Hydroxypyridinethione Inhibitors of Human Insulin-Degrading Enzyme

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Insulin-degrading enzyme (IDE) is a human mononuclear Zn^{2+}-dependent metalloenzyme that is widely regarded as the primary peptidase responsible for insulin degradation. Despite its name, IDE is also critically involved in the hydrolysis of several other disparate peptide hormones, including glucagon, amylin, and the amyloid β-protein. As such, the study of IDE inhibition is highly relevant to deciphering the role of IDE in conditions such as type-2 diabetes mellitus and Alzheimer disease. There have been few reported IDE inhibitors, and of these, inhibitors that directly target the active-site Zn^{2+} ion have yet to be fully explored. In an effort to discover new, zinc-targeting inhibitors of IDE, a library of ~350 metal-binding pharmacophores was screened against IDE, resulting in the identification of 1-hydroxypyridine-2-thione (1,2-HOPTO) as an effective Zn^{2+}-binding scaffold. Screening a focused library of HOPTO compounds identified 3-sulfonamide derivatives of 1,2-HOPTO as inhibitors of IDE (K_i values of ~50 μM). Further structure-activity relationship studies yielded several thiophene-sulfonamide HOPTO derivatives with good, broad-spectrum activity against IDE that have the potential to be useful pharmacological tools for future studies of IDE.

Introduction

Insulin-degrading enzyme (IDE, EC 3.4.24.56), also known as insulysin and insulinase, is found across nearly all domains of life, and within mammals, is widely considered to be the primary protease responsible for insulin degradation.[1] IDE belongs to the inverzincin family of metalloproteases, which use an active-site Zn^{2+} metal ion to catalyze the hydrolysis of peptide bonds.[1] The protease is also inhibited by thiol-modifying compounds, and was at one point miscategorized as a cysteine-dependent protease, though it is now known that the cysteine residues within IDE are not involved directly in the proteolytic activity of the enzyme.[1]

Human IDE is a ~113-kDa Zn^{2+}-metalloendopeptidase (Figure 1) that is classified as cryptidase-type protease, as it uses a clamshell-like mechanism to encapsulate substrate peptides within its abnormally large, 13000-Å^3 internal chamber.[2] IDE is comprised of two bowl-shaped N- and C-terminal domains (NTD, CTD) linked by a hinge that can adopt "open" and "closed" conformations.[2] Notably, the active site is bipartite, comprised of regions within both the NTD and CTD, and is fully formed only when the protease is in the closed conformation.[2,3] Because substrates must fit completely within the internal chamber to be hydrolyzed, this constrains the size of the substrates that can be processed, rendering IDE a true peptidase and also resulting in exquisite substrate selectivity.[4–6] The catalytic Zn^{2+} is coordinated by His108, His112, and Glu188, with an axially bound water (which is also H-bonded to Glu111) that completes the tetrahedral coordination geometry of the metal center (Figure 1b).[2] To aid in substrate binding, IDE uses an exosite ~30 Å away from the catalytic site to anchor the N-terminal domain of various substrates and facilitate substrate unfolding.[7–8] Mechanistically, IDE is believed to operate by first enveloping its peptide substrates, with hydrolysis occurring only upon complete encapsulation of the substrate.[7–8] In the case of insulin, IDE is able to perform multiple peptide cleavages without impacting the disulfide bonds that hold the

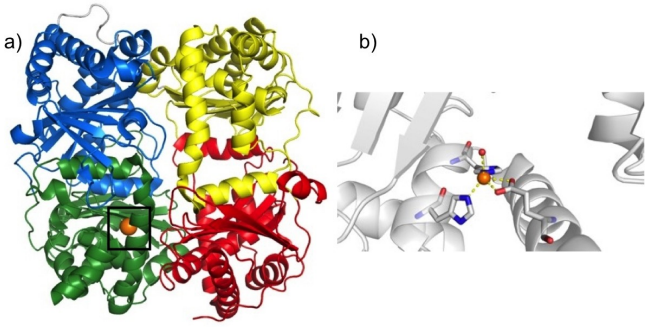

Figure 1. a) The complete structure of IDE, with the NTD in blue and green, the CTD in yellow and red, and the connecting hinge in white. b) Image of the buried catalytic Zn^{2+} ion and metal-coordinating residues within the active site (highlighted in black box in (a)), with the Zn^{2+} ion shown as an orange sphere, water as a red sphere, and coordination bonds as yellow dashes. Images produced from PDB ID: 4NXO.

