2H), 2.76-2.59 (m, 2H), 2.14 – 1.88 (m, 6H).

**N-hydroxybenzimidamide:** To 3-(5-(benzyloxy)-3-((1-benzylpiperidin-4-yl)methyl)-2-oxotetrahydropyrimidin-1(2H)-yl)benzonitrile (190 mg, 0.384 mmol) dissolved in Ethanol (7683 µl), hydroxylamine hydrochloride (53.4 mg, 0.768 mmol) followed by Hunig'sBase (134 µl, 0.768 mmol) were added at room temperature. The combined orange colored reaction mixture stirred for 6 h at 85 °C followed by overnight stirring at RT. After reaction was complete, as monitored by TLC and LCMS analysis, the solvent was removed and the resulting crude material was purified

by silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$, to give the desired product as an off-white color solid (145 mg, 72% yield).

**HRMS**: for $C_{31}H_{37}N_5O_3$ calculated $(M+H)^+$ = 528.29692 m/z; found $(M+H)^+$ = 528.29651 m/z.

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 10.70 (br.S, 2H), 9.62 (s, 1H), 7.64-7.55 (m, 2H), 7.53 (s, 1H), 7.48-7.39 (m, 4H), 7.36 – 7.24 (m, 7H), 4.59 (s, 2H), 4.20 (s, 2H), 4.06 – 3.97 (m, 1H), 3.89 (d, *J* = 12.2 Hz, 1H), 3.72 (d, J = 11.3 Hz, 1H), 3.60 (d, *J* = 12.4 Hz, 1H), 3.28 – 3.06 (m, 4H), 2.85-2.65 (m, 2H), 1.90-1.70 (m, 3H), 1.68-1.40 (m, 3H).

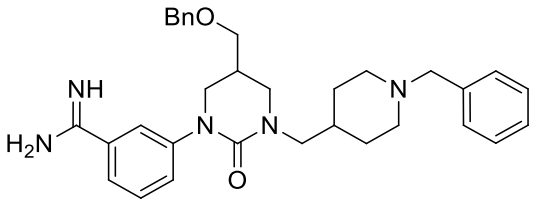
**Compound 8e**: To the oxime, 3-(5-(benzyloxy)-3-((1-benzylpiperidin-4-yl)methyl)-2-oxotetrahydropyrimidin-1(2H)-yl)-N-hydroxybenzimidamide (134 mg, 0.254 mmol) dissolved in ethanol (6 ml), Raney nickel (149 mg, 2.54 mmol) followed by Acetic Acid (462 µl) were added at room temperature and the suspension was shaken overnight on a Parr apparatus under $H_2$ atmosphere at 50 psi. After reaction completion, the crude was purged with Ar gas then filtered through a Celite pad with copious washing with methanol. Further purification of the product by silica gel column chromatography on an Isco CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, provided the desired product (37 mg, 28% yield) as a white solid.

**HRMS**: for $C_{31}H_{37}N_5O_2$ calculated $(M+2H)^{+2}$ = 256.64736 m/z; found $(M+2H)^{+2}$ = 256.65229 m/z.

**Purity**: 89%, retention time 5.12 min.

**$^1$H NMR** (400 MHz, Methanol-d4) δ 7.63 (dt, J = 1.9, 1.2 Hz, 1H), 7.61 - 7.54 (m, 3H), 7.37 - 7.24 (m, 10H), 4.64 (d, J = 1.0 Hz, 2H), 4.09 (p, J = 3.1 Hz, 1H), 3.97 (dd, J = 12.5, 2.6 Hz, 1H), 3.81 (ddd, J = 12.6, 3.6, 2.6 Hz, 1H), 3.71 - 3.64 (m, 3H), 3.53 (dt, J = 12.9, 2.9 Hz, 1H), 3.34 (dd, J = 7.9, 6.0 Hz, 1H), 3.22 (dd, J = 13.9, 6.8 Hz, 1H), 3.03 - 2.93 (m, 2H), 2.26 - 2.09 (m, 3H), 1.77 (td, J = 11.1, 10.6, 5.1 Hz, 3H), 1.34 (tdd, J = 16.5, 14.4, 12.2, 6.0 Hz, 3H).

<u>3-(5-((benzyloxy)methyl)-3-((1-benzylpiperidin-4-yl)methyl)-2-oxotetrahydropyrimidin-1(2H)-yl)benzimidamide (**8f**)</u>




**8f**

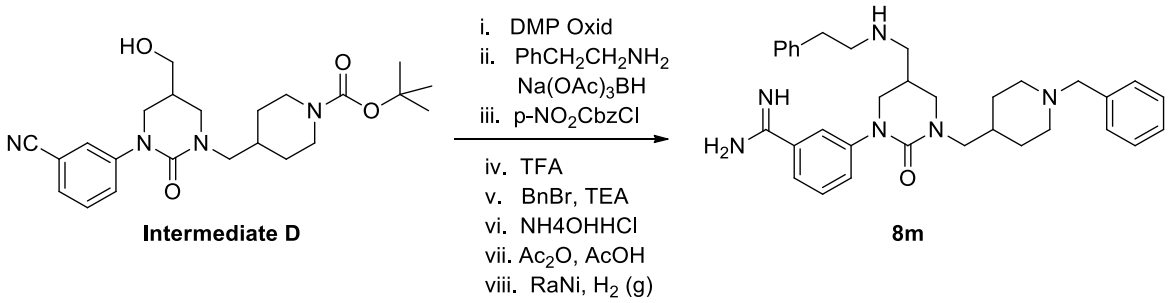
**Compound 8f** was synthesized from **intermediate D** following similar a synthetic sequence as described for **Compound 8d**. After purification by silica gel column chromatography on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, the product was obtained as a white solid in 6% yield (11 mg).

**HRMS**: $C_{32}H_{39}N_5O_2$ Calculated $(M+H)^+$ = 526.31765 m/z, found $(M+H)^+$ = 526.31701 m/z.

**Purity**: 100%, retention time 5.19 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.64 (br.s, 1H), 7.61 – 7.58 (m, 3H), 7.52-7.45 (m, 5H), 7.34 – 7.25 (m, 5H), 4.54 (s, 2H), 4.22 (s, 2H), 3.85 (dd, *J* = 11.6, 4.2 Hz, 1H), 3.68 (dd, *J* = 11.7, 7.5 Hz, 1H), 3.61 (d, *J* = 6.7 Hz, 2H), 3.57 (dd, *J* = 12.0, 4.7 Hz, 1H), 3.45 – 3.34 (m, 5H), 2.90 (br.s, 2H), 2.63-2.51 (m, 1H), 2.05-1.98 (m, 1H), 1.92 (t, *J* = 12.6 Hz, 2H), 1.5-1.45 (m, 2H).

3-(3-((1-benzylpiperidin-4-yl)methyl)-2-oxo-5-((phenethylamino)methyl)tetrahydropyrimidin-1(2H)-yl)benzimidamide (**8m**)




**DMP Oxidation**: To a stirred solution of tert-butyl 4-((3-(3-cyanophenyl)-5-(hydroxymethyl)-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (277 mg, 0.646 mmol) in $CH_2Cl_2$ (3.5 ml), Dess-Martin periodinane (548 mg, 1.293 mmol) followed by TFA (149 µl, 1.939 mmol) were added at RT and stirred for 90 min. After 90 min the reaction was quenched with 10% of $Na_2S_2O_3$ (5 ml) followed by sat. $NaHCO_3$ solution (5 ml) and stirred vigorously for 30 min. Afterwards the reaction mixture was transferred to a separatory funnel and the separated aqueous layer washed thrice with $CH_2Cl_2$ (10 ml). The combined organic layers were dried over MgSO4 and concentrated under reduced pressure. After purification by silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ there was obtained the desired product as a gummy solid in quantitative yields (275 mg).

**HRMS**: for hemiacetal $C_{24}H_{34}N_4O_5$: Calculated $(M+Na)^+$ = 481.24214 m/z, found $(M+Na)^+$ = 481.24163 m/z

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 9.79 (s, 1H), 7.60 – 7.58 (m, 1H), 7.54 (dtd, *J* = 7.0, 2.3, 0.8 Hz, 1H), 7.47 – 7.43 (m, 2H), 4.18 – 3.95 (m, 4H), 3.80 (ddd, *J* = 12.1, 4.4, 1.4 Hz, 1H), 3.72 – 3.66 (m, 1H), 3.55 – 3.08 (m, 2H), 3.04 – 2.95 (m, 1H), 2.70 (t, *J* = 12.7 Hz, 2H), 1.91 (qdt, *J* = 10.6, 7.1, 3.9 Hz, 2H), 1.65 (t, *J* = 12.4 Hz, 2H), 1.59 – 1.54 (m, 1H), 1.45 (s, 9H), 1.29 – 1.11 (m, 2H).

**Reductive Amination**: To a stirred solution of tert-butyl 4-((3-(3-cyanophenyl)-5-formyl-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (490 mg, 1.149 mmol) in $CH_2Cl_2$ (5744 µl) was added 2-phenylethanamine (144 µl, 1.149 mmol) followed by sodium (triacetoxy)borohydride (365 mg, 1.723 mmol). This mixture was stirred at room temperature; after 90 min, the reaction was quenched with sat. $NaHCO_3$. The solution was extracted with $CH_2Cl_2$ (3 X 15 ml) and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. There was obtained a light brown solid which was purified by silica gel chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ to provide the desired product as a viscous oil (140 mg, 23% yield).

**HRMS**: for $C_{31}H_{41}N_5O_3$ calculated $(M+H)^+$ = 532.32822 m/z; found $(M+H)^+$ = 532.32846 m/z.

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.58 – 7.56 (m, 1H), 7.54 (ddd, *J* = 5.2, 4.0, 2.3 Hz, 1H), 7.40 – 7.37 (m, 2H), 7.31 – 7.26 (m, 2H), 7.24 – 7.17 (m, 3H), 4.09 (s, 2H), 3.72 (ddd, *J* = 11.5, 4.2, 1.3 Hz, 1H), 3.50 (dd, *J* = 11.4, 8.3 Hz, 1H), 3.45 – 3.39 (m, 1H), 3.19 (dd, *J* = 11.8, 8.2 Hz, 2H), 2.93 – 2.88 (m, 2H), 2.83 – 2.77 (m, 2H), 2.70 (t, *J* = 7.3 Hz, 4H), 2.31 (tt, *J* = 7.5, 3.9 Hz, 1H), 1.94-1.71 (m, 4H), 1.44 (s, 9H), 1.23-1.09 (m, 3H).

