


Discovery of sultam-containing small-molecule disruptors of the huntingtin–calmodulin protein–protein interaction

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Abstract

The aberrant protein–protein interaction between calmodulin and mutant huntingtin protein in Huntington’s disease patients has been found to contribute to Huntington’s disease progression. A high-throughput screen for small molecules capable of disrupting this interaction revealed a sultam series as potent small-molecule disruptors. Diversification of the sultam scaffold afforded a set of 24 analogs for further evaluation. Several structure–activity trends within the analog set were found, most notably a negligible effect of absolute stereochemistry and a strong beneficial correlation with electron-withdrawing aromatic substituents. The most promising analogs were profiled for off-target effects at relevant kinases and, ultimately, one candidate molecule was evaluated for neuroprotection in a neuronal cell model of Huntington’s disease.

Keywords Huntington’s disease · High-throughput screening · Neurodegeneration · Structure–activity relationship studies

Introduction

Progressive neurodegenerative disorders such as Parkinson’s disease, Alzheimer’s disease, and Huntington’s disease (HD) have remained challenging therapeutic areas for drug discovery in large part due to poor understanding of

the molecular basis for disease progression (Hague et al. 2005; Marsh 2019). The underlying pathology of HD arises from an underlying gene mutation that encodes for a mutant Huntingtin protein (mHtt) whose subsequent aggregation leads to neuronal death. Currently available therapeutic agents for the treatment of HD and other neurodegenerative diseases treat only the symptoms associated with the disease, leaving the underlying pathology and progression unaffected. The treatment disparity is especially apparent for HD where both the underlying cause and the molecular basis of the disease progression has been established, yet the FDA-approved drugs available, such as tetrabenazine, merely dampen the involuntary movements (chorea) associated with HD (Paleacu 2007). Currently, the majority of developmental HD drugs that aim to treat disease progression are macromolecular biologics that will require invasive routes of administration (i.e., intrathecal or intracerebroventricular) for these agents to reach the CNS (Berg 2017). This would be particularly problematic for the aging population that exhibits the highest penetrance of the disease. Accordingly, small-molecule therapeutics that directly address the primary HD lesion are highly sought. To this end, herein we report preliminary efforts toward validating drug-like agents that focus on a target protein–protein interaction (PPI) that has previously been validated using a polypeptide inhibitor.

We dedicate this paper to Professor Robert Hanzlik in recognition of his many scientific achievements.

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A current hypothesis for the cause of mHtt aggregation is transglutaminase (TG) upregulation that catalyzes the calcium-dependent cross-linking (transamidation) of peptide-bound glutamine residues on substrate proteins to the ϵ -amino-group of lysine residues on either the same or different proteins (Folk and Finlayson 1977; Karpuj et al. 1999). These cross-linkages are implicated in tau protein oligomerization in Alzheimer's disease patients and there is also evidence to suggest that aberrant TG activity contributes to the HD phenotype (Tucholski 1999). TG mRNA, protein levels and activity have all been shown to be upregulated in HD cell models (Karpuj et al. 1999; Lesort 1999, Zainelli et al. 2005), and TG proteins co-localize with mHtt in inclusion bodies found in HD patients (Zainelli et al. 2003). Furthermore, inhibiting or knocking out TG in mouse models increases their survival (Dedeoglu et al. 2002; Mastroberardino et al. 2002) while inhibiting TG in a cellular model also decreases cross-linking of mHtt and the cytotoxicity associated with mHtt expression (Zainelli et al. 2005).

Calmodulin (CaM) is a calcium (Ca^{2+})-binding protein that, amongst its other roles in the cell, regulates TG activity (Cheung 1982; Puszkin and Raghuraman 1985). Studies have shown that CaM and TG proteins co-immunoprecipitate with mHtt in transfected cell culture models as well as intranuclear inclusions found in HD brain samples (Zainelli et al. 2004). Furthermore, inhibition of CaM in cells expressing mHtt and TG resulted in a decrease of TG-catalyzed mHtt aggregation (Zainelli et al. 2004). Interestingly, although wild-type huntingtin does not interact with CaM, mHtt has a much higher affinity for CaM (Bao et al. 1996). The increased affinity for CaM by mHtt alters CaM function and leads to aberrant biochemical processes within the cell, including upregulation of TG activity. Based on the above data, we believe that an underlying driver of HD progression is the sequestration and upregulation of TG by the mHtt–CaM heterodimer leading to an increase of TG-catalyzed mHtt cross-linking. Previous studies using short peptide sequences borrowed from CaM have provided substantial evidence that the disruption of the mHtt–CaM PPI is an attractive target for the treatment of HD in vitro, in vivo, and in mouse models (Dudek et al. 2008, 2010; Dai et al. 2009). In biochemical and cell-based assays, disruption of this system leads to both decreased TG-catalyzed modifications of mHtt and mHtt-associated cytotoxicity (Dudek et al. 2008, 2010); in mHtt mouse models, administration of the CaM fragment significantly reduced body weight loss and improved motor function as indicated by improved rotarod performance, longer stride length, lower stride frequency, fewer low mobility bouts, and longer travel distance than HD controls (Dai et al. 2009). The inhibition of the mHtt–CaM PPI could be a viable target for small molecules (Berg 2017), and complementary to macromolecular therapies in

development, such as antisense mRNA or zinc-finger protease inhibitors, that attempt to interrupt the production of mHtt at the transcriptional or translational level. Accordingly, we sought to develop small-molecule probes capable of disrupting this PPI to validate this interaction as a viable small-molecule target.

Materials and methods

Chemistry

See SI for the synthesis, characterization, ^1H and ^{13}C NMR spectra of all intermediates and ^1H and ^{13}C NMR spectra of final compounds.

General Procedure A: HATU-promoted amide synthesis

A 1-dram vial equipped with a stir bar was charged with 40 mg of carboxylic acid **6–8**, HATU (1.2 equiv), and MeCN (10 mL per mmol acid) and the reaction stirred at rt for 20 min. The requisite amine component (2.0 equiv) was added and the reaction stirred for an additional 20 min. DIPEA (3.0 equiv) was then added and the reaction stirred overnight for 12–19 h. The reaction mixture was diluted with EtOAc, washed with saturated, aqueous sodium bicarbonate solution, and then brine. The organic layer was dried over anhydrous Na_2SO_4 , filtered, concentrated, and purified via flash chromatography (30% EtOAc in hexanes) to afford the amide product as a colorless oil.

(S)-N-Benzyl-4-methyl-2-(5-methylene-1,1-dioxido-4,5-dihydrobenzo[f][1,2]thiazepin-2(3H)-yl)pentanamide (**9b**)

Carboxylic acid **6** and benzylamine were reacted according to General Procedure A to afford the amide product **9b** as a colorless oil (45.1 mg, 0.109 mmol, 85% yield). $R_f = 0.4$ (20% EtOAc in hexanes). ^1H NMR (400 MHz, CDCl_3) δ 7.82 (*dd*, $J = 8.0, 1.4$ Hz, 1H), 7.42 (*td*, $J = 7.5, 1.4$ Hz, 1H), 7.30 (*ddd*, $J = 7.4, 6.5, 1.5$ Hz, 2H), 7.26–7.16 (*m*, 3H), 7.09–7.04 (*m*, 2H), 6.13 (*s*, 1H), 5.21 (*dd*, $J = 24.0, 1.2$ Hz, 2H), 4.43 (*dd*, $J = 8.5, 6.4$ Hz, 1H), 4.28 (*dd*, $J = 14.8, 5.9$ Hz, 1H), 4.16 (*dd*, $J = 14.8, 5.7$ Hz, 1H), 3.71 (*ddd*, $J = 13.4, 9.2, 3.8$ Hz, 1H), 3.51 (*ddd*, $J = 14.1, 6.3, 4.2$ Hz, 1H), 2.59 (*ddd*, $J = 13.9, 6.3, 3.8$ Hz, 1H), 2.45 (*ddd*, $J = 13.7, 9.2, 4.2$ Hz, 1H), 1.71 (*ddd*, $J = 14.4, 8.3, 6.4$ Hz, 1H), 1.37 (*ddd*, $J = 14.0, 8.5, 5.6$ Hz, 1H), 1.30–1.15 (*m*, 1H), 0.74 (*d*, $J = 4.4$ Hz, 3H), and 0.72 (*d*, $J = 4.6$ Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 169.9, 146.9, 140.0, 139.7, 137.7, 132.8, 130.6, 128.8, 127.7, 127.6, 127.6, 126.0, 119.8, 59.2, 45.5, 43.8, 37.8, 35.7, 24.7, 22.8, and 22.0. FTIR (thin film) 2955, 2359, 2341, 1661, and 1332 cm^{-1} . $[\alpha]_D^{22} = 77.7$ (*c* 1.0, CHCl_3). HRMS (ESI, m/z): $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{29}\text{N}_2\text{O}_3\text{S}^+$ 413.1893, found 413.1868.