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hormone peptide together.[7] Following substrate cleavage, IDE re-opens to release the hydrolyzed products, and the cycle begins anew.[7–8]

Homologues of IDE are found across all domains of life, but within humans, misregulation of this mononuclear Zn^{2+} metallopeptidase has been implicated in both type-2 diabetes mellitus (T2DM) and Alzheimer disease (AD).[9–10] While the biological roles of IDE are not yet fully understood, this metalloenzyme is expressed throughout the body, and is found in both the intra- and extra-cellular compartments.[11] IDE functions to hydrolyze a broad range of peptide hormones, yet paradoxically only hydrolyzes certain members of these disparate peptide families.[2] Chief among the known IDE substrates are insulin, glucagon, amylin, and the amyloid β-protein (Aβ; Figure 2).[12–13] Of these, three are relevant to T2DM, as amylin promotes satiety,[14] and insulin and glucagon work in tandem to down- and up-regulate blood glucose, respectively.[15] Additionally, Aβ accumulates abnormally in AD.[16–17]

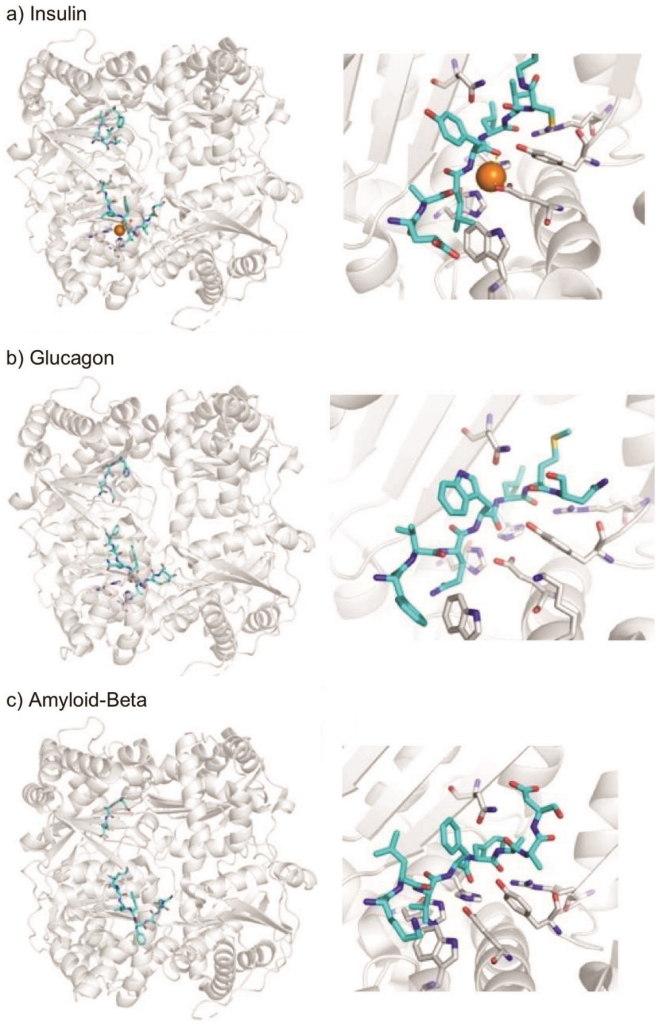


Figure 2. Binding of IDE to a broad range of substrates, as exemplified by: a) insulin, b) glucagon, and c) Aβ.[2] The full structure of IDE bound to each substrate is shown on the left, with close-ups of the catalytic site shown on the right. The zinc ion is depicted as an orange sphere in (a). Images produced from PDB IDs 2G54, 2G49, and 2G47, respectively.

