**Supporting Information**

# Irreversible 4-Aminopiperidine Transglutaminase 2 Inhibitors for Huntington's Disease

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## Contents

Included is a general disclosure describing the synthesis of inhibitors followed by experimental details for the synthesis of compounds **8a** from Scheme 1 and **9h** from Scheme 2. In addition, experimental details are provided for the solubility, microsomal stability, MDCK-MDR1, and plasma stability assays. This is followed by a table of transglutaminase profiling data on all compounds.

## List of Schemes

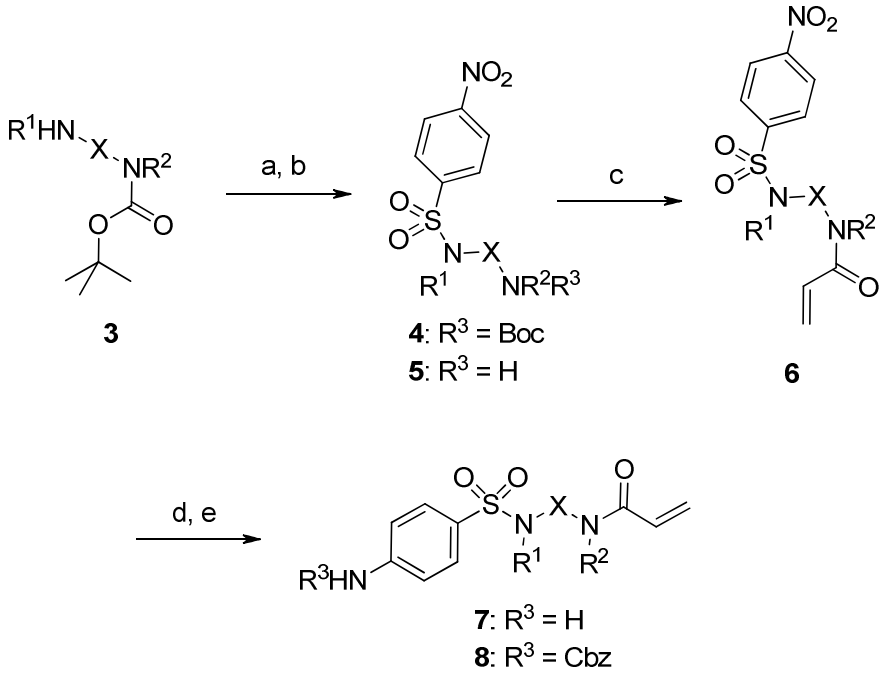


## List of Figures and Tables



A general synthesis of this initial series of compounds is shown in Scheme 1. Sulfonylation of a mono-Boc-protected diamine **3** with 4-nitrophenylsulfonyl chloride followed by deprotection using HCl in dioxane afforded amine **5**. Acylation with acryloyl chloride gave **6**, which was treated to iron mediated reduction of the nitro group and acylation of the resulting aniline to afford the final products (**8**).

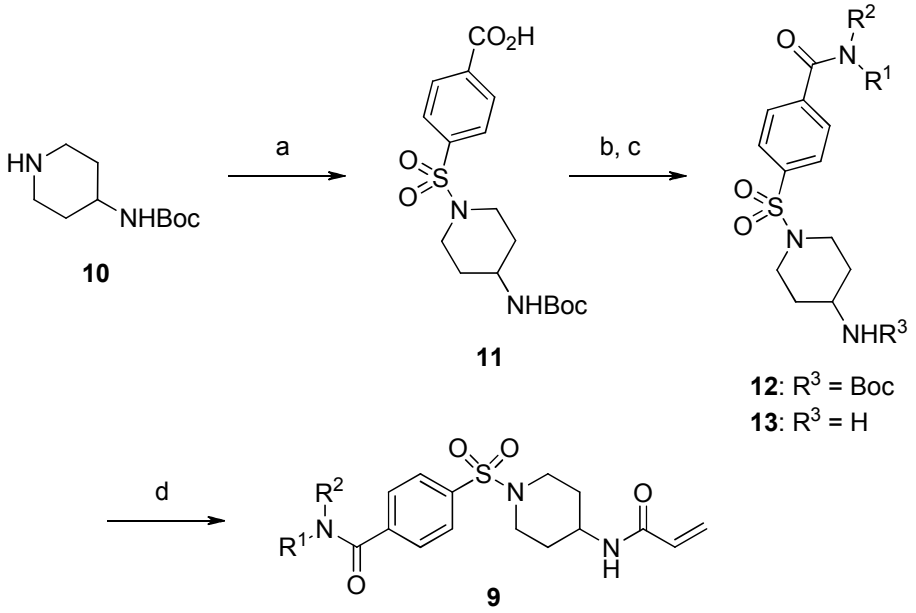
**Scheme 1**. General synthesis of acrylamide aniline replacement TG2 inhibitors **8**[a]




[a]Reagents and conditions: (a) 4-nitrophenylsulfonyl chloride, DIPEA, $CH_2Cl_2$, RT, 1 h; (b) HCl-dioxane, RT, 3 h; (c) acryloyl chloride, DIPEA, $CH_2Cl_2$, 3 h; (d) Fe, $NH_4Cl$ (aq), EtOH/$H_2O$ (5:1), 80 °C, 3 h; (e) CbzCl, THF, DIPEA, RT, 3 h.