(S)-N-(4-Chlorobenzyl)-4-methyl-2-(5-methylene-1,1-dioxido-4,5-dihydrobenzo[f][1,2]thiazepin-2(3H)-yl)pentanamide (9c)

Carboxylic acid **6** and 4-chlorobenzylamine were reacted according to General Procedure A to afford the amide product **9c** as a colorless oil (51.3 mg, 0.115 mmol, 93% yield). $R_f=0.3$ (20% EtOAc in hexanes). ^1H NMR (400 MHz, CDCl_3) δ 7.81 (*dd*, $J=8.2, 1.4$ Hz, 1H), 7.47–7.39 (*m*, 1H), 7.30 (*ddd*, $J=7.8, 6.3, 1.5$ Hz, 2H), 7.22–7.15 (*m*, 2H), 7.01 (*d*, $J=8.4$ Hz, 2H), 6.24 (*s*, 1H), 5.25 (*d*, $J=1.1$ Hz, 1H), 5.19 (*d*, $J=1.2$ Hz, 1H), 4.43 (*dd*, $J=8.5, 6.4$ Hz, 1H), 4.27–4.10 (*m*, 2H), 3.71 (*ddd*, $J=13.5, 9.4, 3.7$ Hz, 1H), 3.48 (*ddd*, $J=14.1, 6.2, 4.2$ Hz, 1H), 2.58 (*ddd*, $J=13.9, 6.2, 3.7$ Hz, 1H), 2.44 (*ddd*, $J=13.7, 9.5, 4.2$ Hz, 2H), 1.70 (*ddd*, $J=14.4, 8.3, 6.4$ Hz, 1H), 1.36 (*ddd*, $J=14.0, 8.5, 5.6$ Hz, 1H), 1.27–1.14 (*m*, 1H), 0.72 (*d*, $J=3.3$ Hz, 3H), and 0.71 (*d*, $J=3.4$ Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 170.1, 146.8, 140.0, 139.6, 136.4, 133.4, 132.8, 130.6, 129.0, 128.9, 127.6, 126.0, 119.9, 59.2, 45.5, 43.0, 37.7, 35.7, 24.7, 22.8, and 21.9. FTIR (thin film) 2955, 2359, 2341, 1663, and 1331 cm^{-1} . $[\alpha]_D^{22.2} - 67.1$ (*c* 1.0, CHCl_3). HRMS (ESI, m/z): m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{28}\text{ClN}_2\text{O}_3\text{S}^+$ 447.1504, found 447.1489.

(S)-N-(4-Chloro-3-(trifluoromethyl)benzyl)-4-methyl-2-(5-methylene-1,1-dioxido-4,5-dihydrobenzo[f][1,2]thiazepin-2(3H)-yl)pentanamide (9d)

Carboxylic acid **6** and 4-chloro-3-(trifluoromethyl)benzylamine were reacted according to General Procedure A to afford the amide product **9d** as a colorless oil (61.7 mg, 0.120 mmol, 97% yield). $R_f=0.3$ (20% EtOAc in hexanes). ^1H NMR (400 MHz, CDCl_3) δ 7.80 (*dd*, $J=8.1, 1.3$ Hz, 1H), 7.46–7.40 (*m*, 2H), 7.35 (*d*, $J=8.2$ Hz, 1H), 7.32–7.26 (*m*, 2H), 7.25–7.20 (*m*, 1H), 6.41 (*s*, 1H), 5.26 (*d*, $J=1.1$ Hz, 1H), 5.20 (*d*, $J=1.2$ Hz, 1H), 4.44 (*dd*, $J=8.3, 6.6$ Hz, 1H), 4.26 (*dd*, $J=6.2, 1.7$ Hz, 2H), 3.72 (*ddd*, $J=13.6, 9.5, 3.6$ Hz, 1H), 3.48 (*ddd*, $J=14.1, 6.1, 4.2$ Hz, 1H), 2.59 (*ddd*, $J=13.9, 6.1, 3.8$ Hz, 1H), 2.44 (*ddd*, $J=14.1, 9.6, 3.9$ Hz, 1H), 1.70 (*ddd*, $J=14.0, 8.1, 6.6$ Hz, 1H), 1.34 (*ddd*, $J=14.0, 8.3, 5.7$ Hz, 1H), 1.25–1.12 (*m*, 1H), 0.72 (*d*, $J=2.2$ Hz, 3H), and 0.70 (*d*, $J=2.4$ Hz, 3H). ^{13}C NMR (176 MHz, CDCl_3) δ 170.3, 146.8, 140.0, 139.4, 137.3, 132.9, 132.0, 131.8, 131.3, 130.6, 128.5 (*q*, $J=31.44$ Hz), 127.6, 126.7 (*q*, $J=5.19$ Hz), 125.9, 122.8 (*q*, $J=273.30$), 119.9, 59.1, 45.5, 42.6, 37.7, 35.7, 24.7, 22.6, and 22.0. FTIR (thin film) 2957, 2359, 2341, 1668, 1318, 1168, and 1130 cm^{-1} . $[\alpha]_D^{22.4} - 51.3$ (*c* 1.0, CHCl_3). HRMS (ESI, m/z): m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{27}\text{ClF}_3\text{N}_2\text{O}_3\text{S}^+$ 515.1378, found 515.1406.

(S)-4-Methyl-N-(4-methylbenzyl)-2-(5-methylene-1,1-dioxido-4,5-dihydrobenzo[f][1,2]thiazepin-2(3H)-yl)pentanamide (9e)

Carboxylic acid **6** and 4-methylbenzylamine were reacted according to General Procedure A to afford the amide product **9e** as a colorless oil (45.8 mg, 0.107 mmol, 87% yield). $R_f=0.3$ (20% EtOAc in hexanes). ^1H NMR (400 MHz, CDCl_3) δ 7.82 (*dd*, $J=8.2, 1.4$ Hz, 1H), 7.42 (*td*, $J=7.4, 1.4$ Hz, 1H), 7.29 (*ddd*, $J=7.8, 6.4, 1.5$ Hz, 2H), 7.03 (*d*, $J=7.7$ Hz, 2H), 6.95 (*d*, $J=8.0$ Hz, 2H), 6.07 (*s*, 1H), 5.24 (*d*, $J=1.1$ Hz, 1H), 5.18 (*d*, $J=1.2$ Hz, 1H), 4.41 (*dd*, $J=8.4, 6.4$ Hz, 1H), 4.23 (*dd*, $J=14.6, 5.9$ Hz, 1H), 4.10 (*dd*, $J=14.6, 5.5$ Hz, 1H), 3.71 (*ddd*, $J=13.4, 9.2, 3.8$ Hz, 1H), 3.51 (*ddd*, $J=14.1, 6.4, 4.2$ Hz, 1H), 2.64–2.52 (*m*, 1H), 2.51–2.39 (*m*, 1H), 2.26 (*s*, 3H), 1.70 (*ddd*, $J=14.4, 8.3, 6.4$ Hz, 1H), 1.37 (*ddd*, $J=14.0, 8.5, 5.6$ Hz, 1H), 1.23 (*dq*, $J=13.1, 6.5$ Hz, 1H), 0.73 (*d*, $J=4.4$ Hz, 3H), and 0.71 (*d*, $J=4.5$ Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 169.8, 147.0, 140.1, 139.7, 137.3, 134.7, 132.8, 130.5, 129.5, 127.7, 127.6, 126.0, 119.8, 59.2, 45.5, 43.5, 37.8, 35.7, 24.7, 22.8, 22.0, and 21.2. FTIR (thin film) 2955, 2359, 2341, 1661, and 1332 cm^{-1} . $[\alpha]_D^{22.4} - 71.2$ (*c* 1.0, CHCl_3). HRMS (ESI, m/z): $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{31}\text{N}_2\text{O}_3\text{S}^+$ 427.2050, found 427.2042.

(S)-N-(4-Methoxybenzyl)-4-methyl-2-(5-methylene-1,1-dioxido-4,5-dihydrobenzo[f][1,2]thiazepin-2(3H)-yl)pentanamide (9f)

Carboxylic acid **6** and 4-methoxybenzylamine were reacted according to General Procedure A to afford the amide product **9f** as a colorless oil (46.6 mg, 0.105 mmol, 85% yield). $R_f=0.2$ (20% EtOAc in hexanes). ^1H NMR (400 MHz, CDCl_3) δ 7.82 (*dd*, $J=8.2, 1.4$ Hz, 1H), 7.42 (*td*, $J=7.4, 1.4$ Hz, 1H), 7.30 (*ddd*, $J=6.9, 6.3, 1.4$ Hz, 2H), 7.02–6.97 (*m*, 2H), 6.78–6.72 (*m*, 2H), 6.06 (*s*, 1H), 5.24 (*d*, $J=1.1$ Hz, 1H), 5.18 (*d*, $J=1.2$ Hz, 1H), 4.41 (*dd*, $J=8.5, 6.4$ Hz, 1H), 4.20 (*dd*, $J=14.5, 5.8$ Hz, 1H), 4.09 (*dd*, $J=14.6, 5.6$ Hz, 1H), 3.73 (*s*, 4H), 3.51 (*ddd*, $J=14.1, 6.3, 4.1$ Hz, 1H), 2.58 (*ddd*, $J=13.9, 6.4, 3.8$ Hz, 1H), 2.45 (*ddd*, $J=13.6, 9.1, 4.0$ Hz, 1H), 1.70 (*ddd*, $J=14.4, 8.3, 6.4$ Hz, 1H), 1.37 (*ddd*, $J=14.0, 8.4, 5.5$ Hz, 1H), 1.23 (*td*, $J=12.9, 12.4, 6.1$ Hz, 1H), 0.73 (*d*, $J=4.5$ Hz, 3H), and 0.71 (*d*, $J=4.5$ Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 169.8, 159.1, 146.9, 140.0, 139.7, 132.8, 130.5, 129.8, 129.0, 127.6, 126.0, 119.8, 114.2, 59.1, 55.4, 45.4, 43.2, 37.8, 35.7, 24.7, 22.8, and 21.9. FTIR (thin film) 2955, 2359, 2341, 1662, and 1512 cm^{-1} . $[\alpha]_D^{22.1} - 66.3$ (*c* 1.0, CHCl_3). HRMS (ESI, m/z): m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{24}\text{H}_{31}\text{N}_2\text{O}_3\text{S}^+$ 433.1999, found 433.1992.