**p-$NO_2$Cbz protection**: To a solution of tert-butyl 4-((3-(3-cyanophenyl)-2-oxo-5-((phenethylamino)methyl)tetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (140 mg, 0.263 mmol) in $CH_2Cl_2$ (5266 µl) was added $Et_3N$ (147 µl, 1.053 mmol) and DMAP (32.2 mg, 0.263 mmol) followed by 4-nitrobenzyl carbonochloridate (171 mg, 0.789 mmol). This mixture was stirred at room temperature for 5 h. After the reaction was complete the solvent was evaporated and the product isolated by silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ as a viscous oil (154 mg, 82% yield).

**LRMS**: $(M+H\text{-}Boc)^+$ = 611.3 m/z and $(M+H\text{-}tBu)^+$ = 655.3 m/z.

**HRMS**: for $C_{39}H_{46}N_6O_7$ calculated $(M+Na)^+$ = 733.33202 m/z; found $(M+Na)^+$ = 733.33084 m/z.

**$^1$H NMR** (400 MHz, Chloroform-d) δ 8.17 - 8.12 (m, 2H), 7.51 - 7.41 (m, 2H), 7.41 - 7.35 (m, 2H), 7.36 - 7.31 (m, 2H), 7.24 - 7.13 (m, 3H), 7.10-6.98 (m, 2H), 5.09 (s, 2H), 4.03 (br.s, 2H), 3.56

(dd, J = 11.4, 3.9 Hz, 1H), 3.52-3.33 (m, 3H), 3.29 (dd, J = 11.8, 4.7 Hz, 1H), 3.20 (d, J = 7.6 Hz, 2H), 3.16-2.98 (m, 3H), 2.86-2.68 (m, 3H), 2.60 (t, J = 12.8 Hz, 2H), 2.50-2.38 (s, 1H), 1.78 (br.s, 2H), 1.38 (s, 9H), 1.08 (s, 2H)

**Boc Deprotection**: A solution of tert-butyl4-((3-(3-cyanophenyl)-5-(((((4-nitrobenzyl)oxy)carbonyl) (phenethyl)amino)methyl)-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (154 mg, 0.217 mmol) dissolved in $CH_2Cl_2$ (1733 µl) and TFA (433 µl) was stirred for 30 min. After the reaction was complete, the solvent was removed and the residue evaporated with chloroform (2x) then with ethanol (2x) to obtain a colorless viscous oil in quantitative yield (132 mg). This material was used without further purification in the next step.
**LRMS**: $(M+H)^+$ = 611.3 m/z.

**N-Benzylation**: The 4-nitrobenzyl ((1-(3-cyanophenyl)-2-oxo-3-(piperidin-4-ylmethyl) hexahydropyrimidin-5-yl)methyl)(phenethyl)carbamate (132 mg, 0.216 mmol) prepared above was dissolved in $CH_2Cl_2$ (2161 µl); (bromomethyl)benzene (28.4 µl, 0.238 mmol) and $Et_3N$ (90 µl, 0.648 mmol) were added at room temperature and stirred for 30 min. After the reaction was complete, the solvent was removed and the residue was purified by silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ to obtain the desired product (100 mg, 66% yield) as a gummy solid.
**LRMS**: $(M+H)^+$ = 701.3 m/z.
**$^1$H NMR** (400 MHz, Chloroform-d) δ 8.22 - 8.18 (m, 2H), 7.57 - 7.47 (m, 2H), 7.47 - 7.42 (m, 2H), 7.42 - 7.18 (m, 10H), 7.18 - 7.05 (m, 2H), 5.15 (s, 2H), 3.68 (d, J = 11.9 Hz, 2H), 3.61 (dd, J = 11.2, 4.2 Hz, 1H), 3.49 (dq, J = 20.5, 11.7, 9.4 Hz, 3H), 3.40 - 3.33 (m, 1H), 3.33 - 3.11 (m, 5H), 3.02 (d, J = 8.8 Hz, 2H), 2.83 (t, J = 7.5 Hz, 2H), 2.48 (p, J = 7.1 Hz, 1H), 2.20 (s, 2H), 1.88-1.63 (m, 3H), 1.54 (s, 2H).

**N-hydroxybenzimidamide:** A combined solution of 4-nitrobenzyl ((1-((1-benzylpiperidin-4-yl)methyl)-3-(3-cyanophenyl)-2-oxohexahydropyrimidin-5-yl)methyl)(phenethyl)carbamate (100 mg, 0.143 mmol), hydroxylamine hydrochloride (19.83 mg, 0.285 mmol) and Hunig's Base (49.8 µl, 0.285 mmol) in ethanol (2854 µl) were stirred for 6 h at 85 °C; this mixture was then stirred overnight at room temperature. After the reaction was complete, the solvent was evaporation to

provide the product as a colorless solid. The crude is carried through next step without purification.

**LRMS**: $(M+H)^+$ = 734.3 m/z.

**O-Acetylation**: 4-Nitrobenzyl ((1-((1-benzylpiperidin-4-yl)methyl)-3-(3-(N-hydroxy carbamimidoyl)phenyl)-2-oxohexahydropyrimidin-5-yl)methyl)(phenethyl)carbamate (105 mg, 0.143 mmol) from the previous reaction was dissolved in acetic acid (1431 µl) to which acetic anhydride (40.6 µl, 0.429 mmol) was added; this mixture was stirred for 15 min. After the reaction was complete, the solvent was removed by evaporation and the residue was purified by column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ to give the desired product (54 mg, 49% yield) as a colorless gummy solid.

**LRMS**: $(M+H)^+$ = 776.3 m/z.

**HRMS**: for $C_{43}H_{47}N_7O_6$ calculated $(M+H)^+$ = 758.36606 m/z; found $(M+H)^+$ = 758.36309 m/z. Exact MS showed the 5-membered cyclization of the side chain which is formed during the solvent evaporation.

**$^1$H NMR** (400 MHz, MeOH-$d_4$) δ 8.18-8.09 (m, 2H), 7.95-7.78 (m, 4H), 7.62-7.53 (m, 3H), 7.53-7.43 (m, 4H), 7.28-7.11 (m, 5H), 5.21-5.09 (m, 2H), 4.32 (s, 2H), 3.64-3.37 (m, 7H), 3.10-2.97 (m, 3H), 2.88 (t, J = 7.4 Hz, 2H), 2.63 (s, 2H), 2.06-1.88 (m, 4H), 1.75 (s, 2H), 1.55 (t, J = 6.2 Hz, 2H), 1.23 (t, J = 7.0 Hz, 3H)

**Compound 8m:** To a combined solution of the carbamate (54 mg, 0.070 mmol) in ethanol (2 ml), Raney Ni (41 mg, 0.70 mmol) and acetic acid (0.5 ml) were added at room temperature. The resulting suspension was shaken under $H_2$ atmosphere at 50 psi in a Parr apparatus for 12-16 h at room temperature. After the reaction was complete, as monitored by LCMS analysis, the solution was purged with $N_2$ and filtered over Celite pad washing with MeOH. The solvent was removed under reduced pressure to provide a solid. Further purification by silica gel column chromatography on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, provided 25 mg of the desired product as a white solid in 67% yield. The product was further purified by column chromatography on silica gel on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive

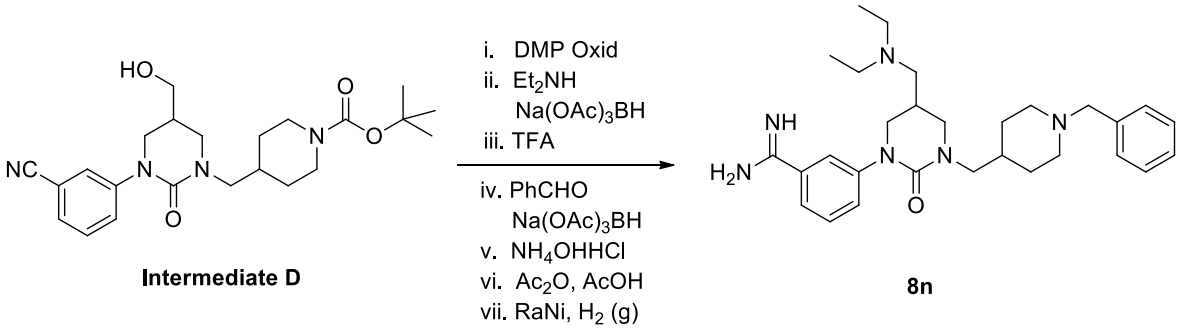
**HRMS**: for $C_{33}H_{42}N_6O$ calculated $(M+H)^+$ = 539.34929 m/z; found 539.34845 m/z.

**Purity**: 97%, retention time 0.23 min and 0.97 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 8.50 (s, 3H), 7.71 (d, *J* = 1.9 Hz, 1H), 7.67 - 7.58 (m, 3H), 7.52 - 7.43 (m, 5H), 7.35 - 7.20 (m, 5H), 4.19 (s, 2H), 4.00 - 3.91 (m, 1H), 3.66 (ddd, *J* = 14.5,

12.2, 6.3 Hz, 2H), 3.38 (ddd, *J* = 16.8, 8.5, 3.4 Hz, 4H), 3.20 (dd, *J* = 9.7, 6.2 Hz, 2H), 3.10 (d, *J* = 6.2 Hz, 2H), 3.00 (dd, *J* = 9.7, 6.2 Hz, 2H), 2.87 (t, *J* = 11.7 Hz, 2H), 2.74 - 2.63 (m, 1H), 2.09 - 1.97 (m, 2H), 2.02 (br.s, 1H), 1.97 - 1.87 (m, 2H), 1.54 (d, *J* = 12.2 Hz, 2H).

3-(3-((1-benzylpiperidin-4-yl)methyl)-5-((diethylamino)methyl)-2-oxotetrahydropyrimidin-1(2H)-yl)benzimidamide (**8n**)




**DMP Oxidation**: To a stirred solution of tert-butyl 4-((3-(3-cyanophenyl)-5-(hydroxymethyl)-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (277 mg, 0.646 mmol) in $CH_2Cl_2$ (3.5 ml), Dess-Martin periodinane (548 mg, 1.293 mmol) followed by TFA (149 µl, 1.939 mmol) were added at RT and stirred for 90 min. After 90 min the reaction was quenched with 10% of $Na_2S_2O_3$ (5 ml) followed by sat. $NaHCO_3$ solution (5 ml) and stirred vigorously for 30 min. Afterwards the reaction mixture was transferred to a separatory funnel and the separated aqueous layer washed (3x) with $CH_2Cl_2$ (10 ml). The combined organic layers were dried over MgSO4 and concentrated under reduced pressure. After purification by silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ the desired product was obtained as a gummy solid in quantitative yield (275 mg).