Considering the relevance of IDE to various disease states, there has been great interest in developing substrate-selective inhibitors of IDE,[18] with a particular emphasis on the development of tool compounds to disentangle the different biological roles of IDE in relation to its various substrates. Among the more sought-after goals related to IDE are compounds that act as activators in respect to Aβ degradation, as a means to decrease levels of this neurotoxic peptide.[16–17] In addition, there is particular interest in the discovery of compounds that inhibit insulin degradation, while retaining IDE activity against other substrates. Such a strategy would theoretically provide a novel pharmacological approach to boosting insulin signaling and thereby treating T2DM.[9,19–20] Selective inhibitors of either glucagon or amylin would be of considerable value as pharmacological tools to aid in evaluating the biological roles of IDE. However, few substrate selective inhibitors of IDE are available. Of the reported IDE inhibitors, most are peptidic and bind at the exosite distal to the catalytic Zn^{2+} (Figure 3). The current best-in-class inhibitor is a small molecule that is both potent and selective for IDE-mediated insulin hydrolysis (Figure 3c);[18] however, this compound is difficult to synthesize and has yet to be examined in cellular studies. Finally, there remains a great experimental need for broad-spectrum inhibitors of IDE that are inexpensive to synthesize at scale.

Metal-binding inhibitors of IDE have the potential to provide alternative avenues of IDE inhibition, but these inhibitors have not been thoroughly explored. Only two hydroxamic acid peptide compounds and 1,10-phenanthroline have been reported as metal-binding inhibitors of IDE (Figure 3).[21–22] Hydroxamic acids may display potential issues with selectivity, pharmacokinetics, and metabolism and 1,10-phenanthroline is a non-selective, metal-stripping agent, leaving ample room for the development of more selective, potent, and druglike inhibitors of IDE.

Herein, a library of metal-binding pharmacophores (MBPs) was screened against IDE. MBPs are small molecules (< 300 MW) containing one or more donor atoms suited for metal binding.[23] Metal binding (a.k.a., dative bonding, coordination bonding) is a strong, but reversible interaction, making MBPs ideal starting points for metalloenzyme drug discovery efforts. The results of screening this library against IDE are described here, revealing that 1,2-hydroxypyridinethione (1,2-HOPTO) is a promising fragment lead against this important metalloenzyme.

Results and Discussion

Screening of the MBP Library

To identify MBPs with the potential for inhibitor development against IDE, a ~350-component library[29–30] was screened against IDE, testing for inhibition against the degradation of several key biological IDE substrates, including insulin, glucagon, amylin, and Aβ, as well as the synthetic fluorogenic peptide, substrate V.[31–32] Compounds were initially screened against all five substrates at fragment concentrations of 500 and 50 μM, then for compounds showing good inhibitory