A general synthesis of the compounds in Tables 2 and 3 is shown in Scheme 2. Sulfonylation of Boc-protected 4-aminopiperidine **10** gave sulfonamide **11**, which was then coupled to a variety of amines to give amide **12**. Deprotection of the amino group under acidic conditions followed by introduction of the acrylamide moiety afforded the final products **9**.

**Scheme 2**. Synthesis of TG2 inhibitors **9**[a]




[a]Reagents and conditions: (a) 4-chlorosulfonylbenzoic acid, DIPEA, THF, RT, 1 h; (b) $NHR^1R^2$, DIPEA, EDC, HOBt, $CH_2Cl_2$, 24 h; (c) HCl-dioxane, RT, 3 h; (d) acryloyl chloride, DIPEA, THF, 3 h.

## General Synthetic Procedures

Commercially available reagents and solvents (HPLC grade) were used without further purification. $^1H$ and $^{13}C$ NMR spectra were recorded on a Bruker DRX 500 MHz spectrometer or Bruker DPX 250 MHz spectrometer in deuterated solvents. Chemical shifts (δ) are in parts per million. Thin-layer chromatography (TLC) analysis was performed with Kieselgel 60 $F_{254}$ plates (Merck) and visualized using UV light.

Analytical HPLC-MS was performed on Shimadzu LCMS-2010EV systems using reverse phase Atlantis dC18 columns (3 μm, 2.1 x 50 mm), gradient 5-100% B (A = water/ 0.1% formic acid, B = acetonitrile/ 0.1% formic acid) over 3 min, injection volume 3 μL, flow = 1.0 mL/min. UV spectra were recorded at 215 nm using a Waters 2788 dual wavelength UV detector. Mass spectra were obtained over the range m/z 150 to 850 at a sampling rate of 2 scans per second using Waters LCT or analytical HPLC-MS on Shimadzu LCMS-2010EV systems using reverse phase Waters Atlantis dC18 columns (3 μm, 2.1 x 100 mm), gradient 5-100% B (A = water/ 0.1% formic acid, B = acetonitrile/ 0.1% formic acid) over 7 min, injection volume 3 μL, flow = 0.6 mL/min. UV spectra were recorded at 215 nm using a Waters 2996 photo diode array. Data were integrated and reported using Shimadzu psiport software. All compounds displayed purity of > 95% as determined by this method, unless stated otherwise.

**Synthesis of [4-(4-Acryloylamino-piperidine-1-sulfonyl)-phenyl]-carbamic acid benzyl ester (8a)**

**[1-(4-Nitro-benzenesulfonyl)-piperidin-4-yl]-carbamic acid *tert*-butyl ester**

Diisopropylethylamine (4.5 mL, 27.0 mmol) was added in one portion to a stirred solution of piperidin-4-yl-carbamic acid *tert*-butyl ester (5.0 g, 20.0 mmol) in DCM (40 mL) at room temperature. To this mixture was added 4-nitrophenyl sulfonyl chloride (6.0 g, 27.0 mmol) in one portion and the mixture was stirred at room temperature under a nitrogen atmosphere for 1 hour. After this time the mixture was diluted with DCM (100 mL) and washed sequentially with HCl (1 M solution, 50 mL), NaOH (1 M solution, 50 mL) and brine (50 mL). The organic layer was separated, dried ($MgSO_4$), filtered and concentrated. The resulting solid was collected by filtration, dried with diethyl ether and dried under vacuum to give the title compound (7.14 g, 74% yield) as a pale yellow solid. *m/z* ($ES^+$) ($M+Na^+$) 408.

**1-(4-Nitro-benzenesulfonyl)-piperidin-4-ylamine**

[1-(4-Nitro-benzenesulfonyl)-piperidin-4-yl]-carbamic acid *tert*-butyl ester (3.5 g, 9.0 mmol) was suspended in a 4M solution of HCl in dioxane (100 mL) and the resulting suspension was stirred at room temperature for 3 hours. After this time the solution was concentrated under vacuum, then azeotropically distilled with methanol and suspended in diethyl ether. The resulting solid precipitate was then collected by filtration, washed with diethyl ether and dried under vacuum to afford the title compound (2.3 g, 86% yield) as a white solid. *m/z* ($ES^+$) $(M+H)^+$ 286.

***N*-[1-(4-Nitro-benzenesulfonyl)-piperidin-4-yl]-acrylamide**

Diisopropylethylamine (6.8 mL, 41.5 mmol) was added in one portion to a stirred solution of 1-(4-nitro-benzenesulfonyl)-piperidin-4-ylamine (2.36 g, 8.3 mmol) in DCM (10 mL) followed by the dropwise addition of acryloyl chloride (0.78 mL, 9.1 mmol) and the mixture was stirred at room temperature under a nitrogen atmosphere for 3 hours. After this time the resulting precipitate was collected by filtration, washed with water and dried under vacuum to give the title compound (2.80 g, 98% yield) as a white solid. $\delta_H$ (500 MHz, DMSO) 8.39 - 8.51 (m, 2H) 7.95 - 8.10 (m, 3H) 6.11 - 6.21 (m, 1H) 6.01 - 6.10 (m, 1H) 5.56 (dd, *J*=10.09, 2.20 Hz, 1H) 3.61 - 3.68 (m, 1H) 3.52 - 3.60 (m, 2H) 2.56 - 2.66 (m, 2H) 1.83 (dd, *J*=13.20, 3.30 Hz, 2H) 1.36 - 1.47 (m, 2H). *m/z* ($ES^+$) $(M+H)^+$ 340.