**HRMS**: for hemiacetal $C_{24}H_{34}N_4O_5$: Calculated $(M+Na)^+$ = 481.24214 m/z, found $(M+Na)^+$ = 481.24163 m/z

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 9.79 (s, 1H), 7.60 – 7.58 (m, 1H), 7.54 (dtd, *J* = 7.0, 2.3, 0.8 Hz, 1H), 7.47 – 7.43 (m, 2H), 4.18 – 3.95 (m, 4H), 3.80 (ddd, *J* = 12.1, 4.4, 1.4 Hz, 1H), 3.72 – 3.66 (m, 1H), 3.55 – 3.08 (m, 2H), 3.04 – 2.95 (m, 1H), 2.70 (t, *J* = 12.7 Hz, 2H), 1.91 (qdt, *J* = 10.6, 7.1, 3.9 Hz, 2H), 1.65 (t, *J* = 12.4 Hz, 2H), 1.59 – 1.54 (m, 1H), 1.45 (s, 9H), 1.29 – 1.11 (m, 2H).

**Reductive Amination with $Et_2NH$**: To a solution of tert-butyl 4-((3-(3-cyanophenyl)-5-formyl-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (550 mg, 1.29 mmol) dissolved in dichloromethane (7 ml), diethylamine (0.133 ml, 1.290 mmol) followed by sodiumtriacetoxyborohydride (410 mg, 1.934 mmol) were added at room temperature and stirred for 90 minutes. After reaction completion as monitored by TLC and LCMS analysis, quenched with sat. $NaHCO_3$, extracted with $CH_2Cl_2$ (3 X 15 ml) and combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure to obtain a light brown colored gummy solid. The crude material was then purified by silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ to obtain the desired product as a yellow color viscous oil (159 mg, 26% yield).

**HRMS**: for $C_{27}H_{41}N_5O_3$ calculated $(M+H)^+$ = 484.32822 m/z, found $(M+H)^+$ = 484.32840 m/z.

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.56-7.54 (m, 1H), 7.51 (dt, *J* = 6.6, 2.8 Hz, 1H), 7.34 – 7.30 (m, 2H), 4.03 (br.s, 2H), 3.70 (dd, *J* = 11.1, 3.8 Hz, 1H), 3.43 (ddd, *J* = 17.4, 11.6, 6.1 Hz, 2H), 3.13 (dd, *J* = 11.8, 7.5 Hz, 2H), 2.63 (t, *J* = 12.7 Hz, 2H), 2.54-2.43 (m, 4H), 2.43-2.26 (m, 3H), 1.83 (ddt, *J* = 11.3, 7.6, 3.8 Hz, 1H), 1.59 (d, *J* = 13.0 Hz, 2H), 1.38 (s, 9H), 1.11 (qd, *J* = 12.1, 4.4 Hz, 3H), 0.94 (t, *J* = 7.1 Hz, 6H).

**Boc Deprotection**: To a stirring solution of tert-butyl 4-((3-(3-cyanophenyl)-5-((diethylamino)methyl)-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (213 mg, 0.440 mmol) in $CH_2Cl_2$ (4 ml), was added TFA (1 ml) at room temperature and stirred for 30 min. After reaction was complete, the crude material was taken up in chloroform and the solvent was evaporated off. EtOH was added and evaporated off several times to give an off-white solid that was carried through next step without purification.

**LRMS**: $(M+H)^+$ = 384.3 m/z.

**Reductive Amination with benzaldehyde**: To a solution of 3-(5-((diethylamino)methyl)-2-oxo-3-(piperidin-4-ylmethyl)tetrahydropyrimidin-1(2H)-yl)benzonitrile (170 mg, 0.443 mmol) dissolved in Acetonitrile (5 mlµl), benzaldehyde (49.8 µl, 0.488 mmol) followed by sodium triacetoxyborohydride (141 mg, 0.665 mmol) were added at RT and stirred for 2 h. After reaction was complete, it was quenched with saturated $NaHCO_3$ solution, extracted with $CHCl_3$ (3 X 10 ml) and combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. After solvent evaporation the resulting crude material was purified by silica gel column

chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ to obtain the desired product as an off-white color viscous oil (68 mg, 32% yield).

**HRMS**: for $C_{29}H_{39}N_5O$ calculated $(M+H)^+$ = 474.32274 m/z, found $(M+H)^+$ = 474.32184 m/z.

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.61-7.59 (m, 1H), 7.57 (ddd, *J* = 6.2, 3.9, 2.3 Hz, 1H), 7.39 – 7.36 (m, 2H), 7.33 – 7.27 (m, 5H), 3.76 – 3.70 (m, 1H), 3.55 (s, 2H), 3.50 – 3.42 (m, 2H), 3.25 (dd, *J* = 7.0, 2.2 Hz, 1H), 3.16 (dd, *J* = 11.6, 7.5 Hz, 1H), 2.93 (br.d, *J* = 11.4 Hz, 2H), 2.50 (qd, *J* = 7.1, 1.5 Hz, 4H), 2.41 (dd, *J* = 7.2, 5.4 Hz, 2H), 2.38-2.29 (m, 1H), 2.02 (s, 3H), 1.68 (d, *J* = 13.1 Hz, 3H), 1.45 – 1.32 (m, 2H), 0.98 (t, *J* = 7.1 Hz, 6H).

**N-hydroxybenzimidamide:** A combined solution of 3-(3-((1-benzylpiperidin-4-yl)methyl)-5-((diethylamino)methyl)-2-oxotetrahydropyrimidin-1(2H)-yl)benzonitrile (95 mg, 0.201 mmol), hydroxylamine hydrochloride (34.8 mg, 0.501 mmol), Hunig'sBase (88 µl, 0.501 mmol) in ethanol (4011 µl) was stirred at 85 °C for 6 h followed by overnight stirring at RT. After the reaction was complete, the solvent was removed by evaporation and the resulting off-white viscous oil was carried through next step without purification.

**LRMS**: $(M+H)^+$ = 507.4 m/z

**O-Acetylation:** Crude 3-(3-((1-benzylpiperidin-4-yl)methyl)-5-((diethylamino)methyl)-2-oxotetrahydropyrimidin-1(2H)-yl)-N-hydroxybenzimidamide (102 mg, 0.201 mmol) from prevous step was dissolved in acetic acid (2013 µl) and acetic anhydride (57.1 µl, 0.604 mmol) was added and the solution was stirred for 15 min. After reaction completion evaporated off the solvents and the resulting crude was purified by silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ to obtain the desired compound as a white solid (61 mg, 55% yield).

**LRMS:** $(M+H)^+$ = 549.3 m/z.

**HRMS**: for $C_{31}H_{42}N_6O_2$ calculated $(M+H)^+$ = 531.34420 m/z; found $(M+H)^+$ = 531.34270 m/z. Exact MS showed the 5-membered cyclization of the side chain which is formed during the solvent evaporation.

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 7.92 (s, 1H), 7.73 (d, J = 7.9 Hz, 1H), 7.61-7.38 (m, 9H), 4.28-4.06 (m, 2H), 3.97-3.81 (m, 2H), 3.65-3.51 (m, 3H), 3.27-3.18 (m, 4H), 3.18-2.93 (m, 4H), 2.66 (s, 3H), 1.94 – 1.73 (m, 4H), 1.62-1.40 (m, 3H), 1.26-0.99 (m, 6H).

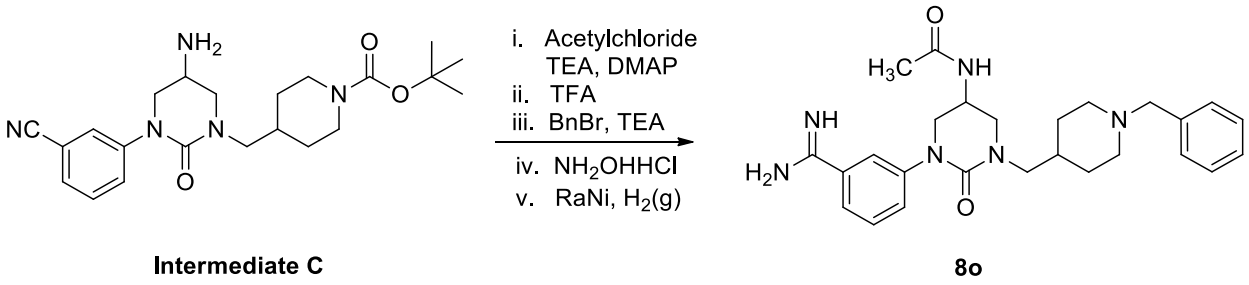
**Compound 8n:** To a combined solution of N-acetoxy-3-(3-((1-benzylpiperidin-4-yl)methyl)-5-((diethylamino)methyl)-2-oxotetrahydropyrimidin-1(2H)-yl)benzimidamide (45 mg, 0.082 mmol) in ethanol (2 ml), Raney Ni (48 mg, 0.820 mmol) and acetic acid (0.5 ml) were added together at room temperature. The resulting suspension was shaken under $H_2$ atmosphere at 50 psi in a Parr apparatus for 12-16 h at room temperature. After the reaction was complete, as monitored by LCMS analysis, the solution was purged with $N_2$ and filtered over Celite pad washing with MeOH. The solvent was removed under reduced pressure to provide a solid. Further purification by silica gel column chromatography on an Isco CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, provided 8 mg of the desired product as an off-white solid in 20% yield.

**HRMS**: for $C_{29}H_{43}N_6O$ calculated $(M+H)^+$ = 491.34929 m/z; found $(M+H)^+$ = 491.34904 m/z.

**Purity**: 100%, retention time 0.281 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 8.57 - 8.42 (m, 2H), 7.69 (dt, *J* = 2.2, 1.1 Hz, 1H), 7.64 (dtd, *J* = 5.8, 4.0, 1.9 Hz, 1H), 7.61 - 7.58 (m, 2H), 7.47 - 7.39 (m, 5H), 4.00 (s, 2H), 3.87 (ddd, *J* = 11.7, 4.2, 1.4 Hz, 1H), 3.66 - 3.54 (m, 2H), 3.34 (d, *J* = 4.0 Hz, 3H), 3.26 (dd, *J* = 10.7, 5.7 Hz, 2H), 2.73 (q, *J* = 7.1 Hz, 4H), 2.69 - 2.59 (m, 4H), 2.52 (ddt, *J* = 12.1, 8.2, 4.0 Hz, 1H), 2.00 - 1.82 (m, 3H), 1.54 - 1.41 (m, 2H), 1.29 (s, 1H), 1.09 (t, *J* = 7.1 Hz, 6H).