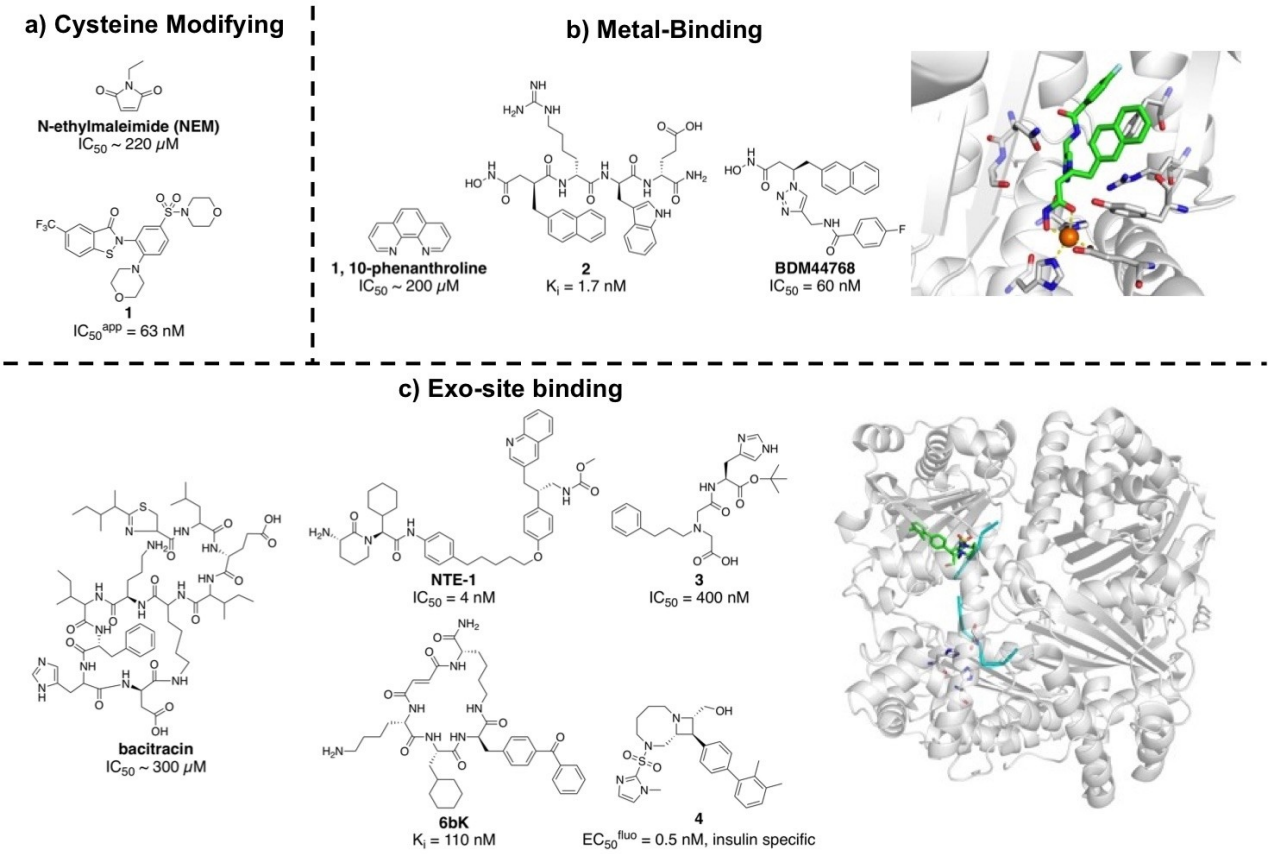


Figure 3. Structures and inhibitory values of reported IDE inhibitors.[3,18,21,22,24–28] a) Cysteine-modifying IDE inhibitors. b) Metal-binding IDE inhibitors; inset: crystal structure of hydroxamate-based compound **2** coordinating to the active site Zn^{2+}, PDB ID: 4NXO.[22] c) Exosite-binding IDE inhibitors; inset: crystal structure of **4** (green) bound to the exosite and co-crystallized with insulin (cyan) PDB ID: 6EDS.[18]

activity, K_i values were established via dose-response experiments. Approximately 14 compounds exhibited average K_i values <100 μM across the five different substrates. These initial hits showed varied degrees of substrate selectivity, including three amylin-sparing compounds and another two showing selectivity for glucagon (data not shown). In addition, consistent with previous reports showing differential activities against short peptide substrates,[32] five compounds were in fact activators of the degradation of substrate V. From among the identified hits, 1,2-hydroxypyridinethione (1,2-HOPTO, **5**, Figure 4), with an average $K_i = 86$ μM against the evaluated substrates, was identified as a fragment lead[33] against IDE based on criteria of potency, synthetic tractability, and the observation that it belonged to a larger family of hits that also contained members exhibiting substrate-selective properties.