***N*-[1-(4-Amino-benzenesulfonyl)-piperidin-4-yl]-acrylamide**

*N*-[1-(4-Nitro-benzenesulfonyl)-piperidin-4-yl]-acrylamide (2.80 g, 8.3 mmol) was suspended in a 5:1 mixture of ethanol and water (30 mL). To this solution was added iron powder (1.20 g, 21.0 mmol) followed by saturated ammonium chloride solution (3 mL) and the mixture was heated to 80 $^oC$ for three hours. After this time, the reaction mixture was cooled to room temperature and filtered through a pad of Celite, the filter pad was washed with ethanol (10 mL) and EtOAc (50 mL), and the solution was concentrated under vacuum. The resulting residue was suspended in water (10 mL) and the solid precipitate collected by filtration and dried under vacuum to give the title compound (1.3 g, 51% yield) as a white solid. *m/z* ($ES^+$) $(M+H)^+$ 310.

**[4-(4-Acryloylamino-piperidine-1-sulfonyl)-phenyl]-carbamic acid benzyl ester (8a)**

*N*-[1-(4-Amino-benzenesulfonyl)-piperidin-4-yl]-acrylamide (0.09 g, 0.3 mmol) was dissolved in THF (5 mL). To this was added diisopropylethylamine (0.1 mL, 0.6 mmol) in one portion followed by the dropwise addition of benzyl chloroformate (0.05 mL, 0.33 mmol). The resulting

mixture was stirred at room temperature under a nitrogen atmosphere for 3 hours. After this time the mixture was diluted with DCM (100 mL) and washed sequentially with HCl (1 M solution, 50 mL), NaOH (1 M solution, 50 mL) and brine (50 mL). The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified using flash column chromatography (elution: 100% EtOAc) to give the title compound (0.04 g, 34% yield) as a white powder. $\delta_H$ (500 MHz, DMSO-d6) 10.31 (s, 1H), 8.05 (d, *J* = 7.46 Hz, 1H), 7.77 – 7.60 (m, 4H), 7.50 – 7.29 (m, 7H), 6.16 (dd, *J* = 10.08, 17.06 Hz, 1H), 6.05 (dd, *J* = 2.25, 17.07 Hz, 1H), 5.56 (dd, *J* = 2.25, 10.07 Hz, 1H), 5.19 (s, 2H), 3.58 (dq, *J* = 5.31, 6.95, 10.42 Hz, 2H), 3.46 (d, *J* = 11.98 Hz, 3H), 2.45 (t, *J* = 10.49 Hz, 3H), 1.87 – 1.75 (m, 3H), 1.51 – 1.34 (m, 3H). $\delta_C$ (126 MHz, DMSO) 164.0, 153.4, 143.6, 136.4, 131.8, 128.9, 128.8, 128.6, 128.4, 125.4, 118.0, 66.3, 44.9, 44.9, 30.7. *m/z* ($ES^+$) $(M+H)^+$ 444. HRMS ($ES^+$) *m/z* 444.1598 (444.1593 Calcd for $C_{22}H_{25}N_3O_5S$ M+H).

**Synthesis of *N*-(2-Cyclohexylethyl)-4-[4-(prop-2-enamido)piperidine-1-sulfonyl]benzamide (9h)**

**4-(4-*tert*-Butoxycarbonylamino-piperidine-1-sulfonyl)-benzoic acid**

Diisopropylethylamine (10.9 mL, 62.4 mmol) was added in one portion to a stirred solution of piperidin-4-yl-carbamic acid *tert*-butyl ester (5.0 g, 20.0 mmol) in THF (150 mL) at room temperature. To this mixture was added 4-(chlorosulfonyl) benzoic acid (5.52 g, 25.0 mmol) portion-wise and the mixture was stirred at room temperature under a nitrogen atmosphere for 1 hour. After this time the mixture was concentrated and the resulting residue suspended in HCl (1 M solution, 100 mL), the resulting solid precipitate was collected by filtration, washed with water and dried under vacuum to give the title compound (9.2 g, 96% yield) as a white solid. *m/z* ($ES^+$) $(M+Na^+)$ 407.

***N*-[1-({4-[(2-Cyclohexylethyl)carbamoyl]benzene}sulfonyl)piperidin-4-yl]carbamic acid *tert*-butyl ester**

Diisopropylethylamine (0.36 mL, 2.1 mmol) was added in one portion to a stirred solution of 4-(4-*tert*-butoxycarbonylamino-piperidine-1-sulfonyl)-benzoic acid (0.2 g, 0.52 mmol), EDC (0.2 g, 1.04 mmol) and HOBT (0.16 g, 1.04 mmol) in DCM (5 mL) at room temperature. The mixture was stirred at room temperature for 10 minutes before 2-cyclohexylethan-1-amine hydrochloride salt (0.13 g, 0.78 mmol) was added in one portion and stirring continued for 24 hours. After this time the mixture was diluted with DCM (50 mL), before being washed sequentially with saturated sodium bicarbonate (20 mL), water (20 mL), and brine (20 mL). The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified using flash column chromatography (elution: 0 to 2% MeOH in DCM) to give the title compound (0.19 g, 76% yield) as a white powder. *m/z* ($ES^+$) $(M+Na^+)$ 516.