(S)-N-(Cyclohexylmethyl)-4-methyl-2-(5-methylene-1,1-dioxido-4,5-dihydrobenzo[f][1,2]thiazepin-2(3H)-yl)pentanamide (9g)

Carboxylic acid **6** and 4-cyclohexylmethylamine were reacted according to General Procedure A to afford the amide product **9g** as a colorless oil (40 mg, 0.096 mmol, 77% yield). $R_f=0.5$ (20% EtOAc in hexanes). ^1H NMR (400 MHz, CDCl_3) δ 7.85 (*dd*, $J=8.2, 1.4$ Hz, 1H), 7.48–7.40 (*m*, 1H), 7.36–7.28 (*m*, 2H), 5.85 (*s*, 1H), 5.27 (*d*, $J=1.1$ Hz, 1H), 5.22 (*d*, $J=1.3$ Hz, 1H), 4.37 (*dd*, $J=8.5, 6.3$ Hz, 1H), 3.73 (*ddd*, $J=14.1, 9.2, 3.7$ Hz, 1H), 3.51 (*ddd*, $J=14.1, 6.4, 4.1$ Hz, 1H), 2.99–2.79 (*m*, 2H), 2.68–2.54 (*m*, 1H), 2.52–2.39 (*m*, 1H), 1.76–1.42 (*m*, 7H), 1.41–0.96 (*m*, 5H), and 0.84–0.66 (*m*, 8H). ^{13}C NMR (101 MHz, CDCl_3) δ 170.0, 147.9, 140.1, 139.7, 132.8, 130.6, 127.6, 126.0, 119.8, 59.2, 45.9, 45.5, 37.8, 35.8, 30.8, 26.4, 25.9, 25.9, 24.7, 22.8, and 22.0. FTIR (thin film) 2922, 2539, 2341, 1661, and 1332 cm^{-1} . $[\alpha]_D^{22.4} - 68.5$ (*c* 1.0, CHCl_3). HRMS (ESI, m/z): $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{35}\text{N}_2\text{O}_3\text{S}^+$ 419.2363, found 419.2361.

(S)-N-(3,4-Dichlorophenethyl)-4-methyl-2-(5-methylene-1,1-dioxido-4,5-dihydrobenzo[f][1,2]thiazepin-2(3H)-yl)pentanamide (9h)

Carboxylic acid **6** and 3,4-dichlorophenethylamine were reacted according to General Procedure A to afford the amide product **9h** as a colorless oil (55 mg, 0.11 mmol, 90% yield). $R_f=0.4$ (30% EtOAc in hexanes). ^1H NMR (400 MHz, CDCl_3) δ 7.88 (*ddd*, $J=7.7, 1.3, 0.6$ Hz, 1H), 7.51 (*td*, $J=7.5, 1.4$ Hz, 1H), 7.41–7.32 (*m*, 3H), 7.24 (*d*, $J=2.1$ Hz, 1H), 6.99 (*dd*, $J=8.2, 2.1$ Hz, 1H), 6.06–5.99 (*m*, 1H), 5.32 (*d*, $J=1.1$ Hz, 1H), 5.27 (*d*, $J=1.2$ Hz, 1H), 4.39 (*dd*, $J=8.2, 6.6$ Hz, 1H), 3.70 (*ddd*, $J=13.5, 9.6, 3.6$ Hz, 1H), 3.47 (*ddd*, $J=14.1, 6.0, 4.2$ Hz, 1H), 3.42–3.30 (*m*, 2H), 2.65 (*ddt*, $J=13.3, 6.1, 3.3$ Hz, 3H), 2.55–2.43 (*m*, 1H), 1.73 (*ddd*, $J=13.9, 8.1, 6.6$ Hz, 1H), 1.33 (*ddd*, $J=14.0, 8.3, 5.7$ Hz, 1H), 1.23–1.14 (*m*, 1H), and 0.75 (*t*, $J=6.8$ Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 170.1, 146.9, 140.0, 139.6, 138.9, 132.9, 132.5, 130.8, 130.7, 130.6, 130.6, 128.3, 127.6, 126.0, 119.8, 59.2, 45.5, 40.5, 37.7, 35.8, 34.8, 24.7, 22.7, and 22.0. FTIR (thin film) 2955, 2868, 1666, 1469, 1331, and 1167 cm^{-1} . $[\alpha]_D^{22.5} - 66.7$ (*c* 1.0, CHCl_3). HRMS (ESI, m/z): m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{29}\text{Cl}_2\text{N}_2\text{O}_3\text{S}^+$ 495.1270, found 495.1262.

(R)-N-(3,4-Dichlorobenzyl)-4-methyl-2-(5-methylene-1,1-dioxido-4,5-dihydrobenzo[f][1,2]thiazepin-2(3H)-yl)pentanamide (10a)

Carboxylic acid **7** and 3,4-dichlorobenzylamine were reacted according to General Procedure A to afford the amide

product **10a** as a colorless oil (54.2 mg, 0.113 mmol, 91% yield). $R_f=0.4$ (25% EtOAc in hexanes). ^1H NMR (400 MHz, CDCl_3) δ 7.80 (*dd*, $J=8.1, 1.3$ Hz, 1H), 7.48–7.38 (*m*, 1H), 7.32–7.26 (*m*, 4H), 6.93 (*dd*, $J=8.2, 2.1$ Hz, 1H), 6.32 (*d*, $J=6.4$ Hz, 1H), 5.26 (*q*, $J=1.0$ Hz, 1H), 5.20 (*d*, $J=1.2$ Hz, 1H), 4.43 (*dd*, $J=8.4, 6.5$ Hz, 1H), 4.18 (*dd*, $J=6.0, 1.5$ Hz, 2H), 3.78–3.66 (*m*, 1H), 3.48 (*ddd*, $J=14.1, 6.2, 4.2$ Hz, 1H), 2.63–2.54 (*m*, 1H), 2.51–2.38 (*m*, 1H), 1.69 (*ddd*, $J=14.1, 8.2, 6.5$ Hz, 1H), 1.36 (*ddd*, $J=14.0, 8.4, 5.6$ Hz, 1H), 1.28–1.13 (*m*, 1H), 0.72 (*d*, $J=2.4$ Hz, 3H), and 0.71 (*d*, $J=2.5$ Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 170.2, 146.8, 140.0, 139.5, 138.2, 132.9, 132.7, 131.6, 130.7, 130.6, 129.7, 127.6, 127.0, 125.9, 119.9, 59.1, 45.5, 42.6, 37.6, 35.7, 24.7, 22.7, and 21.9. FTIR (thin film) 2956, 2359, 2341, 1668, 1333, and 1169 cm^{-1} . $[\alpha]_D^{21.9} + 58.8$ (*c* 1.0, CHCl_3). HRMS (ESI, m/z): m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{27}\text{Cl}_2\text{N}_2\text{O}_3\text{S}^+$ 481.1114, found 481.1114.

(R)-N-Benzyl-4-methyl-2-(5-methylene-1,1-dioxido-4,5-dihydrobenzo[f][1,2]thiazepin-2(3H)-yl)pentanamide (10b)

Carboxylic acid **7** and benzylamine were reacted according to General Procedure A to afford the amide product **10b** as a colorless oil (42.1 mg, 0.102 mmol, 83% yield). $R_f=0.4$ (25% EtOAc in hexanes). ^1H NMR (400 MHz, CDCl_3) δ 7.82 (*dd*, $J=8.1, 1.4$ Hz, 1H), 7.46–7.38 (*m*, 1H), 7.32–7.26 (*m*, 2H), 7.26–7.16 (*m*, 3H), 7.09–7.03 (*m*, 2H), 6.14 (*s*, 1H), 5.24 (*d*, $J=1.2$ Hz, 1H), 5.18 (*d*, $J=1.2$ Hz, 1H), 4.43 (*dd*, $J=8.5, 6.4$ Hz, 1H), 4.28 (*dd*, $J=14.8, 5.9$ Hz, 1H), 4.16 (*dd*, $J=14.8, 5.7$ Hz, 1H), 3.71 (*ddd*, $J=13.4, 9.2, 3.8$ Hz, 1H), 3.51 (*ddd*, $J=14.1, 6.4, 4.2$ Hz, 1H), 2.58 (*ddd*, $J=13.9, 6.4, 3.7$ Hz, 1H), 2.52–2.39 (*m*, 1H), 1.71 (*ddd*, $J=14.4, 8.3, 6.4$ Hz, 1H), 1.37 (*ddd*, $J=14.0, 8.5, 5.6$ Hz, 1H), 1.31–1.16 (*m*, 1H), 0.73 (*d*, $J=4.4$ Hz, 3H), and 0.72 (*d*, $J=4.6$ Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 169.9, 146.9, 140.0, 139.7, 137.7, 132.8, 130.6, 128.8, 127.7, 127.6, 127.6, 126.0, 119.8, 59.2, 45.5, 43.8, 37.8, 35.7, 24.7, 22.8, and 22.0. FTIR (thin film) 2955, 2359, 2341, 1662, 1335, and 1169 cm^{-1} . $[\alpha]_D^{21.6} + 67.5$ (*c* 1.0, CHCl_3). HRMS (ESI, m/z): m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{29}\text{N}_2\text{O}_3\text{S}^+$ 413.1893, found 413.1870.

(R)-N-(4-Chlorobenzyl)-4-methyl-2-(5-methylene-1,1-dioxido-4,5-dihydrobenzo[f][1,2]thiazepin-2(3H)-yl)pentanamide (10c)

Carboxylic acid **7** and 4-chlorobenzylamine were reacted according to General Procedure A to afford the amide product **10c** as a colorless oil (48.4 mg, 0.124 mmol, 88%). $R_f=0.4$ (25% EtOAc in hexanes). ^1H NMR (400 MHz, CDCl_3) δ 7.81 (*dd*, $J=8.1, 1.4$ Hz, 1H), 7.43 (*td*, $J=7.5,$

1.3 Hz, 1H), 7.30 (*ddd*, $J = 7.9, 6.2, 1.5$ Hz, 2H), 7.19 (*s*, 1H), 7.17 (*d*, $J = 2.0$ Hz, 1H), 7.01 (*d*, $J = 8.4$ Hz, 2H), 6.25 (*d*, $J = 6.9$ Hz, 1H), 5.25 (*d*, $J = 1.2$ Hz, 1H), 5.19 (*d*, $J = 1.2$ Hz, 1H), 4.43 (*dd*, $J = 8.5, 6.4$ Hz, 1H), 4.26–4.13 (*m*, 2H), 3.71 (*ddd*, $J = 13.5, 9.4, 3.7$ Hz, 1H), 3.48 (*ddd*, $J = 14.0, 6.2, 4.2$ Hz, 1H), 2.58 (*ddd*, $J = 13.9, 6.2, 3.7$ Hz, 1H), 2.51–2.38 (*m*, 1H), 1.70 (*ddd*, $J = 14.4, 8.3, 6.4$ Hz, 1H), 1.36 (*ddd*, $J = 14.1, 8.5, 5.5$ Hz, 1H), 1.21 (*dt*, $J = 14.0, 6.1$ Hz, 2H), 0.72 (*d*, $J = 3.3$ Hz, 3H), and 0.71 (*d*, $J = 3.4$ Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 170.1, 146.8, 140.0, 139.6, 136.4, 133.4, 132.8, 130.6, 129.0, 128.9, 127.6, 126.0, 119.9, 59.2, 45.5, 43.0, 37.7, 35.7, 24.7, 22.8, and 21.9. FTIR (thin film) 2956, 1663, 1333, and 1168 cm^{-1} . $[\alpha]_D^{20.9} + 69.2$ (*c* 1.0, CHCl_3). HRMS (ESI, m/z): m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{28}\text{ClN}_2\text{O}_3\text{S}^+$ 447.1504, found 447.1476.