<u>N-(1-((1-benzylpiperidin-4-yl)methyl)-3-(3-carbamimidoylphenyl)-2-oxohexahydropyrimidin-5-yl)acetamide (**8o**)</u>




**Intermediate C** **8o**

**Acetamide Synthesis**: Combined solution of tert-butyl 4-((5-amino-3-(3-cyanophenyl)-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (360 mg, 0.871 mmol), triethylamine (364 µl, 2.61 mmol) and N,N-dimethylpyridin-4-amine (10.64 mg, 0.087 mmol) in $CH_2Cl_2$ (4353 µl) stirred at 0 °C for 15 min, then acetyl chloride (74.3 µl, 1.045 mmol) was added and the solution was stirred for an additional 30 min 0 °C followed by 90 min reaction at room temperature. After reaction completion, solvents were evaporated off and the crude material was

purified by silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ to obtain the desired product as a yellow color viscous oil (268 mg, 68% yield).
**HRMS**: for $C_{24}H_{33}N_5O_4$ calculated $(M+Na)^+$ = 478.24248 m/z; found $(M+Na)^+$ = 478.24246 m/z
**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.60-7.56 (m, 1H), 7.43 – 7.40 (m, 1H), 7.37 – 7.32 (m, 2H), 6.66 (s, 1H), 4.47 (dt, *J* = 7.8, 3.9 Hz, 1H), 4.02 (br.s, 2H), 3.88 (dd, *J* = 11.8, 3.4 Hz, 1H), 3.61 (dd, *J* = 12.2, 3.9 Hz, 1H), 3.54 (ddd, *J* = 11.8, 4.5, 1.9 Hz, 1H), 3.35-3.24 (m, , 2H), 3.06 (br.s, 1H), 2.68-2.56 (m, 2H), 1.94 (s, 3H), 1.85 – 1.72 (m, 1H), 1.63-1.51 (m, 2H), 1.36 (s, 9H), 1.16 – 1.02 (m, 2H).

**Boc Deprotection**: tert-Butyl 4-((5-acetamido-3-(3-cyanophenyl)-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (268 mg, 0.588 mmol) dissolved in $CH_2Cl_2$ (6 ml), TFA (453 µl, 5.88 mmol) was added and stirred at room temperature for 20 min. After the reaction was complete, the crude reaction mixture was diluted with chloroform and evaporated. This procedure was repeated several times with ethanol to remove all of the TFA. The resulting yellow viscous oil was carried through next step without any further purification.
**LRMS**: $(M+H)^+$ = 356.2 m/z.

**N-Benzylation**: The combined solution of N-(1-(3-cyanophenyl)-2-oxo-3-(piperidin-4-ylmethyl)hexahydropyrimidin-5-yl)acetamide (209 mg, 0.588 mmol), (bromomethyl)benzene (77 µl, 0.647 mmol) and TEA (246 µl, 1.764 mmol) in $CH_2Cl_2$ (6 ml) stirred at room temperature for 30 min. After the reaction was complete, the solvent was evaporated off and the crude material was purified by silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$. The desired compound was obtained as an off-white viscous oil in 86% yield (225 mg).
**HRMS**: for $C_{26}H_{31}N_5O_2$ calculated $(M+H)^+$ = 446.25505 m/z; found $(M+H)^+$ = 446.25440 m/z
**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.64 (s, 1H), 7.55 – 7.41 (m, 4H), 7.40 – 7.32 (m, 4H), 4.71 (s, 1H), 4.56-4.46 (m, 1H), 4.02 – 3.89 (m, 2H), 3.80 (tt, *J* = 11.7, 6.7 Hz, 2H), 3.58 – 3.45 (m, 2H), 3.35 (q, *J* = 7.3 Hz, 2H), 3.30-3.18 (s, 2H), 3.07 (d, *J* = 14.2 Hz, 1H), 2.62 (br.s, 1H), 2.05 (s, 3H), 1.98-1.78 (m, 2H), 1.48-1.41 (m, 3H).

**N-hydroxybenzimidamide:** Combined solution of N-(1-((1-benzylpiperidin-4-yl)methyl)-3-(3-cyanophenyl)-2-oxohexahydropyrimidin-5-yl)acetamide (225 mg, 0.505 mmol),

hydroxylamine hydrochloride (70.2 mg, 1.010 mmol) and Hunig'sBase (0.176 ml, 1.010 mmol) in Ethanol (10.100 ml) stirred for 6 h at 85 °C followed by overnight reaction at RT. After the reaction was complete the solvents were removed by evaporation to afford an off-white viscous oil. Further purification by silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ provided the desired compound as a white solid (180 mg, 75% yield).

**HRMS**: for $C_{26}H_{34}N_6O_3$ calculated $(M+K)^+$ = 517.23240 m/z; found $(M+K)^+$ = 517.23227 m/z

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 10.41 (s, 1H), 9.59 (s, 1H), 8.29 (d, J = 6.9 Hz, 1H), 7.62 - 7.49 (m, 3H), 7.48 - 7.37 (m, 4H), 7.28 (d, J = 5.4 Hz, 2H), 5.78 (s, 2H), 4.33 - 4.11 (m, 2H), 3.79 (dd, J = 11.5, 3.9 Hz, 1H), 3.52 (td, J = 12.3, 5.1 Hz, 2H), 3.16 (q, J = 7.2 Hz, 2H), 3.11-3.02 (m, 2H), 2.08 (d, J = 0.7 Hz, 1H), 1.85 (s, 3H), 1.78 (br.s, 2H), 1.47 (br.s, 2H), 1.30 (t, J = 7.2 Hz, 3H).

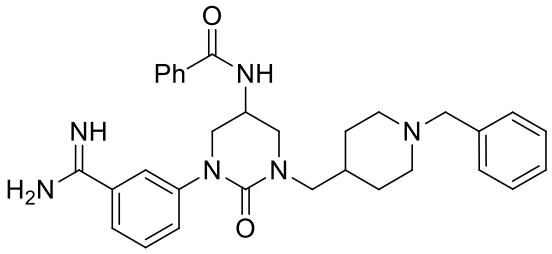
**Compound 8o:** To a combined solution of N-(1-((1-benzylpiperidin-4-yl)methyl)-3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-oxohexahydropyrimidin-5-yl)acetamide (180 mg, 0.376 mmol) in ethanol (5 ml), Raney Ni (221 mg, 3.76 mmol) was added at room temperature. The resulting suspension was shaken under $H_2$ atmosphere at 50 psi in a Parr apparatus for 12-16 h at room temperature. After the reaction was complete, as monitored by LCMS analysis, the solution was purged with $N_2$ and filtered over Celite pad washing with MeOH. The solvent was removed under reduced pressure to provide a solid. Further purification by silica gel column chromatography on an Isco CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, provided 25 mg of the desired product as an off-white solid in 14% yield.

**HRMS**: $C_{26}H_{34}N_6O_2$ calculated $(M+2H)^{+2}$ = 232.14444 m/z, found $(M+2H)^{+2}$ = 232.14516 m/z

**Purity**: 100%, retention time: 0.536 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.74-7.72 (M, 1H)7.68 – 7.63 (m, 1H), 7.62 – 7.59 (m, 2H), 7.54 (d, *J* = 1.5 Hz, 1H), 7.49 (dq, *J* = 5.5, 3.4, 2.9 Hz, 2H), 7.46 – 7.43 (m, 3H), 4.41 (tt, *J* = 5.7, 4.1 Hz, 1H), 3.98 (ddd, *J* = 11.8, 3.8, 0.9, 1H), 3.77 – 3.68 (m, 2H), 3.50 (dd, *J* = 13.9, 7.4 Hz, 2H), 3.44 – 3.38 (m, 1H), 3.38 – 3.35 (m, 1H), 3.27 (d, *J* = 7.3 Hz, 1H), 3.26 – 3.22 (m, 1H), 3.21 (d, *J* = 7.3 Hz, 1H), 2.85 – 2.75 (m, 2H), 2.00 (s, 3H), 1.52 (q, *J* = 12.3, 11.7 Hz, 3H), 1.43 (tt, *J* = 7.3, 1.7 Hz, 2H).

N-(1-((1-benzylpiperidin-4-yl)methyl)-3-(3-carbamimidoylphenyl)-2-oxohexahydropyrimidin-5-yl)benzamide (**8q**)



**8q**

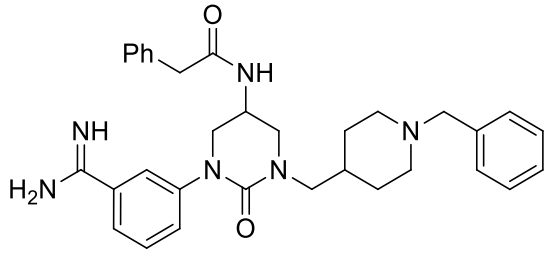
**Compound 8q** was synthesized from **Intermediate C** following similar a synthetic sequence as described for **Compound 8o**, wherein acetyl chloride replaced by benzoyl chloride. After purification by silica gel column chromatography on an Isco CombiFlash $R_f^{®}$ using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, the final product was obtained as a white solid in 4% yield (17 mg).

**HRMS**: $C_{31}H_{36}N_6O_2$ calculated $(M+2H)^{+2}$ = 263.15226 m/z, found $(M+2H)^{+2}$ = 263.15277 m/z.

**Purity**: 100%, retention time: 1.223 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.86 – 7.85 (m, 1H), 7.84 (d, *J* = 1.4 Hz, 1H), 7.75 (dt, *J* = 2.3, 1.0 Hz, 1H), 7.69 (ddd, *J* = 5.8, 3.1, 2.1 Hz, 1H), 7.63 – 7.59 (m, 2H), 7.59 – 7.56 (m, 1H), 7.56 – 7.52 (m, 2H), 7.50 – 7.46 (m, 5H), 4.65 (tt, *J* = 7.1, 4.5 Hz, 1H), 4.30 (s, 2H), 4.03 (ddd, *J* = 11.7, 4.2, 1.2 Hz, 1H), 3.94 (ddd, *J* = 11.6, 7.2, 0.9 Hz, 1H), 3.78 (ddd, *J* = 12.0, 4.7, 1.2 Hz, 1H), 3.68 – 3.62 (m, 1H), 3.53 – 3.41 (m, 3H), 3.08 – 2.93 (m, 2H), 2.00 (d, *J* = 14.7 Hz, 4H), 1.60 (t, *J* = 12.6 Hz, 2H).

N-(1-((1-benzylpiperidin-4-yl)methyl)-3-(3-carbamimidoylphenyl)-2-oxohexahydropyrimidin-5-yl)-2-phenylacetamide (**8h**)



**8h**

**Compound 8h** was synthesized from **Intermediate C** following a similar synthetic sequence as described for **Compound 8o**, where acetyl chloride replaced by phenyl acetyl chloride. After

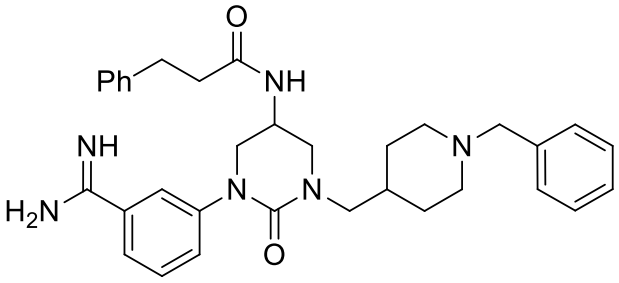
purification by silica gel column chromatography on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, the final product was obtained as a white solid in 19% yield (23 mg).