***N*-(2-Cyclohexylethyl)-4-[4-(prop-2-enamido)piperidine-1-sulfonyl]benzamide (9h)**

*N*-[1-({4-[(2-Cyclohexylethyl)carbamoyl]benzene}sulfonyl)piperidin-4-yl]carbamic acid *tert*-butyl ester (0.19 g, 0.39 mmol) was suspended in a 4M solution of HCl in dioxane (10 mL). The resulting suspension was stirred at room temperature for 3 hours. After this time the solution was concentrated under vacuum, then azeotropically distilled with methanol before being re-dissolved in THF (7 mL). Diisopropylethylamine (0.33 mL, 1.9 mmol) was then added in one

portion, followed by the dropwise addition of acryloyl chloride (0.03 mL, 0.43 mmol) and the mixture was stirred at room temperature under a nitrogen atmosphere for 3 hours. After this time the mixture was diluted with DCM (100 mL) and washed sequentially with HCl (1 M solution, 50 mL), NaOH (1 M solution, 50 mL), and brine (50 mL). The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified using flash column chromatography (elution: 2% MeOH, 98% DCM) to give the title compound (0.06 g, 33% yield) as a white powder. $\delta_H$ (500 MHz, DMSO-$d_6$) 8.69 (t, $J$ = 5.54 Hz, 1H), 8.11 – 7.99 (m, 3H), 7.83 (d, $J$ = 8.41 Hz, 2H), 6.16 (dd, $J$ = 10.07, 17.06 Hz, 1H), 6.04 (dd, $J$ = 2.22, 17.07 Hz, 1H), 5.56 (dd, $J$ = 2.22, 10.07 Hz, 1H), 3.68 – 3.59 (m, 2H), 3.53 (d, $J$ = 12.10 Hz, 2H), 3.33 – 3.24 (m, 2H), 2.54 (d, $J$ = 11.55 Hz, 2H), 1.87 – 1.78 (m, 2H), 1.75 – 1.56 (m, 5H), 1.42 (td, $J$ = 5.40, 13.93 Hz, 4H), 1.31 (ddq, $J$ = 3.65, 7.21, 10.79 Hz, 1H), 1.18 (dq, $J$ = 12.17, 23.45 Hz, 4H), 0.90 (q, $J$ = 9.62, 10.66 Hz, 2H). $\delta_C$ (126 MHz, DMSO-$d_6$) 164.6, 163.7, 138.4, 137.7, 131.5, 128.0, 127.3, 125.1, 44.6, 44.5, 37.0, 36.4, 34.6, 32.5, 30.4, 26.0, 25.6. *m/z* ($ES^+$) $(M+H)^+$ 448. HRMS ($ES^+$) *m/z* 448.2273 (448.227 Calcd for $C_{23}H_{33}N_3O_4S$ M+H).