(R)-N-(4-Chloro-3-(trifluoromethyl)benzyl)-4-methyl-2-(5-methylene-1,1-dioxido-4,5-dihydrobenzo[*f*][1,2]thiazepin-2(3H)-yl)pentanamide (10d)

Carboxylic acid **7** and 4-chloro-3-(trifluoromethyl)benzylamine were reacted according to General Procedure A to afford the amide product **10d** as a colorless oil (54.9 mg, 0.124 mmol, 86%). $R_f = 0.4$ (25% EtOAc in hexanes). ^1H NMR (400 MHz, CDCl_3) δ 7.80 (*dd*, $J = 8.1, 1.3$ Hz, 1H), 7.46–7.40 (*m*, 2H), 7.35 (*d*, $J = 8.2$ Hz, 1H), 7.32–7.26 (*m*, 2H), 7.22 (*dd*, $J = 8.2, 2.2$ Hz, 1H), 6.41 (*s*, 1H), 5.26 (*d*, $J = 1.1$ Hz, 1H), 5.20 (*d*, $J = 1.2$ Hz, 1H), 4.44 (*dd*, $J = 8.3, 6.6$ Hz, 1H), 4.32–4.17 (*m*, 2H), 3.72 (*ddd*, $J = 13.5, 9.5, 3.6$ Hz, 1H), 3.48 (*ddd*, $J = 14.1, 6.1, 4.2$ Hz, 1H), 2.59 (*ddd*, $J = 13.9, 6.1, 3.6$ Hz, 1H), 2.52–2.39 (*m*, 1H), 1.70 (*ddd*, $J = 14.4, 8.1, 6.6$ Hz, 1H), 1.34 (*ddd*, $J = 14.0, 8.3, 5.7$ Hz, 1H), 1.27–1.11 (*m*, 1H), 0.72 (*d*, $J = 2.2$ Hz, 3H), and 0.70 (*d*, $J = 2.3$ Hz, 3H). ^{13}C NMR (176 MHz, CDCl_3) δ 170.4, 146.8, 140.0, 139.4, 137.3, 132.9, 132.0, 131.8, 131.3, 130.6, 128.6 (*q*, $J = 32.08$ Hz), 127.6, 126.8 (*q*, $J = 5.25$ Hz), 125.9, 122.5 (*q*, $J = 273.35$ Hz), 119.9, 59.1, 45.5, 42.6, 37.6, 35.8, 24.7, 22.6, and 22.0. FTIR (thin film) 2957, 1667, 1318, 1169, and 1130 cm^{-1} . $[\alpha]_D^{20.9} + 52.4$ (*c* 1.0, CHCl_3). HRMS (ESI, m/z): m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{27}\text{ClF}_3\text{N}_2\text{O}_3\text{S}^+$ 515.1378, found 515.1413.

(R)-4-Methyl-N-(4-methylbenzyl)-2-(5-methylene-1,1-dioxido-4,5-dihydrobenzo[*f*][1,2]thiazepin-2(3H)-yl)pentanamide (10e)

Carboxylic acid **7** and 4-methylbenzylamine were reacted according to General Procedure A to afford the amide product **10e** as a colorless oil (50.8 mg, 0.119 mmol, 96% yield). $R_f = 0.4$ (25% EtOAc in hexanes). ^1H NMR (400 MHz, CDCl_3) δ 7.81 (*dd*, $J = 8.1, 1.6$ Hz, 1H), 7.42

(*ddt*, $J = 7.8, 7.2, 0.9$ Hz, 1H), 7.32–7.26 (*m*, 2H), 7.05–7.00 (*m*, 2H), 6.95 (*d*, $J = 7.9$ Hz, 2H), 6.08 (*s*, 1H), 5.24 (*d*, $J = 1.2$ Hz, 1H), 5.18 (*d*, $J = 1.3$ Hz, 1H), 4.41 (*dd*, $J = 8.5, 6.4$ Hz, 1H), 4.23 (*dd*, $J = 14.7, 5.9$ Hz, 1H), 4.10 (*dd*, $J = 14.6, 5.5$ Hz, 1H), 3.71 (*ddd*, $J = 13.4, 9.2, 3.8$ Hz, 1H), 3.51 (*ddd*, $J = 14.1, 6.4, 4.2$ Hz, 1H), 2.59 (*ddd*, $J = 13.9, 6.4, 3.8$ Hz, 1H), 2.45 (*ddd*, $J = 13.8, 9.3, 4.2$ Hz, 1H), 2.26 (*s*, 3H), 1.70 (*ddd*, $J = 14.3, 8.3, 6.4$ Hz, 1H), 1.37 (*ddd*, $J = 14.0, 8.5, 5.6$ Hz, 1H), 1.30–1.15 (*m*, 1H), 0.73 (*d*, $J = 4.3$ Hz, 3H), and 0.71 (*d*, $J = 4.5$ Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 169.8, 147.0, 140.0, 139.7, 137.3, 134.7, 132.8, 130.5, 129.5, 127.7, 127.6, 126.0, 119.8, 59.2, 45.5, 43.5, 37.8, 35.7, 24.7, 22.8, 22.0, and 21.2. FTIR (thin film) 2923, 2359, 23412, 1668, 1334, and 1168 cm^{-1} . $[\alpha]_D^{21.1} + 68.7$ (*c* 1.0, CHCl_3). HRMS (ESI, m/z): m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{31}\text{N}_2\text{O}_3\text{S}^+$ 427.2050, found 427.2048.

(R)-N-(4-Methoxybenzyl)-4-methyl-2-(5-methylene-1,1-dioxido-4,5-dihydrobenzo[*f*][1,2]thiazepin-2(3H)-yl)pentanamide (10f)

Carboxylic acid **7** and 4-methoxybenzylamine were reacted according to General Procedure A to afford the amide product **10f** as a colorless oil (52.3 mg, 0.124 mmol, 96%). $R_f = 0.3$ (25% EtOAc in hexanes). $[\alpha]_D^{21.1} + 64.0$ (*c* 1.0, CHCl_3). ^1H NMR (400 MHz, CDCl_3) δ 7.81 (*dd*, $J = 8.1, 1.4$ Hz, 1H), 7.45–7.39 (*m*, 1H), 7.33–7.26 (*m*, 2H), 7.01–6.97 (*m*, 2H), 6.77–6.73 (*m*, 2H), 6.07 (*s*, 1H), 5.24 (*d*, $J = 1.1$ Hz, 1H), 5.18 (*d*, $J = 1.3$ Hz, 1H), 4.40 (*dd*, $J = 8.5, 6.4$ Hz, 1H), 4.20 (*dd*, $J = 14.6, 5.8$ Hz, 1H), 4.09 (*dd*, $J = 14.6, 5.6$ Hz, 1H), 3.74–3.66 (*complex*, 4H), 3.51 (*ddd*, $J = 14.1, 6.4, 4.2$ Hz, 1H), 2.58 (*ddd*, $J = 13.5, 6.3, 3.7$ Hz, 1H), 2.50–2.39 (*m*, 1H), 1.69 (*ddd*, $J = 14.3, 8.3, 6.4$ Hz, 1H), 1.37 (*ddd*, $J = 14.0, 8.5, 5.6$ Hz, 1H), 1.22 (*td*, $J = 12.5, 11.5, 5.8$ Hz, 1H), 0.73 (*d*, $J = 4.2$ Hz, 3H), and 0.71 (*d*, $J = 4.4$ Hz, 4H). ^{13}C NMR (101 MHz, CDCl_3) δ 169.8, 159.1, 146.9, 140.0, 139.7, 132.8, 130.5, 129.8, 129.0, 127.6, 126.0, 119.8, 114.2, 59.2, 55.4, 45.4, 43.2, 37.8, 35.7, 24.7, 22.8, and 22.0. FTIR (thin film) 2966, 1662, 1512, and 1333 cm^{-1} . $[\alpha]_D^{21.1} + 64.0$ (*c* 1.0, CHCl_3). HRMS (ESI, m/z): m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{31}\text{N}_2\text{O}_4\text{S}^+$ 443.1999, found 443.1973.

(R)-N-(Cyclohexylmethyl)-4-methyl-2-(5-methylene-1,1-dioxido-4,5-dihydrobenzo[*f*][1,2]thiazepin-2(3H)-yl)pentanamide (10g)

Carboxylic acid **7** and 4-cyclohexylmethylamine were reacted according to General Procedure A to afford the amide product **10g** as a colorless oil (43 mg, 0.103 mmol, 83% yield). $R_f = 0.4$ (25% EtOAc in hexanes). ^1H NMR (400 MHz, CDCl_3) δ 7.86–7.82 (*m*, 1H), 7.47–7.41 (*m*, 1H),

7.35–7.29 (complex, 2H), 5.85 (*m*, 1H), 5.27 (*d*, *J* = 1.1 Hz, 1H), 5.22 (*d*, *J* = 1.3 Hz, 1H), 4.37 (*dd*, *J* = 8.5, 6.4 Hz, 1H), 3.78–3.68 (*m*, 1H), 3.55–3.45 (*m*, 1H), 2.99–2.78 (*m*, 2H), 2.67–2.54 (*m*, 1H), 2.53–2.38 (*m*, 1H), 1.76–1.45 (complex, 7H), 1.40–0.98 (complex, 5H), and 0.84–0.66 (complex, 8H). ¹³C NMR (101 MHz, CDCl₃) δ 170.0, 147.0, 140.1, 139.7, 132.8, 130.6, 127.6, 126.0, 119.8, 59.2, 45.9, 45.5, 37.8, 35.8, 30.8, 26.4, 25.9, 25.9, 24.7, 22.8, and 22.0. FTIR (thin film) 2922, 2851, 2359, 2341, 1661, and 1334 cm⁻¹. [α]_D^{22.6} + 67.5 (*c* 1.0, CHCl₃). HRMS (ESI, *m/z*): *m/z*: [M + H]⁺ calcd for C₂₃H₃₅N₂O₃S⁺ 419.2363, found 419.2344.