**HRMS**: $C_{32}H_{38}N_6O_2$ calculated $(M+H)^+$ = 539.31290 m/z, found $(M+H)^+$ = 539.31331 m/z.

**Purity**: 100%, retention time: 1.255 min.

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 8.53 (d, *J* = 7.3 Hz, 1H), 7.63 – 7.56 (m, 2H), 7.52 – 7.44 (m, 2H), 7.35 – 7.16 (m, 9H), 3.87 (dd, *J* = 11.6, 3.5 Hz, 1H), 3.64 – 3.53 (m, 2H), 3.48 – 3.42 (m, 4H), 3.31 – 3.21 (m, 3H), 3.09 (dd, *J* = 13.7, 6.4 Hz, 1H), 2.77 (s, 2H), 1.87 (t, *J* = 11.6 Hz, 2H), 1.58 (d, *J* = 12.8 Hz, 2H), 1.14 (t, *J* = 11.4 Hz, 2H).

N-(1-((1-benzylpiperidin-4-yl)methyl)-3-(3-carbamimidoylphenyl)-2-oxohexahydropyrimidin-5-yl)-3-phenylpropanamide (**8r**)




**8r**

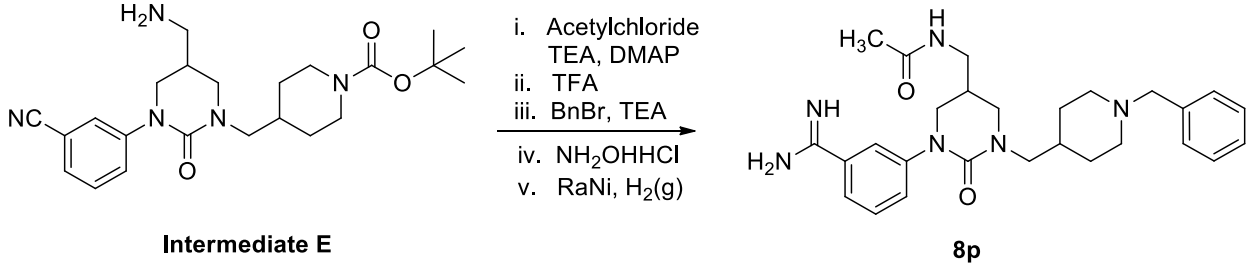
**Compound 8r** was synthesized from **Intermediate C** following a similar synthetic sequence as described for **Compound 8o**, where acetyl chloride replaced by 3-(phenyl)propionyl chloride. After purification by silica gel column chromatography on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, the final product was obtained as a white crystalline solid in 36% yield (38 mg).

**HRMS**: $C_{33}H_{40}N_6O_2$ calculated $(M+H)^+$ = 553.32855 m/z, found $(M+H)^+$ = 553.32884 m/z. $^1$H

**Purity**: 100%, retention time: 1.335 min.

**NMR** (400 MHz, DMSO-$d_6$) δ 10.67 (s, 1H), 9.38 (s, 1H), 9.13 (s, 1H), 8.35 (d, *J* = 7.1 Hz, 1H), 7.70 – 7.57 (m, 4H), 7.53 (d, *J* = 7.7 Hz, 2H), 7.48 – 7.41 (m, 2H), 7.28 – 7.13 (m, 5H), 4.27 (dd, *J* = 14.9, 5.3 Hz, 3H), 3.83 (dd, *J* = 11.6, 3.9 Hz, 1H), 3.55 (ddd, *J* = 14.8, 11.7, 5.2 Hz, 2H), 3.32-3.16 (m, 3H), 3.12 – 3.02 (m, 2H), 2.79 (t, *J* = 7.8 Hz, 4H), 2.44 (dd, *J* = 8.9, 6.7 Hz, 2H), 1.80 (s, 3H), 1.54 (d, *J* = 13.3 Hz, 2H).

N-((1-((1-benzylpiperidin-4-yl)methyl)-3-(3-carbamimidoylphenyl)-2-oxohexahydropyrimidin-5-yl)methyl)acetamide (**8p**)




**Intermediate E** **8p**

**Acetamide Synthesis**: To a reaction flask purged with argon was added tert-butyl 4-((5-(aminomethyl)-3-(3-cyanophenyl)-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (192 mg, 0.449 mmol) was dissolved in $CH_2Cl_2$ (4491 µl). To this clear solution, $Et_3N$ (188 µl, 1.347 mmol) followed by DMAP (5.49 mg, 0.045 mmol) were added at room temperature and stirred for 15 min. After 15 min, acetyl chloride (38.3 µl, 0.539 mmol) was added at once and the mixture was allowed to react for 30 min. After reaction completion solvents were evaporated off to afford a yellow colored crude solid. Further purification by silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ provided the desired compound as a yellow color solid (132 mg, 63% yield).

**HRMS**: for $C_{25}H_{36}5_6O_4$ calculated $(M+Na)^+$ = 492.25813 m/z, found $(M+Na)^+$ = 492.23793 m/z.

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.62-7.60 (m, 1H), 7.58-7.54 (m, 1H), 7.41 – 7.37 (m, 2H), 4.08 (br.s, 3H), 3.74 – 3.68 (m, 1H), 3.55 (dd, *J* = 11.5, 8.8 Hz, 1H), 3.45 – 3.38 (m, 1H), 3.38 – 3.29 (m, 2H), 3.29 – 3.20 (m, 2H), 2.68 (t, *J* = 12.6 Hz, 3H), 2.01 (s, 3H), 1.87 (tt, *J* = 7.7, 3.6 Hz, 1H), 1.63 (d, *J* = 12.9 Hz, 3H), 1.43 (s, 9H), 1.21 – 1.09 (m, 1H).

**Boc Deprotection**: Combined solution of tert-butyl 4-((5-(acetamidomethyl)-3-(3-cyanophenyl)-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (132 mg, 0.281 mmol), $CH_2Cl_2$ (2 mL) and TFA (1 mL) stirred for 30 min at RT. After reaction completion solvent evaporated off by diluting with chloroform followed by ethanol to obtain an offwhite color viscous oil in quantitative yields. The crude material was carried through the next step without further purification.

**LRMS**: $(M+H)^+$ = 370.2 m/z

**N-Benzylation**: N-((1-(3-cyanophenyl)-2-oxo-3-(piperidin-4-ylmethyl)hexahydropyrimidin-5-yl)methyl)acetamide (104 mg, 0.281 mmol) was dissolved in acetonitrile (2815 µl) then Hunig's Base (73.7 µl, 0.422 mmol) was added followed by (bromomethyl)benzene (36.8 µl, 0.310 mmol). This mixture was stirred at room temperature for 30 min. After the reaction was complete, the solvent was evaporated and the crude material was purified by silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$. The desired compound was obtained as an off-white viscous oil (60 mg, 47% yield).
**LRMS:** $(M+H)^+$ = 460.3 m/z.

**N-hydroxybenzimidamide:** A combined solution of N-((1-((1-benzylpiperidin-4-yl)methyl)-3-(3-cyanophenyl)-2-oxohexahydropyrimidin-5-yl)methyl)acetamide (60 mg, 0.131 mmol), $NH_2OHHCl$ (27.2 mg, 0.392 mmol) and Hunig's Base (68.4 µl, 0.392 mmol) in ethanol (3 ml) was stirred for 6 h at 85 °C, then left to stir at room temperature overnight. After the reaction was complete, the solvent was removed under reduced pressure and the resulting crude off-white gummy solid was carried through to the next step without purification.
**LRMS**: $(M+H)^+$ = 493.3 m/z.

**O-Acetylation**: Crude N-((1-((1-benzylpiperidin-4-yl)methyl)-3-(3-(N-hydroxycarbamimidoyl) phenyl)-2-oxohexahydropyrimidin-5-yl)methyl)acetamide (65 mg, 0.132 mmol) from previous reaction was redissolved in AcOH (2639 µl) to which acetic anhydride (37.4 µl, 0.396 mmol) was added; this mixture was stirred for 15 min. After the reaction was complete, the solvent was evaporation and the residue was purified by silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$. The desired product was isolated as a gummy solid (25 mg, 36%).
**LRMS**: $(M+H)^+$ = 535.3 m/z.

**Compound 8p:** To a combined solution of N-((1-(3-(N-acetoxycarbamimidoyl)phenyl)-3-((1-benzylpiperidin-4-yl)methyl)-2-oxohexahydropyrimidin-5-yl)methyl)acetamide (25 mg, 0.047 mmol) in ethanol (2 ml), Raney Ni (20 mg, 0.47 mmol) followed by acetic acid (0.1 ml) were added at room temperature. The resulting suspension was shaken under $H_2$ atmosphere at 50 psi in a Parr apparatus for 12-16 h at room temperature. After the reaction was complete, as monitored by LCMS analysis, the solution was purged with $N_2$ and filtered over Celite pad washing with

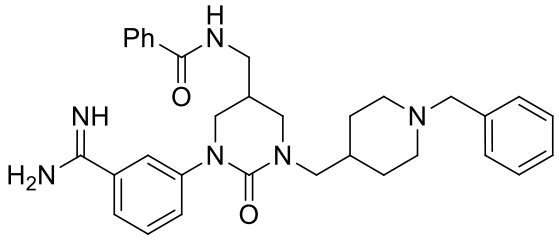
MeOH. The solvent was removed under reduced pressure to provide a solid. Further purification by silica gel column chromatography on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, provided 7 mg of the desired product as an off-white solid in 31% yield.

**HRMS**: for $C_{27}H_{36}N_6O_2$ calculated $(M+H)^+$ = 477.29725 m/z, found $(M+H)^+$ = 477.29684 m/z.

**Purity**: 100%, retention time: 0.122 min and 3.941 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.74 – 7.70 (m, 1H), 7.69 – 7.60 (m, 3H), 7.43 (tt, *J* = 8.3, 3.6 Hz, 5H), 3.93 (s, 2H), 3.85 (dd, *J* = 11.7, 4.0 Hz, 1H), 3.66 – 3.54 (m, 2H), 3.39-3.26 (m, 3H), 3.23 (dd, *J* = 11.4, 3.8 Hz, 2H), 2.60 – 2.50 (m, 2H), 2.50 – 2.39 (m, 1H), 1.97 (d, *J* = 3.2 Hz, 5H), 1.91 – 1.81 (m, 2H), 1.54 – 1.39 (m, 2H), 1.32 (s, 1H).