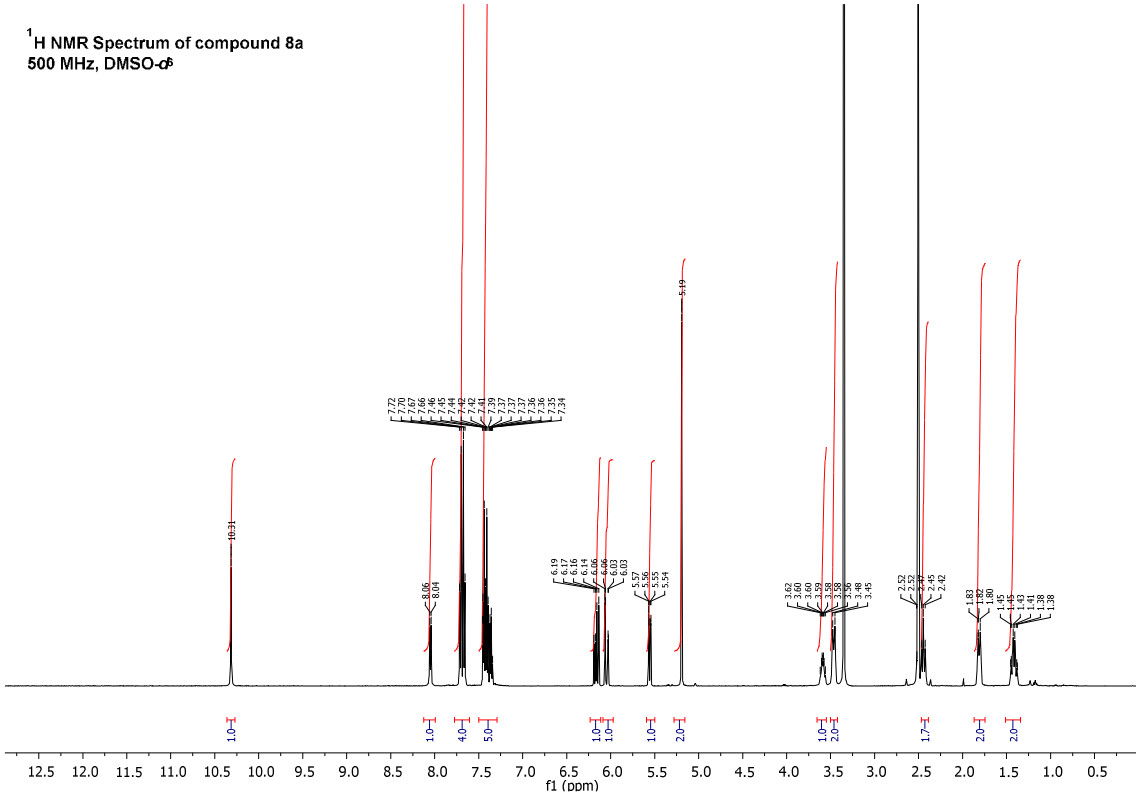



**Figure 1**. 500 MHz $^1$H NMR spectrum of **8a**.

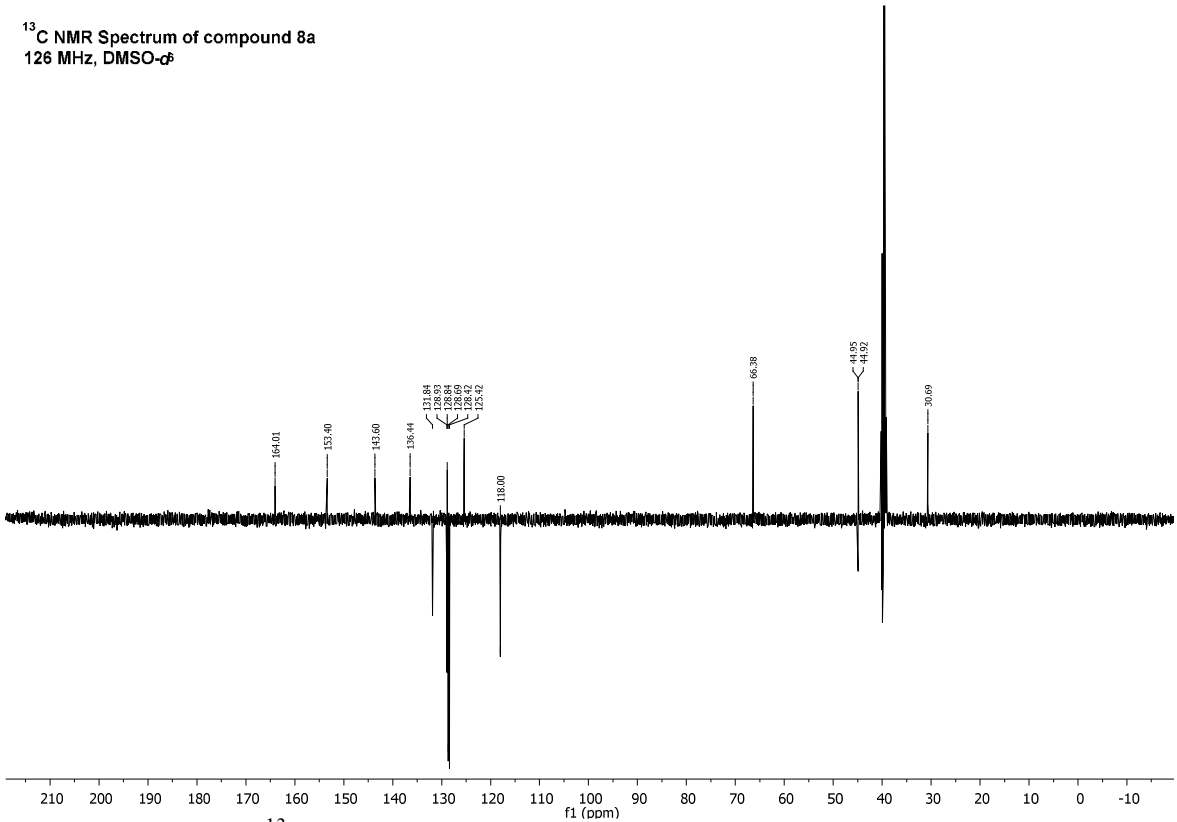



**Figure 2**. 126 MHz $^{13}$C NMR spectrum of **8a**.

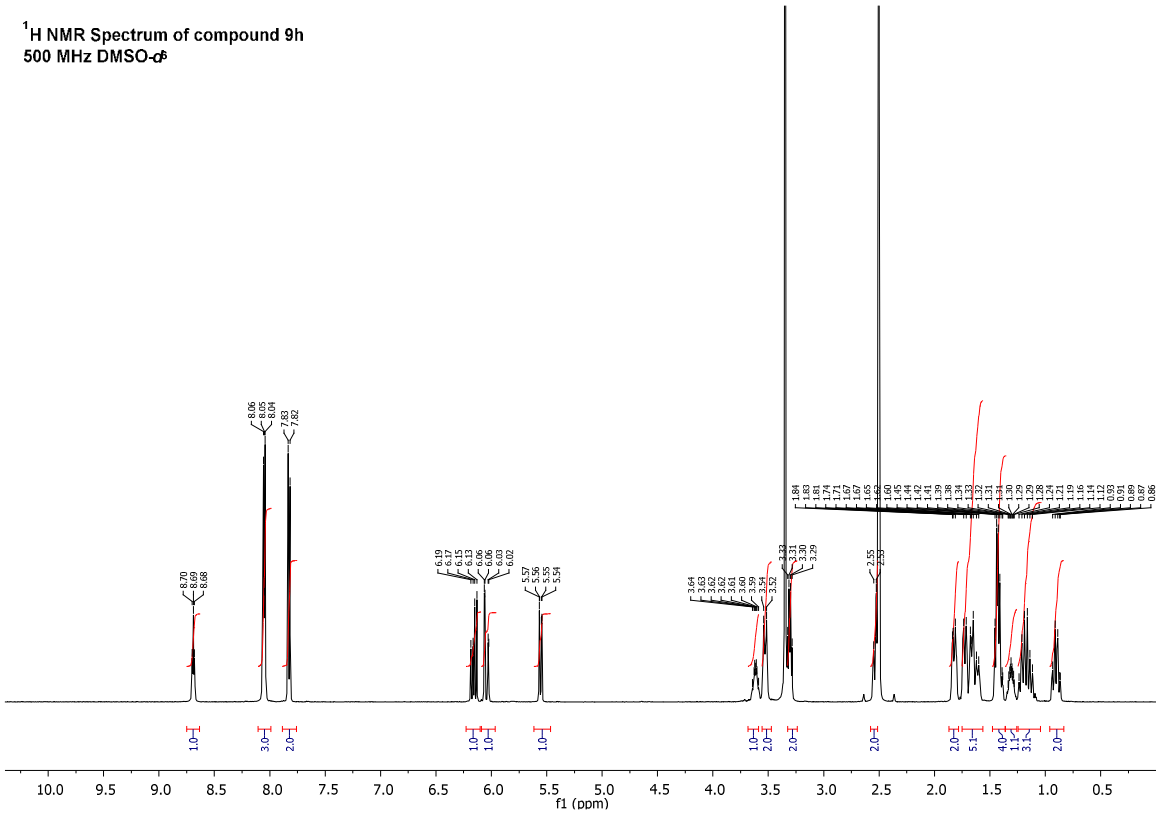



**Figure 3**. 500 MHz $^{1}$H NMR spectrum of **9h**.

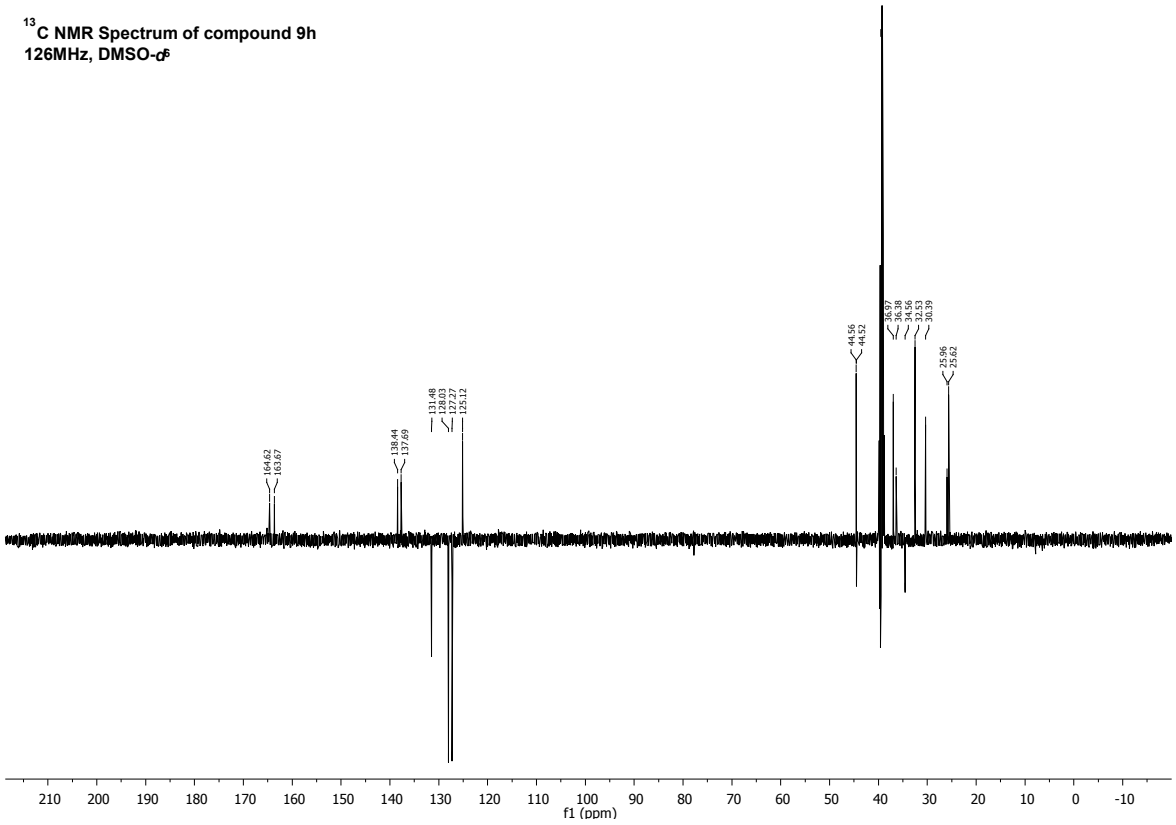



**Figure 4**. 126 MHz $^{13}$C NMR spectrum of **9h**.

## Kinetic Solubility (2% DMSO)

Using a 10 mM stock solution of each compound in 100% DMSO, dilutions are prepared to a theoretical concentration of 200 µM in both Phosphate Buffered Saline (PBS), pH 7.4 (2% DMSO final concentration) and in 100% DMSO. An aliquot of the 200 µM DMSO solution is then further diluted to 10 µM and all dilutions (n = 2 in 96-well plates) allowed to equilibrate at room temperature on an orbital shaker for two hours. The PBS dilutions are filtered using a Multiscreen HTS solubility filter plate (Millipore) and filtrates are analyzed by LC-UV and LC-MS. The concentration of compound in the PBS filtrate is determined by comparing the UV absorbance peak with that of the two DMSO dilutions which are used as calibration standards. Mass spectrometry is used to confirm the presence of the expected molecular ion in the UV peak measured. The effective range of the assay is 5 - 200 µM and compounds returning values close to the upper limit may have much higher solubility.

## Plasma Stability

Incubations of test compound (1 µM initial concentration, n = 2) were carried out with pooled plasma or BSA (45 mg/mL in 0.1 M phosphate buffered saline pH 7.4). The incubations were performed at 37 °C. Samples (50 µL) were obtained from the incubation at 0, 10, 30, 120, 240, 360 and 1440 min, and added to 150 µL of acetonitrile containing carbamazepine as analytical

internal standard to terminate the reaction. Samples were centrifuged and the supernatant fractions analyzed using LC-MS/MS.