(*R*)-*N*-(3,4-Dichlorophenethyl)-4-methyl-2-(5-methylene-1,1-dioxido-4,5-dihydrobenzo[f][1,2]thiazepin-2(3H)-yl)pentanamide (10h)

Carboxylic acid **7** and 3,4-dichlorophenethylamine were reacted according to General Procedure A to afford the amide product **10h** as a colorless oil (58.2 mg, 0.117 mmol, 95%). *R*_f = 0.4 (25% EtOAc in hexanes). ¹H NMR (400 MHz, CDCl₃) δ 7.84–7.80 (*m*, 1H), 7.44 (*td*, *J* = 7.6, 1.4 Hz, 1H), 7.35–7.26 (*m*, 3H), 7.17 (*d*, *J* = 2.1 Hz, 1H), 6.93 (*dd*, *J* = 8.2, 2.1 Hz, 1H), 5.97 (*s*, 1H), 5.25 (*t*, *J* = 1.0 Hz, 1H), 5.21 (*d*, *J* = 1.2 Hz, 1H), 4.33 (*dd*, *J* = 8.3, 6.6 Hz, 1H), 3.64 (*ddd*, *J* = 13.5, 9.5, 3.6 Hz, 1H), 3.45–3.21 (*m*, 3H), 2.59 (*ddt*, *J* = 13.3, 6.0, 3.3 Hz, 3H), 2.41 (*dddd*, *J* = 13.8, 9.6, 4.2, 1.0 Hz, 1H), 1.66 (*ddd*, *J* = 14.0, 8.1, 6.6 Hz, 1H), 1.27 (*ddd*, *J* = 14.0, 8.3, 5.7 Hz, 1H), 1.20–1.07 (*m*, 1H), and 0.68 (*t*, *J* = 6.8 Hz, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 170.0, 146.7, 139.9, 139.5, 138.7, 132.7, 132.4, 130.6, 130.5, 130.5, 130.4, 128.2, 127.4, 125.8, 119.6, 59.1, 45.3, 40.3, 37.5, 35.6, 34.6, 24.5, 22.5, and 21.8. FTIR (thin film) 2956, 1667, 1332, and 1168 cm⁻¹. [α]_D^{20.8} + 63.9 (*c* 1.0, CHCl₃). HRMS (ESI, *m/z*): *m/z*: [M + H]⁺ calcd for C₂₄H₂₉Cl₂N₂O₃S⁺ 495.1270, found 495.1250.

(*S*)-*N*-(3,4-Dichlorobenzyl)-2-(1,1-dioxido-5-oxo-4,5-dihydrobenzo[f][1,2]thiazepin-2(3H)-yl)-4-methylpentanamide (11a)

Carboxylic acid **8** and 3,4-dichlorobenzylamine were reacted according to General Procedure A to afford the amide product **11a** as a colorless oil (38.5 mg, 0.080 mmol, 65% yield). *R*_f = 0.3 (25% EtOAc in hexanes). ¹H NMR (400 MHz, CDCl₃) δ 7.87 (*dd*, *J* = 7.7, 1.2 Hz, 1H), 7.76 (*dd*, *J* = 7.6, 1.4 Hz, 1H), 7.61 (*dt*, *J* = 22.0, 7.5, 1.4 Hz, 2H), 7.34 (*d*, *J* = 8.2 Hz, 1H), 7.24 (*d*, *J* = 2.1 Hz, 1H), 6.99 (*dd*, *J* = 8.2, 2.1 Hz, 1H), 6.27 (*t*, *J* = 6.0 Hz, 1H), 4.52 (*dd*, *J* = 8.5, 6.6 Hz, 1H), 4.29–4.16 (*m*, 2H), 3.67–3.55 (*m*, 1H), 3.57–3.46 (*m*, 1H), 3.39–3.27 (*m*, 1H), 3.28–3.16 (*m*, 1H), 1.77–1.65 (*m*, 1H), 1.58–1.46 (*m*, 1H), 1.44–1.32 (*m*, 1H),

0.86 (*d*, *J* = 5.1 Hz, 3H), and 0.84 (*d*, *J* = 5.0 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 199.4, 169.3, 139.2, 137.8, 136.1, 132.9, 132.6, 131.8, 131.6, 130.6, 129.6, 129.6, 126.9, 125.5, 58.9, 42.4, 42.4, 39.7, 37.2, 24.6, 22.6, and 21.7. FTIR (thin film) 3365, 2957, 1663, 1638, and 1338 cm⁻¹. [α]_D^{21.8} – 41.4 (*c* 1.0, CHCl₃). HRMS (ESI, *m/z*): *m/z*: [M + H]⁺ calcd for C₂₂H₂₅Cl₂N₂O₄S⁺ 483.0907; found 483.0907.

(*S*)-*N*-Benzyl-2-(1,1-dioxido-5-oxo-4,5-dihydrobenzo[f][1,2]thiazepin-2(3H)-yl)-4-methylpentanamide (11b)

Carboxylic acid **8** and benzylamine were reacted according to General Procedure A to afford the amide product **11b** as a colorless oil (35.0 mg, 0.123 mmol, 68.7%). *R*_f = 0.3 (25% EtOAc in hexanes). ¹H NMR (400 MHz, CDCl₃) δ 7.82 (*dd*, *J* = 7.5, 1.6 Hz, 1H), 7.70 (*dd*, *J* = 7.5, 1.5 Hz, 1H), 7.54 (*ddd*, *J* = 19.5, 7.5, 1.5 Hz, 2H), 7.26–7.17 (complex, 4H), 7.08–7.05 (*m*, 2H), 6.07–5.99 (*m*, 2H), 4.45 (*dd*, *J* = 8.6, 6.6 Hz, 1H), 4.26 (*dd*, *J* = 14.7, 5.9 Hz, 1H), 4.14 (*dd*, *J* = 14.7, 5.6 Hz, 1H), 3.60–4.52 (*m*, 1H), 3.51–3.42 (*m*, 1H), 3.29–3.20 (*m*, 1H), 3.18–3.08 (*m*, 1H), 1.69–1.59 (*m*, 1H), 1.52–1.42 (*m*, 1H), 1.41–1.27 (*m*, 1H), 0.80 (*d*, *J* = 2.5 Hz, 3H), and 0.78 (*d*, *J* = 2.4 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 199.8, 169.2, 139.3, 137.5, 136.4, 133.0, 131.9, 129.7, 128.9, 127.8, 125.8, 59.1, 43.7, 42.6, 39.8, 37.6, 24.8, 22.8, and 21.8. FTIR (thin film): 3320, 2956, 2926, 1359, and 1682 cm⁻¹. [α]_D^{21.4} – 43.6 (*c* 1.0, CHCl₃). HRMS (ESI, *m/z*): *m/z*: [M + H]⁺ calcd for C₂₂H₂₇N₂O₄S⁺ 415.1686, found 415.1686.

(*S*)-*N*-(4-Chlorobenzyl)-2-(1,1-dioxido-5-oxo-4,5-dihydrobenzo[f][1,2]thiazepin-2(3H)-yl)-4-methylpentanamide (11c)

Carboxylic acid **8** and 4-chlorobenzylamine were reacted according to General Procedure A to afford the amide product **11c** as a colorless oil (33.0 mg, 0.068 mmol, 56% yield). *R*_f = 0.3 (25% EtOAc in hexanes). ¹H NMR (400 MHz, CDCl₃) δ 7.88 (*dd*, *J* = 7.8, 1.3 Hz, 1H), 7.77 (*dd*, *J* = 7.6, 1.4 Hz, 1H), 7.61 (*ddd*, *J* = 22.5, 7.5, 1.4 Hz, 2H), 7.28–7.21 (*m*, 2H), 7.11–7.03 (*m*, 2H), 6.18 (*t*, *J* = 5.8 Hz, 1H), 4.51 (*dd*, *J* = 8.5, 6.6 Hz, 1H), 4.32–4.16 (*m*, 2H), 3.67–3.56 (*m*, 1H), 3.57–3.46 (*m*, 1H), 3.37–3.28 (*m*, 1H), 3.27–3.15 (*m*, 1H), 1.77–1.65 (*m*, 1H), 1.58–1.46 (*m*, 1H), 1.46–1.31 (*m*, 1H), 0.85 (*d*, *J* = 4.0 Hz, 3H), and 0.84 (*d*, *J* = 3.9 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 199.6, 169.4, 139.5, 136.3, 136.2, 133.6, 133.0, 131.9, 129.8, 129.1, 129.0, 125.7, 59.1, 43.0, 42.6, 39.8, 37.5, 24.8, 22.8, and 21.8. FTIR (thin film) 3366, 2957, 1663, 1533, and 1336 cm⁻¹. [α]_D^{21.8} – 45.9 (*c* 1.0, CHCl₃). HRMS (ESI, *m/z*): *m/z*: [M + H]⁺ calcd for C₂₄H₂₅ClN₂O₄SH 449.1224; found 449.1296.