N-((1-((1-benzylpiperidin-4-yl)methyl)-3-(3-carbamimidoylphenyl)-2-oxohexahydropyrimidin-5-yl)methyl)benzamide (**8g**)




**8g**

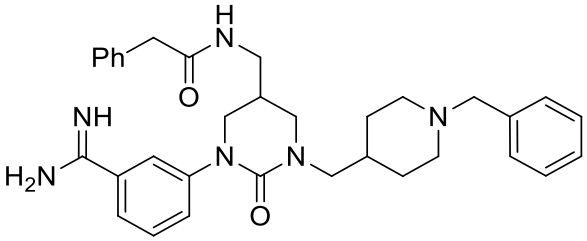
**Compound 8g** was synthesized from **Intermediate E** following a similar synthetic sequence as described for **Compound 8p**, where acetyl chloride replaced by benzoyl chloride. After purification by silica gel column chromatography on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, the final product was obtained as a white solid in 20% yield (5 mg).

**HRMS**: for $C_{32}H_{38}N_6O_2$ calculated $(M+H)^+$ = 539.31290 m/z; found $(M+H)^+$ = 539.31234 m/z.

**Purity**: 97%, retention time: 4.841.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.85 – 7.79 (m, 2H), 7.68 (dq, *J* = 1.9, 0.9 Hz, 1H), 7.61 – 7.44 (m, 6H), 7.37 – 7.26 (m, 5H), 3.91 – 3.83 (m, 1H), 3.68 – 3.55 (m, 4H), 3.54 (s, 2H), 3.42 – 3.35 (m, 2H), 2.94 (dd, *J* = 10.6, 4.5 Hz, 2H), 2.61 (ddd, *J* = 7.2, 3.6, 3.0 Hz, 1H), 2.04 (tt, *J* = 11.8, 2.3 Hz, 2H), 1.76 (dqd, *J* = 20.4, 7.0, 6.5, 3.4 Hz, 3H), 1.43 – 1.28 (m, 4H).

N-((1-((1-benzylpiperidin-4-yl)methyl)-3-(3-carbamimidoylphenyl)-2-oxohexahydropyrimidin-5-yl)methyl)-2-phenylacetamide (**8i**)



**8i**

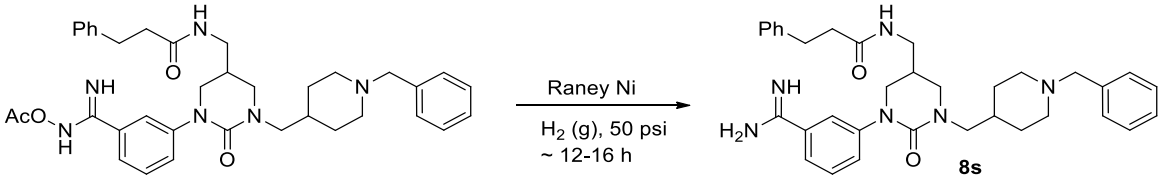
**Compound 8i** was synthesized from **Intermediate E** following a similar synthetic sequence as described for **Compound 8p**, where acetyl chloride replaced by phenyl acetyl chloride. After purification by silica gel column chromatography on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, the final product was obtained as a white crystalline solid in 18% yield (15 mg).

**HRMS**: for $C_{33}H_{40}N_6O_2$ calculated $(M+H)^+$ = 553.32855 m/z; found $(M+H)^+$ = 553.32858 m/z.

**Purity**: 98%, retention time: 4.826 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.67 (q, *J* = 1.5 Hz, 1H), 7.62 – 7.54 (m, 5H), 7.51 – 7.48 (m, 3H), 7.30 – 7.18 (m, 5H), 4.30 (s, 2H), 3.80 (dd, *J* = 11.7, 4.0 Hz, 1H), 3.52 (d, *J* = 1.7 Hz, 3H), 3.51 – 3.47 (m, 1H), 3.44 (d, *J* = 11.4 Hz, 2H), 3.39 (d, *J* = 7.2 Hz, 2H), 3.25 (dd, *J* = 12.0, 6.7 Hz, 2H), 3.05 – 2.92 (m, 2H), 2.44 (ddt, *J* = 8.5, 6.8, 3.5 Hz, 1H), 1.97 – 1.87 (m, 3H), 1.61 – 1.44 (m, 2H), 1.29 (s, 2H).

N-((1-((1-benzylpiperidin-4-yl)methyl)-3-(3-carbamimidoylphenyl)-2-oxohexahydropyrimidin-5-yl)methyl)-3-phenylpropanamide (**8s**)



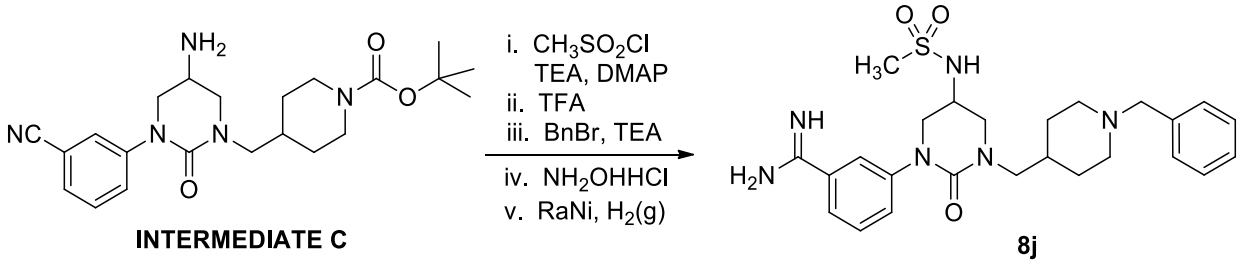

**Compound 8s** was synthesized from **Intermediate E** following similar a synthetic sequence as described for **Compound 8p**, where acetyl chloride replaced by 3-(phenyl)propionyl chloride. After purification by silica gel column chromatography on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, the final product was obtained as a white crystalline solid in 82% yield (60 mg).

**HRMS**: for $C_{34}H_{42}N_6O_2$ calculated $(M+H)^+$ = 567.34420 m/z; found $(M+H)^+$ = 567.34314 m/z.

**Purity**: 95%, retention time: 5.002 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.69 (t, *J* = 1.6 Hz, 1H), 7.62 (td, *J* = 5.5, 4.9, 3.5 Hz, 3H), 7.47 - 7.35 (m, 5H), 7.23 - 7.15 (m, 4H), 7.11 (ddd, *J* = 8.8, 5.7, 2.7 Hz, 1H), 3.67 (dd, *J* = 11.7, 4.1 Hz, 1H), 3.44 - 3.24 (m, 6H), 3.21 - 3.09 (m, 3H), 3.00 (q, *J* = 7.3 Hz, 1H), 2.91 (t, *J* = 7.3 Hz, 2H), 2.53 (t, *J* = 7.3 Hz, 2H), 2.46 (tt, *J* = 12.2, 3.2 Hz, 2H), 2.30 (ddt, *J* = 10.6, 7.3, 3.8 Hz, 1H), 1.88 - 1.76 (m, 3H), 1.51 - 1.37 (m, 2H), 1.34 - 1.27 (m, 1H).

<u>3-(3-((1-benzylpiperidin-4-yl)methyl)-5-(methylsulfonamido)-2-oxotetrahydropyrimidin-1(2H)-yl)benzimidamide (**8j**)</u>




**Sulfonylation**: To tert-butyl 4-((5-amino-3-(3-cyanophenyl)-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (350 mg, 0.846 mmol) in $CH_2Cl_2$ (8464 µl), TEA (354 µl, 2.54 mmol) and DMAP (10.34 mg, 0.085 mmol) were added at 0 °C, then the mixture was stirred for 15 min. After 15 min, methanesulfonyl chloride (79 µl, 1.016 mmol) was added and stirring was continued at 0 °C for an additional 30 min, followed by stirring at room temperature for 1 h. After the reaction was complete, the solvent was removed by evaporation and the product was isolated by silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$, as an amorphous solid (373 mg, 90% yield).

**HRMS**: for $C_{23}H_{33}N_5O_5S$ calculated $(M+Na)^+$ = 514.20976 m/z, found $(M+Na)^+$ = 514.20946 m/z.

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.60 (dt, *J* = 2.2, 0.9 Hz, 1H), 7.56 – 7.50 (m, 1H), 7.47 – 7.40 (m, 2H), 5.73 – 5.61 (m, 1H), 4.07 (dq, *J* = 8.5, 4.3 Hz, 3H), 3.94 (dd, *J* = 11.8, 3.4 Hz, 1H), 3.77 – 3.60 (m, 2H), 3.39 (ddd, *J* = 12.3, 4.8, 1.9 Hz, 2H), 3.02 (d, *J* = 0.8 Hz, 3H), 2.69 (t, *J* = 12.7 Hz, 2H), 1.86 (ddp, *J* = 11.1, 7.3, 3.7 Hz, 1H), 1.72 (br.s, 1H), 1.62 (t, *J* = 13.3 Hz, 2H), 1.44 (s, 9H), 1.23 – 1.09 (m, 2H).

**Boc Deprotection**: tert-Butyl 4-((3-(3-cyanophenyl)-5-(methylsulfonamido)-2-oxotetrahydro pyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (373 mg, 0.759 mmol) in $CH_2Cl_2$ (7587 µl) and TFA (585 µl, 7.59 mmol) were stirred for 30 min at room temperature. After the reaction was complete the solvent was evaporated and the solid crude product was purified further by trituration with chloroform, followed by ethanol. This material was used directly in the next step without further purification.

**LRMS**: $(M+H)^+$ = 392.1 m/z.

**N-Benzylation**: N-(1-(3-cyanophenyl)-2-oxo-3-(piperidin-4-ylmethyl)hexahydropyrimidin-5-yl)methanesulfonamide (300 mg, 0.766 mmol) was dissolved in $CH_2Cl_2$ (8 ml), then (bromomethyl)benzene (100 µl, 0.843 mmol) and TEA (320 µl, 2.299 mmol) were added at room temperature . This mixture was stirred for 15 min then the solvent was evaporated and the crude product was by purified by silica gel column chromatography on an ISCO CombiFlash Rf® using a gradient of MeOH in $CHCl_3$ (370 mg, quantitative yield).

**HRMS**: for $C_{25}H_{31}N_5O_3S$ calculated $(M+H)^+$ = 482.22204 m/z, found $(M+H)^+$ = 482.22215 m/z.

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.70 – 7.67 (m, 1H), 7.62-7.57 (m, 1H), 7.54 – 7.48 (m, 2H), 7.39 (qd, *J* = 6.2, 5.6, 2.5 Hz, 5H), 7.10 (s, 1H), 4.12 (d, *J* = 5.5 Hz, 2H), 4.04 (s, 1H), 3.89 (s, 2H), 3.62 (t, *J* = 5.6 Hz, 3H), 3.47 (d, *J* = 35.4 Hz, 3H), 3.07 (s, 3H), 2.76 (s, 2H), 2.10-1.83 (m, 3H), 1.39 (s, 2H).