**Liver Microsomal Stability**

Incubations of test compound (1 μM initial concentration, n = 2) were carried out with pooled hepatic liver microsomes (0.25 mg protein/mL in 0.1 M phosphate buffer pH7.4). NADPH (1 mM) was added to initiate the reactions. The incubations were performed at 37 °C. Samples (100 μL) were taken from the incubation at 0, 5, 10, 20 and 40 min and added to 100 μL of acetonitrile containing carbamazepine as analytical internal standard, to terminate the reaction. Samples were centrifuged and the supernatant fractions analyzed using LC-MS/MS.

Determinations of Analyte Stability in Plasma and Liver Microsomes

For all incubations, the instrument response (i.e. chromatographic peak area or peak height, normalized by internal standard), at each time-point were referenced to the zero time-point samples (as 100%) in order to determine the percentage of compound remaining at that time-point. Plots of the natural logarithm (Ln) of the percent of parent remaining for each compound, versus time, were used to determine the half-life in the incubation of interest.

Half-life values (t1/2) were calculated from the relationship: t1/2 (min) = -0.693/λ; where λ was the slope of the Ln concentration versus time curve.

For incubations in hepatic liver microsomes, the *in vitro* intrinsic clearance, Clint (μL/min/mg microsomal protein), was calculated using the following formula:

Clint = (0.693/t1/2 microsomal)*(ml incubation/mg microsomal protein)*(mg microsomal protein/g liver)*(g liver/kg body weight)

When quantification was required, calibration standards for parent compound and metabolites were prepared in control hepatic liver microsomes and extracted and analyzed as described for the study samples. Quantification of parent compound or metabolite was by extrapolation from the calibration line.