(S)-N-(4-Chloro-3-(trifluoromethyl)benzyl)-2-(1,1-dioxido-5-oxo-4,5-dihydrobenzof[1,2]thiazepin-2(3H)-yl)-4-methylpentanamide (11d)

Carboxylic acid **8** and 4-chloro-3-(trifluoromethyl)benzylamine were reacted according to General Procedure A to afford the amide product **11d** as a colorless oil (44.0 mg, 0.085 mmol, 69% yield). $R_f = 0.3$ (25% EtOAc in hexanes). $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.92–7.87 (*m*, 1H), 7.80–7.75 (*m*, 1H), 7.62 (*dtd*, $J = 19.3, 7.5, 1.4$ Hz, 2H), 7.49 (*d*, $J = 2.1$ Hz, 1H), 7.42 (*d*, $J = 8.2$ Hz, 1H), 7.29 (*dd*, $J = 8.4, 2.2$ Hz, 1H), 6.32 (*t*, $J = 6.0$ Hz, 1H), 4.53 (*dd*, $J = 8.4, 6.8$ Hz, 1H), 4.40–4.23 (*m*, 2H), 3.66–3.55 (*m*, 1H), 3.57–3.45 (*m*, 1H), 3.41–3.29 (*m*, 1H), 3.28–3.16 (*m*, 1H), 1.79–1.67 (*m*, 1H), 1.56–1.44 (*m*, 1H), 1.43–1.31 (*m*, 1H), and 0.88–0.80 (*m*, 6H). $^{13}\text{C NMR}$ (176 MHz, CDCl_3) δ 199.6, 169.6, 139.4, 137.1, 136.3, 133.0, 132.1, 132.0, 131.9, 131.5, 129.7, 128.7 (q , $J = 31.43$ Hz), 126.8 (q , $J = 5.19$ Hz), 125.5, 122.7 (q , $J = 273.33$ Hz), 59.0, 42.6, 42.5, 39.9, 37.4, 24.8, 22.7, and 21.8. FTIR (thin film) 3398, 2959, 1664, 1532, and 1319 cm^{-1} . $[\alpha]_D^{21.9} - 46.2$ (*c* 1.0, CHCl_3). HRMS (ESI, m/z): m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{25}\text{ClF}_3\text{N}_2\text{O}_4\text{S}^+$ 517.1170; found 517.1171.

(S)-2-(1,1-Dioxido-5-oxo-4,5-dihydrobenzof[1,2]thiazepin-2(3H)-yl)-4-methyl-N-(4-methylbenzyl)pentanamide (11e)

Carboxylic acid **8** and 4-methylbenzylamine were reacted according to General Procedure A to afford the amide product **11e** as a colorless oil (37.9 mg, 0.088 mmol, 72% yield). $R_f = 0.3$ (25% EtOAc in hexanes). $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.88 (*dd*, $J = 7.6, 1.4$ Hz, 1H), 7.77 (*dd*, $J = 7.4, 1.6$ Hz, 1H), 7.61 (*dtd*, $J = 19.7, 7.5, 1.4$ Hz, 2H), 7.10 (*d*, $J = 8.3$ Hz, 2H), 7.02 (*d*, $J = 8.0$ Hz, 2H), 6.04 (*s*, 1H), 4.49 (*dd*, $J = 8.6, 6.6$ Hz, 1H), 4.29 (*dd*, $J = 14.6, 5.8$ Hz, 1H), 4.15 (*dd*, $J = 14.6, 5.5$ Hz, 1H), 3.69–3.57 (*m*, 1H), 3.59–3.48 (*m*, 1H), 3.37–3.26 (*m*, 1H), 3.26–3.14 (*m*, 1H), 2.33 (*s*, 3H), 1.77–1.65 (*m*, 1H), 1.59–1.47 (*m*, 1H), 1.47–1.35 (*m*, 1H), 0.86 (*d*, $J = 2.3$ Hz, 3H), and 0.84 (*d*, $J = 2.2$ Hz, 3H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 199.7, 169.0, 139.2, 137.3, 136.3, 134.3, 132.8, 131.7, 129.6, 129.4, 127.6, 125.6, 59.0, 43.4, 42.5, 39.7, 37.4, 24.6, 22.7, 21.7, and 21.0. FTIR (thin film) 3314, 2956, 2924, 2869, 1662, and 1339. $[\alpha]_D^{21.7} - 46.8$ (*c* 1.0, CHCl_3). HRMS (ESI, m/z): m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{29}\text{N}_2\text{O}_4\text{S}^+$ 429.1843; found 429.1843.

(S)-2-(1,1-Dioxido-5-oxo-4,5-dihydrobenzof[1,2]thiazepin-2(3H)-yl)-N-(4-methoxybenzyl)-4-methylpentanamide (11f)

Carboxylic acid **8** and 4-methoxybenzylamine were reacted according to General Procedure A to afford the amide product **11f** as a colorless oil (40.7 mg, 0.092 mmol, 75%

yield). $R_f = 0.2$ (25% EtOAc in hexanes). $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.88 (*dd*, $J = 7.6, 1.4$ Hz, 1H), 7.77 (*dd*, $J = 7.6, 1.5$ Hz, 1H), 7.61 (*dtd*, $J = 19.1, 7.5, 1.5$ Hz, 2H), 7.10–7.02 (*m*, 2H), 6.85–6.78 (*m*, 2H), 6.03 (*t*, $J = 5.7$ Hz, 1H), 4.49 (*dd*, $J = 8.6, 6.6$ Hz, 1H), 4.25 (*dd*, $J = 14.5, 5.8$ Hz, 1H), 4.13 (*dd*, $J = 14.5, 5.5$ Hz, 1H), 3.79 (*s*, 3H), 3.68–3.57 (*m*, 1H), 3.59–3.47 (*m*, 1H), 3.37–3.25 (*m*, 1H), 3.26–3.14 (*m*, 1H), 1.77–1.64 (*m*, 1H), 1.59–1.47 (*m*, 1H), 1.46–1.34 (*m*, 1H), 0.86 (*d*, $J = 2.4$ Hz, 3H), and 0.84 (*d*, $J = 2.3$ Hz, 3H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 199.8, 169.1, 159.2, 139.3, 136.4, 132.9, 131.9, 129.7, 129.6, 129.2, 125.8, 114.2, 59.1, 55.4, 43.2, 42.6, 39.8, 37.6, 24.8, 22.8, and 21.8. FTIR (thin film) 3318, 2956, 1660, and 1512 cm^{-1} . $[\alpha]_D^{21.8} - 40.1$ (*c* 1.0, CHCl_3). HRMS (ESI, m/z): m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{29}\text{N}_2\text{O}_5\text{S}^+$ 445.1792; found 445.1792.

(S)-N-(Cyclohexylmethyl)-2-(1,1-dioxido-5-oxo-4,5-dihydrobenzof[1,2]thiazepin-2(3H)-yl)-4-methylpentanamide (11g)

Carboxylic acid **8** and 4-cyclohexylmethylamine were reacted according to General Procedure A to afford the amide product **11g** as a colorless oil (28.0 mg, 0.123 mmol, 54% yield). $R_f = 0.3$ (25% EtOAc in hexanes). $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.90–7.86 (*m*, 1H), 7.74–7.70 (*m*, 1H), 7.62–7.53 (*m*, 2H), 5.76 (*s*, 1H), 4.40 (*dd*, $J = 8.5, 6.6$ Hz, 1H), 3.58 (*ddd*, $J = 12.9, 7.9, 4.8$ Hz, 1H), 3.47 (*ddd*, $J = 13.1, 8.1, 4.8$ Hz, 1H), 3.28 (*ddd*, $J = 14.8, 8.1, 4.8$ Hz, 1H), 3.14 (*ddd*, $J = 14.8, 7.9, 4.8$ Hz, 1H), 2.96–2.78 (*m*, 2H), 1.69–1.39 (complex, 8H), 1.38–0.97 (complex, 6H), 0.79 (*d*, $J = 1.4$ Hz, 3H), and 0.78 (*d*, $J = 1.3$ Hz, 3H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 199.8, 169.3, 139.5, 136.5, 132.9, 131.9, 129.8, 125.7, 59.2, 45.8, 42.7, 39.8, 37.7, 37.6, 30.8, 26.4, 25.8, 24.8, 22.8, and 21.9. FTIR (thin film): 3382, 2923, 2851, 1660, and 1431. $[\alpha]_D^{21.7} - 42.2$ (*c* 0.50, CHCl_3). HRMS (ESI, m/z): m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{22}\text{H}_{33}\text{N}_2\text{O}_4\text{S}^+$ 421.2156; found 421.2154.

(S)-N-(3,4-Dichlorophenethyl)-2-(1,1-dioxido-5-oxo-4,5-dihydrobenzof[1,2]thiazepin-2(3H)-yl)-4-methylpentanamide (11h)

Carboxylic acid **8** and 3,4-dichlorophenethylamine were reacted according to General Procedure A to afford the amide product **11h** as a colorless oil (53.3 mg, 0.107 mmol, 87% yield). $R_f = 0.2$ (25% EtOAc in hexanes). $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.94–7.89 (*m*, 1H), 7.81–7.77 (*m*, 1H), 7.68–7.60 (*m*, 2H), 7.35 (*d*, $J = 8.2$ Hz, 1H), 7.22 (*d*, $J = 2.1$ Hz, 1H), 6.98 (*dd*, $J = 8.2, 2.1$ Hz, 1H), 5.93–5.80 (*m*, 1H), 4.41 (*dd*, $J = 8.3, 6.8$ Hz, 1H), 3.59–3.26 (complex, 5H), 3.18 (*ddd*, $J = 14.6, 7.8, 4.8$ Hz, 1H), 2.76–2.59 (*m*, 2H), 1.68 (*ddd*, $J = 14.0, 8.1, 6.8$ Hz,

1H), 1.42 (*ddd*, $J = 14.1, 8.3, 5.8$ Hz, 1H), 1.34–1.22 (*m*, 1H), and 0.81 (*d*, $J = 6.6$ Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 199.6, 169.4, 139.6, 138.7, 136.3, 133.0, 132.6, 132.0, 130.8, 130.8, 130.7, 129.8, 128.2, 125.8, 59.2, 42.6, 40.4, 39.8, 37.4, 34.7, 24.8, 22.7, and 21.9. FTIR (thin film) 3381, 2957, 1682, 1532, and 1336 cm^{-1} . $[\alpha]_D^{21.9} - 35.7$ (c 1.0, CHCl_3). HRMS (ESI, m/z): m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{27}\text{Cl}_2\text{N}_2\text{O}_4\text{S}^+$ 497.0990; found 497.1063.