**N-hydroxybenzimidamide:** N-(1-((1-benzylpiperidin-4-yl)methyl)-3-(3-cyanophenyl)-2-oxo hexahydropyrimidin-5-yl)methanesulfonamide (405 mg, 0.841 mmol), hydroxylamine hydrochloride (117 mg, 1.682 mmol) and Hunig's Base (0.294 ml, 1.682 mmol) in ethanol (16.800 ml) were stirred at 85 °C for 6 h, then overnight at room temperature. After the reaction was complete, the solvent was evaporated and the product isolated by silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$to give the desired product (170 mg, 39%) as a light green color solid.

**HRMS**: for $C_{25}H_{34}N_6O_4S$ calculated $(M+H)^+$ = 515.24350 m/z, found $(M+H)^+$ = 515.24254 m/z.

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 10.95 (s, 2H), 7.70 (dd, *J* = 6.3, 2.1 Hz, 1H), 7.66 – 7.55 (m, 3H), 7.45 (dtt, *J* = 6.3, 4.7, 2.8 Hz, 5H), 7.32 (d, *J* = 2.1 Hz, 1H), 7.19 (d, *J* = 2.3 Hz, 1H), 4.25 (d, *J* = 4.7 Hz, 2H), 3.96 (p, *J* = 5.3 Hz, 1H), 3.89 (dd, *J* = 11.8, 3.6 Hz, 1H), 3.64 (dd, *J* = 11.7, 6.2 Hz, 1H), 3.58 (dd, *J* = 11.8, 4.2 Hz, 1H), 3.28 (s, 3H), 3.18 (dt, *J* = 13.6, 6.8 Hz, 1H), 3.07 (s, 1H),

3.00 (d, *J* = 2.1 Hz, 3H), 2.84 (q, *J* = 12.2, 11.6 Hz, 2H), 1.82 (t, *J* = 13.4 Hz, 3H), 1.57 (q, *J* = 12.8 Hz, 2H).

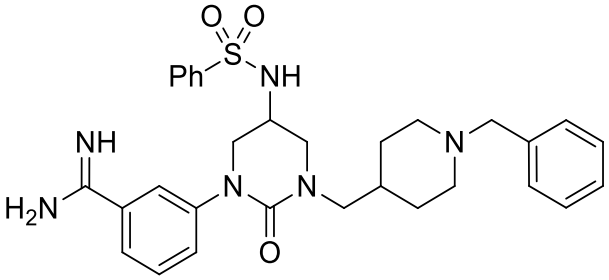
**Compound 8j:** To 3-(3-((1-benzylpiperidin-4-yl)methyl)-5-(methylsulfonamido)-2-oxotetra hydropyrimidin-1(2H)-yl)-N-hydroxybenzimidamide (85 mg, 0.125 mmol), in ethanol (6 ml), Raney nickel (100 mg, 1.652 mmol) was added; this suspension was shaken overnight in a Parr apparatus under $H_2$ (gas) atmosphere at 50 psi. After the reaction was complete, it was purged with Ar gas then filtered through a Celite pad washing with methanol. Further purification of the crude product by silica gel column chromatography on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, provided the desired product (41 mg, 50% yield) as an off-white colored solid.

**HRMS**: $C_{25}H_{34}N_6O_3S$ calculated $(M+2H)^{+2}$ = 250-12793 m/z, found $(M+2H)^{+2}$ = 250.12872 m/z.

**Purity**: 99%, retention time: 0.248 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.74 (dt, *J* = 2.3, 0.9 Hz, 1H), 7.68 (ddd, *J* = 5.5, 3.4, 2.1 Hz, 1H), 7.62 – 7.59 (m, 2H), 7.49 – 7.40 (m, 5H), 4.06 (s, 4H), 3.80 – 3.71 (m, 2H), 3.52 – 3.45 (m, 1H), 3.39 – 3.35 (m, 1H), 3.35 – 3.32 (m, 1H), 3.20 (q, *J* = 7.3 Hz, 1H), 3.05 (s, 3H), 2.70 (dt, *J* = 13.9, 10.6 Hz, 2H), 1.97 (d, *J* = 4.8 Hz, 2H), 1.93 – 1.87 (m, 1H), 1.49 (q, *J* = 11.3, 10.8 Hz, 3H).

3-(3-((1-benzylpiperidin-4-yl)methyl)-2-oxo-5-(phenylsulfonamido)tetrahydropyrimidin-1(2H)-yl)benzimidamide (**8k**)




**8k**

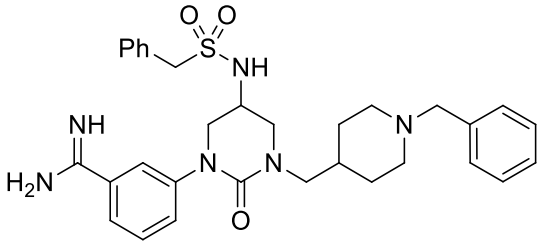
**Compound 8k** was synthesized from **Intermediate C** following a similar synthetic sequence as described for **Compound 8j**, where methanesulfonyl chloride replaced by phenylsulfonyl chloride. After purification by silica gel column chromatography on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, the final product was obtained as a white solid in 12% yield (42 mg).

**HRMS**: $C_{30}H_{36}N_6O_3S$ calculated $(M+2H)^{+2}$ = 281.13802 m/z, found $(M+2H)^{+2}$ = 281.13765

**Purity**: 100%, retention time: 1.273 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.93 – 7.88 (m, 2H), 7.68 – 7.65 (m, 1H), 7.63 – 7.54 (m, 5H), 7.54 – 7.46 (m, 6H), 4.22 (s, 2H), 3.94 – 3.82 (m, 2H), 3.65 – 3.59 (m, 1H), 3.55 (ddd, *J* = 11.9, 4.4, 2.1 Hz, 1H), 3.43 – 3.35 (m, 3H), 3.24 – 3.16 (m, 1H), 2.90 (s, 2H), 2.00 – 1.86 (m, 4H), 1.49 (s, 2H).

3-(3-((1-benzylpiperidin-4-yl)methyl)-2-oxo-5-((phenylmethyl)sulfonamido) tetrahydropyrimidin-1(2H)-yl)benzimidamide (**8l**)




**8l**

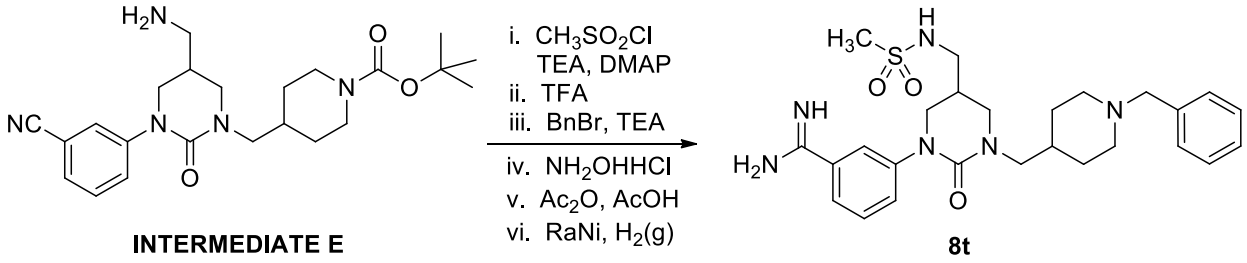
**Compound 8l** was synthesized from **Intermediate C** following a similar synthetic sequence as described for **Compound 8j**, where methanesulfonyl chloride replaced by phenylmethanesulfonyl chloride. After purification by silica gel column chromatography on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, the final product was obtained as a white solid in 7% yield (11 mg).

**HRMS**: $C_{31}H_{38}N_6O_3S$ calculated $(M+2H)^{+2}$ = 288.14358 m/z, found $(M+2H)^{+2}$ = 288.14362 m/z.

**Purity**: 94%, retention time: 1.312 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.66 (s, 1H), 7.59 (d, *J* = 2.4 Hz, 3H), 7.44 (qd, *J* = 3.8, 1.8 Hz, 2H), 7.38 – 7.26 (m, 8H), 4.42 (s, 2H), 3.81 (dd, *J* = 11.6, 3.4 Hz, 1H), 3.70 (s, 2H), 3.58 – 3.49 (m, 2H), 3.37 (s, 1H), 3.29 – 3.22 (m, 1H), 3.20 – 3.11 (m, 1H), 2.99 (d, *J* = 11.7 Hz, 2H), 2.16 (s, 3H), 1.74 (d, *J* = 10.1 Hz, 3H), 1.40 – 1.27 (m, 3H).

3-(3-((1-benzylpiperidin-4-yl)methyl)-5-(methylsulfonamidomethyl)-2-oxotetrahydropyrimidin-1(2H)-yl)benzimidamide (**8t**)




**Sulfonylation**: A stirred solution of amine (143 mg, 0.334 mmol) in DCM (3.5 mL), triethylamine (0.14 ml, 1.003 mmol) and DMAP (4.09 mg, 0.033 mmol) were stirred for 15 min at room temperature, then methanesulfonylchloride (0.029 ml, 0.368 mmol) was added. This mixture was stirred for an additional 30 min. After the reaction was complete, the solvent was evaporated under reduced pressure to give an off-white solid. Further purification by a silica gel (4 g column) on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ provided 86 mg (51% yield) of the desired product as a colorless solid.

**LRMS**: $(M+H\text{-}Boc)^+$ = 406.2 m/z and **LRMS**: $(M+H\text{-}^tBu)^+$ = 450.2 m/z.

**HRMS:** for $C_{24}H_{35}N_5O_5S$ calculated $(M+Na)^+$ = 528.22511 m/z; found $(M+Na)^+$ = 528.22422 m/z.

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.60 (dq, *J* = 2.2, 1.1 Hz, 1H), 7.56 (dddd, *J* = 5.2, 3.7, 2.9, 1.2 Hz, 1H), 7.41 (dt, *J* = 5.0, 1.1 Hz, 2H), 4.10 (s, 2H), 3.88 – 3.76 (m, 1H), 3.58 (dd, *J* = 11.6, 7.9 Hz, 1H), 3.52 (dd, *J* = 12.1, 4.8 Hz, 1H), 3.33 – 3.19 (m, 4H), 3.12 (qd, *J* = 7.3, 4.0 Hz, 4H), 2.97 (d, *J* = 1.1 Hz, 3H), 2.76 – 2.63 (m, 2H), 2.50 (td, *J* = 8.1, 7.6, 4.0 Hz, 1H), 1.88 (ddp, *J* = 12.0, 8.2, 3.9, 3.3 Hz, 1H), 1.67 – 1.57 (m, 2H), 1.44 (d, *J* = 1.1 Hz, 9H).