**Permeability and Effective Efflux Ratio in MDCK-MDR1**

The MDR1-MDCKII and wild type MDCKII cell lines were cultured in accordance with the guidelines provided by Solvo Biotechnology. Both wild-type MDCK and MDR1-MDCK cells were seeded at a cell density of $2.3 \times 10^5$ cells/well into 24-well Transwell plates and cultured for three days to form monolayers. Test compound was loaded into the donor compartments of the Transwell plate (24-well) bearing MDR1-MDCK or wild type MDCK monolayers. Test compound was added to either the apical or basolateral chambers of the Transwell plate assembly at a concentration of 10 μM in Hanks' Balanced Salt Solution containing 25 mM HEPES (pH 7.4). Lucifer Yellow was added to the apical buffer in all wells and its permeation monitored to assess integrity of the cell layer. As Lucifer Yellow (LY) cannot freely permeate lipophilic barriers, a high degree of LY transport indicates poor integrity of the cell layer and wells with LY permeability above 100 nm/s are rejected.

After one hour incubation at 37 °C, aliquots were taken from both chambers and added to acetonitrile containing analytical internal standard (carbamazepine) in a 96-well plate. Concentrations of compound in the samples were measured using LC-MS/MS. Concentrations of LY in the samples were measured using a fluorescence plate reader.

The apparent permeability ($P_{app}$) values of test compound were determined for both the apical to basal (A>B) and basal to apical (B>A) permeation and the efflux ratio (B>A: A>B) determined in both the wild type MDCK and MDR1-MDCK cells.

Apparent permeability ($P_{app}$) values were calculated from the relationship:

$$P_{app}\left(cm/sec \times 10^{-6}\right) = \left[\frac{compound_{acceptor\,final} \times V_{acceptor} \times V_{donor}}{compound_{donor\,initial} \times V_{donor} \times T_{inc} \times surface\,area}\right] \times 10^{6}$$

Where V = chamber volume and $T_{inc}$ = incubation time in seconds.

Donor = Chamber of Transwell to which compound is dosed: apical for A>B experiments and basal for B>A experiments.