Biology

Compounds

All synthesized compounds were solvated in 100% DMSO. For the AlphaScreen and cytotoxicity assays, compounds (final concentrations, 300, 100, 33.3, 11.1, 3.7, 1.2, 0.4, 0.1, 0.046, 0.015, 0.005, 0.002, and 0 μM) were transferred acoustically using ECHO 555 (Labcyte Inc.) to 384-well white Alpha assay plates (PerkinElmer) or to white polystyrene plates for cytotoxicity screens (Griener). All assays were performed in triplicate.

CaM/mHtt Alpha-Screen binding assay

The compound activity was tested in triplicate in the His-mHtt and GST-CaM AlphaScreen assay that was optimized in the KU-HTS lab. All assays were performed in 384-well formats in a final volume of 30 μL /well and 0.6% DMSO. All proteins and beads were diluted in the interaction buffer (IB) containing 10 mM Tris-HCl, pH 8.0, 1 mM CaCl_2 , 150 mM NaCl, 0.1% BSA, and 20% glycerol. Briefly, 10 nM His-mu-mHtt was pre-incubated with library compounds (15 μM) for 30 min followed by the addition of GST-CAM (10 nM). After 60 min at room temperature, a mixture of the acceptor Histidine (Nickel Chelate, 10 $\mu\text{g}/\text{mL}$ and donor Glutathione beads (15 $\mu\text{g}/\text{mL}$) was added together. After 2 h at room temperature, Alpha counts were measured using Enspire Multilabel Plate Reader (PerkinElmer Inc.) using AlphaScreen default label. Percent inhibition of the AlphaScreen assay was normalized to DMSO positive and negative controls.

Counterscreen assay 1: His-GST protein interaction assay

The purified His-tagged GST protein (15 nM) was incubated with the compounds for 30 min at room temperature in IB containing 10 mM Tris-HCl, pH 8.0, 1 mM CaCl_2 , 150 mM NaCl, 0.1% BSA, and 20% glycerol. A mixture of the acceptor Histidine (Nickel Chelate, 5 $\mu\text{g}/\text{mL}$ and donor Glutathione beads (5 $\mu\text{g}/\text{mL}$) was added together. The plates were read using Enspire Plate reader.

Counterscreen assay 2: AlphaScreen TruHits™ assay

The AlphaScreen TruHits kit (PerkinElmer) was used to identify compound interference using the vendor protocol. The Streptavidin donor beads (10 $\mu\text{g}/\text{mL}$) and the biotin acceptor beads (5 $\mu\text{g}/\text{mL}$) were diluted in the IB containing 10 mM Tris-HCl, pH 8.0, 1 mM CaCl_2 , 150 mM NaCl, 0.1% BSA, and 20% glycerol. The TruHits bead mix (30 μL) was incubated with the compounds for 2 h at room temperature. The assay plates were read using the Enspire Plate reader (PerkinElmer) using AlphaScreen default label.

Cytotoxicity assay

The SH-SY5Y cells (ATCC® CRL-2266) were plated in 384-well microplates at 6000 cells/well in 1:1 mixture of ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 302003, and F12 Medium in 10% FBS. The PC12 73Q cell line was grown in Kaighn's modification of Ham's F12 media (ATCC#30-2004) supplemented with 15% horse serum and 2.5% FBS in the presence of G418 and Zeocin. The PC12 73Q cells were seeded at 8000 cells/well in poly-D-lysine coated plates (Corning). The PC12 73Q cell line can be induced to express full-length human huntingtin protein with 73 glutamine repeats under the control of a RheoSwitch (HD Community Biorepository, CHDI Foundation, and the Coriell Institute for Medical Research). In this assay, cells were not induced to express huntingtin protein. Media and vehicle control wells were included in each assay plate. After 24 h of incubation with compounds at 37 °C, cytotoxicity was measured on Enspire plate reader (PerkinElmer) using the luminescence-based CellTiter-Glo reagent (Promega Inc.). Percent cytotoxicity was normalized to DMSO controls.

CaM-dependent kinase assay

To determine if the compounds inhibit the functioning of CaM, the activity of two CaM-dependent enzymes, calcium/CaM-dependent kinase 2 γ (CaMK2 γ , Human, recombinant; C-terminal truncation), and death-associate protein kinase 1 (DAPK1, Human, recombinant; amino acids 1–363) were assessed. The assay was performed in white opaque 384-well plates (Corning, NY) using the ADP-Glo kinase activity assay kit (Promega, Madison, WI) and DAPK1 and CAMK2 γ kinase enzyme system kit (Promega, Madison, WI). The assay was carried out according to the manufacturer's protocol with slight modifications. The DAPK1 reaction was initiated with 25 ng DAPK1, 5 μM ATP, and 0.1 $\mu\text{g}/\mu\text{L}$ myelin basic protein (native Swine) substrate in the presence of test compounds **9c**, **9d**, **9h**, **10c**, and **10h** and incubated at room temperature for 60 min. The CaMK2 γ reaction was initiated with 12 ng

CaMK2 γ , 25 μM ATP, and 0.2 $\mu\text{g}/\mu\text{L}$. Autocamtide-2 substrate in the presence of test compounds **9c**, **9d**, **9h**, **10c**, and **10h** and incubated at room temperature for 60 min. Each test compound was tested at a single concentration of 100 μM (dissolved in DMSO). Kinase enzyme activity was measured by detecting newly synthesized ATP via a luminescent readout.

Results and discussion

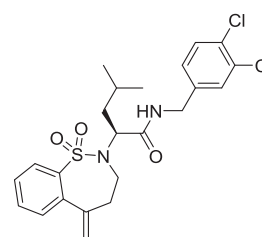
High-throughput screening

To identify small-molecule disruptors of the mHtt–CaM PPI, the University of Kansas High-Throughput Screening Laboratory interrogated their in-house compound collection for hit molecules that inhibited the binding of mHtt to CaM using an AlphaScreen platform (Ullman et al. 1994). The compound collection (ca. 225,000 compounds) comprised small molecules sourced from numerous vendors and collections, including the University of Kansas Chemical Methodology and Library Development (KU CMLD) Center. The KU CMLD center was a synthetic chemistry initiative whose *raison d'être* was to pioneer new synthetic methods for parallel synthesis and demonstrate their utility toward the construction of novel compound sets. The utility of such compound sets to identify tractable hits for various therapeutic targets has been demonstrated by us (Frankowski et al. 2011) and others (Basu et al. 2011; Brown et al. 2011; Huryn et al. 2011).

Among the most potent inhibitors of the CaM–mHtt interaction was a compound synthesized in the KU CMLD, KUC102204N (Fig. 1). KUC102204N is an amino acid-derived sultam core synthesized via a “Click, Click, Cyclize” reaction sequence developed in the laboratory of KU CMLD investigator Paul Hanson (Rayabarapu et al. 2009; Zhou et al. 2009). We used the reported route (Rayabarapu et al. 2009) to resynthesize the hit compound for activity and selectivity confirmation, as well as for the synthesis of new analogs to explore the structure–activity relationship (SAR) trends of the series.

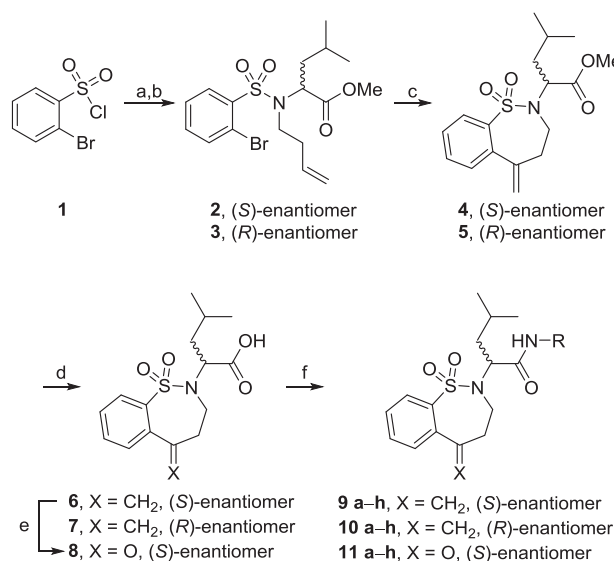
SAR studies

The hit compound, KUC102204N, was synthesized using the protocols developed by Hanson and coworkers with only minor modifications (Scheme 1) (see the Supporting Information for synthetic details of all intermediates). Bromobenzylsulfonyl chloride was coupled to the methyl ester of either (*R*)- or (*S*)-leucine to afford a sulfonamide, which was subsequently alkylated under Mitsunobu conditions to produce olefins **2** and **3**. Microwave irradiation in the presence of Pd(OAc)₂ effected an intramolecular Heck



KUC102204N, **9a**
CaM–mHtt IC₅₀ = 0.83 μM

Fig. 1 Structure and high-throughput screening activity of the sultam hit, KUC102204N, **9a**



Scheme 1 General synthesis of sultam analogs, reagents, and conditions: (a) L- or D-methyl leucinate, Et₃N, DCM, 2 h, 59–61% yield (b) PPh₃, DIAD, but-3-en-1-ol, DCM, 3 h, 80–95% yield (c) Pd(OAc)₂, PPh₃, Et₃N, MeCN, μW , 1 h, 74–84% yield (d) LiOH, 3:1:1 THF: MeOH:H₂O, 2 h, 70–85% yield (e) O₃, Me₂S, DCM, 2 min, 94% yield (f) HATU, DIPEA, NH₂R, MeCN, 18 h, 54–97% yield

cyclization to produce the bicyclic scaffold that was saponified with LiOH to provide carboxylic acids **6** and **7**. The olefin-containing acid **6** was converted to the ketone **8** via ozonolysis and HATU-promoted amide coupling afforded the target analogs.