**Boc Deprotection**: To a stirring solution of tert-butyl 4-((3-(3-cyanophenyl)-5-(methylsulfonamidomethyl)-2-oxotetrahydropyrimidin-1(2H)-yl)methyl)piperidine-1-carboxylate (85 mg, 0.168 mmol) in $CH_2Cl_2$ (504 µl), TFA (336 µl) was added at room temperature and this mixture was stirred for 30 min. After the reaction was complete, chloroform was added and the solvent was evaporated. This process was repeated with ethanol to ensure removal of residual TFA; there was obtained a viscous oil which is directly used in next step.

**LRMS**: $(M+H)^+$ = 406.2 m/z.

**N-Benzylation**: The material from previous step, N-((1-(3-cyanophenyl)-2-oxo-3-(piperidin-4-ylmethyl)hexahydropyrimidin-5-yl)methyl)methanesulfonamide (100 mg, 0.247 mmol), was

dissolved in acetonitrile (2466 µl), then Hunig's Base (64.6 µl, 0.370 mmol) was added followed by benzylbromide (32.3 µl, 0.271 mmol). This reaction mixture was stirred for 1 h at ambient temperature after which the solvent was removed under reduced pressure to provide the product as a viscous oil. Further purification by silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ gave 43 mg (35% yield over two steps) of the desired product as a colorless viscous oil.

**LRMS**: $(M+H)^+$ = 496.2 m/z.

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ 7.58 – 7.55 (m, 1H), 7.52 – 7.45 (m, 1H), 7.43 – 7.36 (m, 7H), 4.19 – 4.07 (m, 2H), 3.77 (dd, *J* = 11.6, 4.0 Hz, 1H), 3.45 (s, 6H), 3.33 (dd, *J* = 13.5, 6.4 Hz, 2H), 3.15 (qd, *J* = 14.6, 13.7, 7.2 Hz, 3H), 2.92 (s, 3H), 2.64 (t, *J* = 11.6 Hz, 1H), 2.46 (s, 1H), 1.74 (d, *J* = 13.3 Hz, 5H).

**N-hydroxybenzimidamide**: To a stirred solution of N-((1-((1-benzylpiperidin-4-yl)methyl)-3-(3-cyanophenyl)-2-oxohexahydropyrimidin-5-yl)methyl)methanesulfonamide (43 mg, 0.087 mmol) in ethanol (1735 µl), hydroxylamine hydrochloride (12.06 mg, 0.174 mmol) followed by Hunig's Base (30.3 µl, 0.174 mmol) were added at room temperature. The combined reaction mixture was stirred for 6 h at 85 °C followed by 15-18 h of stirring at room temperature. After the reaction was complete, the solvent was evaporated under reduced pressure to give a viscous oil, this material was used in next step without further purification.

**LRMS**: $(M+H)^+$ = 529.3 m/z.

**O-Acetylation**: 3-(3-((1-benzylpiperidin-4-yl)methyl)-5-(methylsulfonamidomethyl)-2-oxotetra hydropyrimidin-1(2H)-yl)-N-hydroxybenzimidamide (50 mg, 0.095 mmol)(from step 1) was dissolved in acetic acid (1892 µl), then acetic anhydride (26.8 µl, 0.284 mmol) was added at once and the mixture was stirred for 20 min at room temperature. After reaction was complete, EtOH was added and the solvent evaporated under reduced pressure to give a viscous oil. Further purification by silica gel column chromatography on an ISCO CombiFlash Rf®, using a gradient of MeOH in $CHCl_3$ provided 30 mg (56% yield over two steps) of the desired compound as a colorless solid.

**LRMS**: $(M+H)^+$ = 571.3 m/z.

**HRMS**: for $C_{28}H_{36}N_6O_4S$ calculated $(M+H)^+$ = 553.25915 m/z; found $(M+H)^+$ = 553.25912 m/z. Exact MS showed the 5-membered cyclization of the side chain which is formed during the solvent evaporation.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.64 (q, *J* = 1.3 Hz, 1H), 7.59 (dt, *J* = 6.6, 2.3 Hz, 1H), 7.43 (dd, *J* = 4.9, 2.3 Hz, 2H), 7.38 – 7.28 (m, 5H), 3.83 (dd, *J* = 11.7, 4.2 Hz, 1H), 3.62 (s, 3H), 3.60 – 3.54 (m, 2H), 3.37 (s, 1H), 3.21 (d, *J* = 7.0 Hz, 2H), 3.00 (dd, *J* = 11.3, 3.8 Hz, 2H), 2.41 (dtd, *J* = 11.8, 8.1, 7.6, 3.3 Hz, 2H), 2.21 (s, 3H), 2.15 (d, *J* = 11.8 Hz, 2H), 1.88 – 1.71 (m, 4H), 1.42 – 1.30 (m, 3H).

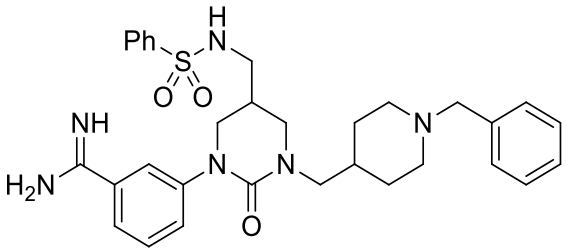
**Compound 8t:** N-acetoxy-3-(3-((1-benzylpiperidin-4-yl)methyl)-5-(methylsulfonamido methyl) -2-oxotetrahydropyrimidin-1(2H)-yl)benzimidamide (30 mg, 0.053 mmol) was dissolved in ethanol (1 ml) and acetic acid (0.2 ml); then Raney Ni (30 mg) was added and the mixture was shaken in a Parr apparatus under an atmosphere of $H_2$ at 40 psi for 16 h. After product formation the reaction was purged with $N_2$ then was filtered through a Celite pad and washed with MeOH. After solvent removal, the resulting crude product was purified by silica gel (4 g column) column chromatography on an ISCO Combiflash $R_f^®$ using a gradient of MeOH in $CHCl_3$ with 2% NH4OH as an additive, which afforded 12 mg of the desired product in 44% yield as a white solid.

**HRMS**: $C_{26}H_{36}N_6O_3S$ calculated $(M+H)^+$ = 513.26424 m/z; found $(M+H)^+$ = 513.26375 m/z.

**Purity**: 99%, retention time: 3.804 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.54 (dt, *J* = 1.9, 1.1 Hz, 1H), 7.47 (ddd, *J* = 5.6, 2.9, 1.8 Hz, 1H), 7.44 – 7.39 (m, 2H), 7.24 – 7.13 (m, 5H), 3.73 (ddd, *J* = 11.9, 4.3, 1.3 Hz, 1H), 3.56 – 3.44 (m, 2H), 3.42 (s, 2H), 3.26 – 3.22 (m, 1H), 3.20 – 3.16 (m, 1H), 3.10 (d, *J* = 7.0 Hz, 2H), 2.85 (d, *J* = 0.5 Hz, 3H), 2.81 (dd, *J* = 8.8, 5.5 Hz, 2H), 2.36 – 2.25 (m, 1H), 1.93 (tat, *J* = 11.8, 2.5 Hz, 2H), 1.70 – 1.56 (m, 3H), 1.29 – 1.16 (m, 3H).

3-(3-((1-benzylpiperidin-4-yl)methyl)-2-oxo-5-(phenylsulfonamidomethyl)tetrahydropyrimidin-1(2H)-yl)benzimidamide (**8u**)



**8u**

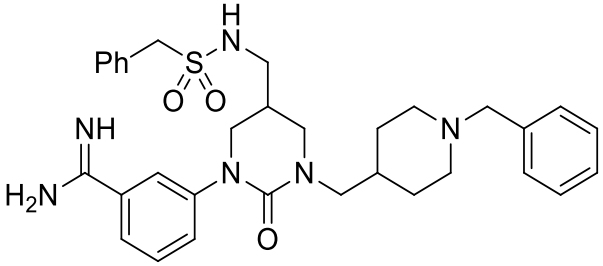
**Compound 8u** was synthesized from **Intermediate E** following similar a synthetic sequence as described for **Compound 8t**, where methanesulfonyl chloride replaced by phenylsulfonyl chloride. After purification by silica gel column chromatography on an ISCO CombiFlash $R_f$® using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, the final product was obtained as a white solid in 21% yield (10 mg).

**HRMS**: $C_{31}H_{38}N_6O_3S$ calculated $(M+H)^+$ = 575.27989 m/z; found $(M+H)^+$ = 575.27950 m/z.

**Purity**: 96%, retention time: 4.923 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 8.54 (s, 2H), 7.86 (t, *J* = 1.3 Hz, 1H), 7.84 (d, *J* = 1.7 Hz, 1H), 7.67 (dt, *J* = 1.9, 1.1 Hz, 1H), 7.65 - 7.53 (m, 6H), 7.46 - 7.37 (m, 5H), 3.96 (d, *J* = 4.8 Hz, 2H), 3.86 - 3.80 (m, 1H), 3.63 - 3.50 (m, 2H), 3.25 (ddd, *J* = 15.7, 9.2, 3.4 Hz, 4H), 3.00 (d, *J* = 7.1 Hz, 2H), 2.58 (t, *J* = 11.8 Hz, 3H), 2.42 - 2.34 (m, 1H), 1.97 - 1.78 (m, 3H), 1.52 - 1.38 (m, 2H).

3-(3-((1-benzylpiperidin-4-yl)methyl)-2-oxo-5-(((phenylmethyl)sulfonamido)methyl) tetrahydropyrimidin-1(2H)-yl)benzimidamide (**8v**)



**8v**

**Compound 8v** was synthesized from **Intermediate E** following a similar synthetic sequence as described for **Compound 8t**, where methanesulfonyl chloride replaced by phenylmethanesulfonyl chloride. After purification by silica gel column chromatography on an ISCO CombiFlash $R_f$®

using a gradient of MeOH in $CHCl_3$ with 2% $NH_4OH$ as an additive, the final product was obtained as an off-white solid in 24% yield (17 mg).

**HRMS**: $C_{32}H_{40}N_6O_3S$ calculated $(M+H)^+$ = 589.29420 m/z; found $(M+H)^+$ = 589.29409 m/z.

**Purity**: 94%, retention time: 4.913 min.

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.66 (dd, *J* = 2.1, 1.0 Hz, 1H), 7.62 - 7.58 (m, 3H), 7.49 - 7.41 (m, 7H), 7.39 - 7.33 (m, 3H), 4.37 (s, 2H), 4.05 (s, 2H), 3.87 - 3.78 (m, 1H), 3.61 - 3.46 (m, 2H), 3.30 - 3.23 (m, 3H), 3.06 (d, *J* = 7.0 Hz, 2H), 2.77 - 2.65 (m, 2H), 2.29 (tt, *J* = 7.0, 4.4 Hz, 1H), 1.94 (s, 2H), 1.90 - 1.81 (m, 2H), 1.54 - 1.42 (m, 2H), 1.29 (s, 1H).