Acceptor = Chamber of Transwell in to which permeation of compound is measured: basal for A>B experiments and apical for B>A experiments.

The Efflux ratios, as an indication of active efflux from the apical cell surface, were calculated using the ratio of $P_{app}$ B>A/ $P_{app}$ A>B.

The effective efflux ratio was also determined from the ratio observed in MDR1-MDCK cells relative to the ratio observed in wild-type cells. Known substrates for human MDR1 typically display effective efflux ratios of greater than two.

**Table 1**. Transglutaminase Inhibitor Selectivity Data[a]

| Cmpd No. | TG2 $IC_{50}$ ± SD (μM) | mTG2 $IC_{50}$ ± SD (μM) | TG1 $IC_{50}$ ± SD (μM) | TG3 $IC_{50}$ ± SD (μM)[b] | TG6 $IC_{50}$ ± SD (μM) | FXIIIa $IC_{50}$ ± SD (μM) |
|---|---|---|---|---|---|---|
| **1** | 0.11 ± 0.073 | 0.77 ± 0.40 | 6.0 ± 2.7 | 110.828 | NT | 0.31 ± 0.0092 |
| **8a** | 0.28 ± 0.064 | NT | 0.91 ± 0.48 | > 80 | 8.9 | 3.6 ± 3.1 |
| **8b** | 5.1 ± 0.20 | NT | NT | NT | NT | NT |
| **8c** | 2.9 ± 0.011 | 5.2 | 2.0 | > 80 | NT | 22 |
| **8d** | 3.3 ± 0.54 | NT | NT | NT | NT | NT |
| **8e** | > 80 | NT | NT | NT | NT | NT |
| **8f** | > 80 | NT | NT | NT | NT | NT |
| **8g** | 56 | NT | NT | NT | NT | NT |
| **8h** | 30 ± 16 | NT | NT | NT | NT | NT |
| **8i** | 1.6 ± 0.085 | NT | NT | NT | NT | NT |
| **14** | 8.7 ± 1.2 | NT | NT | NT | NT | NT |
| **15** | 1.6 ± 0.056 | NT | NT | NT | NT | NT |
| **9a** | 0.18 ± 0.029 | NT | 0.905 | NT | 38 | 9.3 |
| **9aa** | 0.18 ± 0.044 | 0.08 | 1.14 | NT | > 80 | 13 |
| **9b** | 0.20 ± 0.024 | 0.084 | 1.5 | NT | 27 | 4.4 |
| **9c** | 0.77 ± 0.16 | NT | 2.18 | NT | > 80 | 18 |
| **9d** | 0.41 ± 0.074 | 0.16 | 1.03 | NT | 13 | 7.9 |
| **9e** | 0.62 ± 0.16 | NT | 0.331 | NT | 40 | 10 |
| **9f** | 0.46 ± 0.093 | NT | 1.06 | NT | 48 | 9.0 |
| **9g** | 0.63 ± 0.079 | NT | 3.12 | NT | 50 | 36 |
| **9h** | 0.14 ± 0.011 | 0.11 | 0.443 | NT | > 80 | 2.0 |
| **9i** | 0.66 ± 0.087 | 0.26 | 1.14 | NT | > 80 | 47 |
| **9j** | 0.36 ± 0.14 | NT | 1.62 | NT | 38 | 5.9 |
| **9k** | 0.38 ± 0.072 | NT | 1.21 | NT | 38 | 4.4 |
| **9l** | 0.17 ± 0.0092 | 0.12 | 0.798 | NT | 51 | 3.1 |
| **9m** | 0.32 ± 0.043 | 0.16 | 1.49 | NT | 31 | 16 |
| **9n** | 0.20 ± 0.013 | 0.17 | 1.28 | NT | 98 | 5.9 |
| **9o** | 0.55 ± 0.049 | 0.16 | 2.91 | NT | 104 | > 80 |
| **9p** | 0.28 ± 0.016 | 0.1 | 1.09 | NT | 22 | 17 |
| **9q** | 0.11 ± 0.0028 | 0.055 | 1.24 | NT | 34 | 6.4 |
| **9r** | 22 ± 15 | NT | NT | NT | NT | NT |
| **9s** | 57 ± 8.8 | NT | NT | NT | NT | NT |
| **9t** | 0.28 ± 0.0071 | 0.093 | 2.08 | NT | > 80 | > 80 |
| **9u** | 0.31 ± 0.016 | 0.11 | 1.13 | NT | 28 | 9.0 |
| **9v** | 0.23 ± 0.016 | NT | 2.04 | NT | 86 | 11 |
| **9w** | 0.46 ± 0.093 | 0.12 | 1.66 | NT | 25 | 11 |
| **9x** | 0.29 ± 0.052 | 0.0828 | 1.15 | NT | 38 | 46 |
| **9y** | 0.26 ± 0.081 | NT | 1.15 | NT | > 80 | 5.2 |
| **9z** | 0.16 ± 0.021 | 0.083 | 0.85 ± 0.32 | NT | 2.7 | 3.8 ± 3.1 |

[a]Values accompanied by standard deviation were averaged from at least two independent experiments; all enzymes are the human form other than mTG2, which is the corresponding enzyme from mouse

[b]Based upon the results of TG3 testing of numerous analogs showing lack of activity, TG3 testing on this chemotype was discontinued