Using the Topliss decision tree (Topliss 1972), a systematic approach was taken to investigate the effects of ring substitution on activity. We constructed a comprehensive matrix by coupling eight different amines with three carboxylic acid scaffolds (the natural and unnatural amino acid-derived exo-methylenes **6** and **7**, and ketone **8**). All analogs were screened for inhibition of CaM–mHtt binding and counterscreened for assay interference and cytotoxicity (Table 1).

The resynthesized sample of the screening hit, KUC102204N (**9a**) retained activity in disrupting the

Table 1 Structure–activity relationship studies for a sultam analog series

Compound	AlphaScreen CaM–mHtt, IC ₅₀ (μM)	Cytotoxicity (SH-SY5Y cells, 24 h) (μM)
9a (KUC102204N)	1.03	>300
10a	0.98	>300
9b	12.4	>300
10b	12.7	87 ± 2
9c	2.8	94 ± 3
10c	3	86 ± 9
9d	1.38	>300
10d	1.8	>300
9e	15	102 ± 7
10e	15.1	64 ± 2.5
9f	>300	>300
10f	>300	>300
9g	16.6	101 ± 5
10g	12.2	99 ± 4
9h	0.55	>300
10h	0.5	>300
11a	2.14	39.8
11b	14.59	43.8
11c	11.19	41.4
11d	2.03	44.2
11e	24.5	43.4
11f	52.56	44.6
11g	31.03	40.1
11h	0.97	32.3

mHtt–CaM PPI and was found to possess no measurable toxicity to SHY5Y cells. We also synthesized and tested the enantiomer of KUC102204N (**10a**), which was found to have comparable activity and toxicity.

We next investigated the effect of modifying the amine side chain. Replacement of the 3,4-dichlorobenzyl group with a benzyl group afforded analogs **9b** and **10b**, which were significantly less potent in disrupting the mHtt–CaM PPI; again, the enantiomeric sets were approximately equipotent. The 4-chlorobenzyl analogs **9c** and **10c** regained much of the lost potency relative to series **b**, though both enantiomers were slightly cytotoxic (ca. 90 μM). The 4-chloro-3-(trifluoromethyl)benzyl analogs **9d** and **10d** further regained potency, though both enantiomers were still less potent than the hit and its enantiomer (**9a** and **10a**, respectively). In contrast to the above-noted electron-withdrawing group-containing congeners, analogs containing either 4-methyl or 4-methoxy were significantly less potent. Thus, the strongly electron-donating 4-methoxybenzyl analogs **9f** and **10f** lost all mHtt–CaM disruption

activity and the 4-methyl analogs **9e** and **10e** were slightly less active than the unsubstituted benzyl. Replacing the unsubstituted benzyl group with a methylenecyclohexyl group led to analogs of comparable potency (cf. **9b** and **10b** vs. **9g** and **10g**). Extending the linker length by replacing 3,4-dichlorobenzyl with 3,4-dichlorophenylethyl led to the analogs **9h** and **10h** with approximately twofold greater potency than the screening hit.

We next synthesized a complementary series where the methylene on the sultam ring was replaced with a ketone. Having established negligible effect from configuration of the amino acid side chain, we only synthesized the L-leucine derivatives. The aryl-based SAR trends identified above were again observed, though the keto-analogs were generally ~0.5-fold as potent as the methylene-containing analogs, with two exceptions. The 4-methoxybenzyl keto-analog **11f** retained marginal activity in disrupting the mHtt–CaM PPI and the methylenecyclohexyl keto-analog **11g** was only 0.5-fold as potent as the benzyl keto-analog **11b**. The keto-analogs **11a** to **11h** were all found to possess greater cytotoxicity than any of the olefin analogs. Lastly, the compounds were tested in assay interference counter-screens and were found to be free of any liabilities (see the Supporting Information, Fig. S-1).

Taken together, the SAR results demonstrate that this series of small molecules sultams is indeed capable of disrupting the mHtt–CaM interaction; the most potent analog **10h** possessed potency of 0.50 μM. Furthermore, several preliminary SAR trends emerged. Electron-withdrawing groups on the aryl group were found to be beneficial for activity, while converting the olefin to a ketone slightly diminished the activity. Interestingly, the configuration of the leucine side chain did not significantly affect activity and we found that the two-carbon linked phenethyl was more favorable than the one-carbon linked benzyl. The SAR trends identified here would be useful in guiding the design of future analogs in this series for disrupting the mHtt–CaM PPI.

A subset of potent analogs that also possessed minimal cytotoxicity (**9c**, **9d**, **9h**, **10c** and **10h**) was also tested for off-target activity against other CaM-dependent kinases to ensure that the innate functions of CaM were not disrupted upon compound treatment. To determine if the test compounds affect Ca⁺²/CaM-dependent DAPK1 and CaMK2γ activity, we performed an in vitro kinase enzyme activity assay for each kinase in the presence of CaM. Percent enzyme activity was calculated by normalizing the enzyme activity obtained for each compound to the enzyme activity obtained in the absence of any compound. Grati-fyingly, the compounds did not inhibit DAPK1 at compound concentrations up to 100 μM and only one analog (**9h**) had a statistically significant impact on CaM2Kγ activity (Fig. 2).

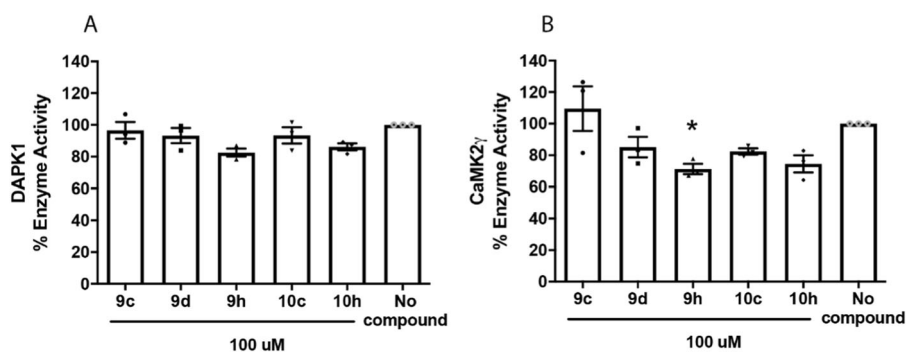
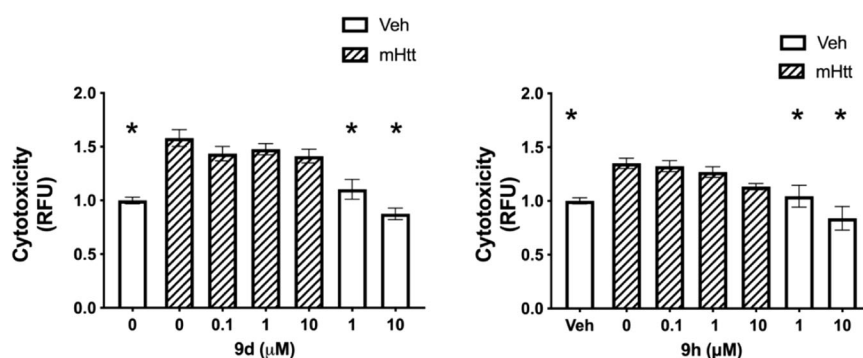


Fig. 2 Compounds were tested in vitro to determine if **a** DAPK1 and **b** CaMK2g enzyme activity was impacted. Each compound was tested at a single concentration of 100 μ M. Data are presented as mean \pm standard error of the mean. Each experiment was run three times with at least two replicates per experiment. Compounds **9c**, **9d**, **10c**, and **10h**

did not significantly inhibit DAPK1 or CaMK2g enzyme activity. Compound **9h** showed a statistically significant effect on inhibition of CaMK2g enzyme activity but not DAPK1 activity as compared with control. * $p = 0.0138$

Fig. 3 Compounds **9d** and **9h** do not show protection against mHtt toxicity



Analog **9d** and **9h** were further evaluated in a neuronal cell model for HD neurodegeneration (Fig. 3). Unfortunately, the activity in the AlphaScreen assay did not translate to therapeutically relevant activity in this cellular model, with barely measurable activity even at 10 μ M. Analog **9h** was evaluated for preliminary pharmacokinetic properties and found to possess a kinetic solubility of 0.6 μ M and permeability (PAMPA assay) of 7.6×10^{-7} cm/s. Based on these data, the poor solubility (extracellular solubility barely above the IC_{50}) and mediocre PAMPA permeability could be sufficient to prevent effective intracellular concentrations and may likely be responsible for the poor correlation between screening assay and cellular model.

Compounds **9d** and **9h** were tested for protection against mHtt toxicity at three different concentrations in PC12 cells. Data are presented as mean \pm standard error of the mean. Each experiment was run at least three times. For compound **9d**, Shapiro–Wilk test showed that data were not normal and log transformation of the data set did not achieve normality. Hence, a Kruskal–Wallis nonparametric test was performed on the data set. Kruskal–Wallis analysis of compound **9d** [(Kruskal–Wallis statistic (7,140) = 81.48, $p < 0.0001$)] showed significant difference among the treatment groups. Dunn’s multiple comparison post hoc test

indicated a significant difference in toxicity due to the induction of mHtt but no significant rescue of the toxicity with compound **9d**. Similarly, one-way ANOVA analysis of compound **9h** [($F(6,99) = 12.46$, $p < 0.0001$)] showed a significant difference among the treatment groups. However, the Dunnett’s multiple comparison test did not show any significant effect of **9h** in rescuing the toxicity associated with mHtt expression although there was a significant toxicity in the presence of mHtt as compared with control.

Conclusion

These results demonstrate that small molecules are capable of disrupting the mHtt–CaM interaction in vitro. Although this particular series did not translate into useful cellular activity, it represents a start toward validating aberrant PPIs as a viable target for small-molecule therapy and paves the way for future investigations into the utilization of small molecules as a treatment for HD.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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