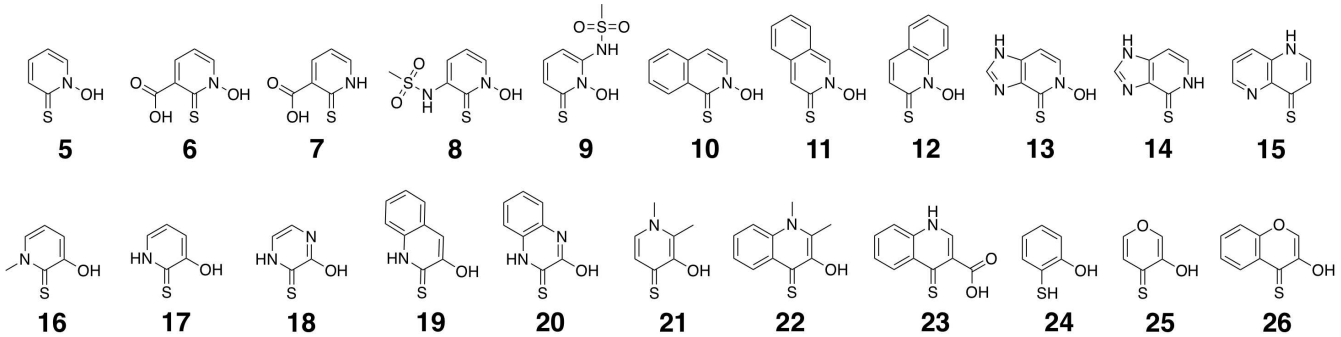


Figure 4. HOPTO derivatives tested for IDE inhibition.

Screening of the elaborated HOPTO sublibrary

HOPTOs are a known class of ligands particularly suited for Zn^{2+} binding based on hard-soft acid-base theory.[34,35] They have been established to affect bidentate binding through a set of oxygen and sulfur donor atoms, where the sulfur has been demonstrated to exist predominately as the thione isomer.[36] In an effort to find HOPTO derivatives as warheads for metalloenzyme inhibition, an expanded library of HOPTO derivatives (Figure 4) was prepared and demonstrated to have druglike qualities while maintaining the core metal-binding capacities.[37] As 1,2-HOPTO was identified as a broad-spectrum scaffold for IDE inhibition, it was decided to employ this elaborated HOPTO sublibrary against IDE to better optimize the MBP warhead before pursuing further inhibitor elaboration. Screening of this focused HOPTO sublibrary revealed both 3-sulfonamide-1,2-HOPTO (**8**) and isoquinoline-1,2-HOPTO (**10**) to have improved inhibition activity, with K_i values of ~50 μM for both compounds. Compound **8** was selected for further development against IDE.

Modeling of 8 in the IDE active site

In the absence of a co-crystal structure, **8** was modeled in the active site of IDE to gain a rudimentary idea of how the compound could potentially bind. The lead fragment **8** has been previously crystallized in a model hydrotris(3,5-phenylmethylpyrazolyl)borate [($Tp^{Ph,Me}$)Zn(**8**)] complex, demonstrating a preferred Zn^{2+} metal-coordination through donor atoms of an axial thione and equatorial hydroxy group.[37] Additionally, there have been previously reported crystal structures of hydroxamic acid-based inhibitors of IDE, showing that these compounds bind the active-site Zn^{2+} through axial carbonyl and equatorial hydroxy groups (Figure 3b).[22] Considering the 1,2-HOPTO MBP core as a cyclized thione analog of a hydroxamic acid, the structure of hydroxamic acid **2** bound to IDE was used to model the binding of **8** in the IDE active site. To prepare the in silico model, the Molecular Operating Environment (MOE, Chemical Computing Group) software suite was employed, using the structure of **8** from the crystal structure of [($Tp^{Ph,Me}$)Zn(**8**)] and superposing it over the hydroxamate of **2** bound in IDE by aligning the thione of **8** with the carbonyl of **2**, and the hydroxy group of **8** with the hydroxy group of **2**. This resulted in the model displayed in Figure 5, with compound **8** being predicted to bind IDE through the thione and hydroxy donor atoms in a manner consistent with both the model [($Tp^{Ph,Me}$)Zn(**8**)] and prior hydroxamic crystal structures.

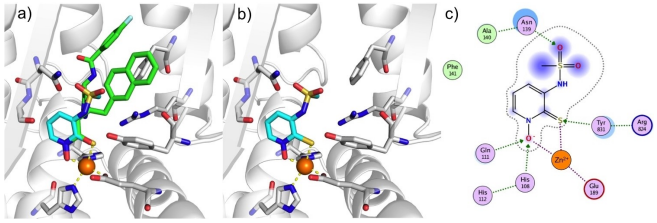
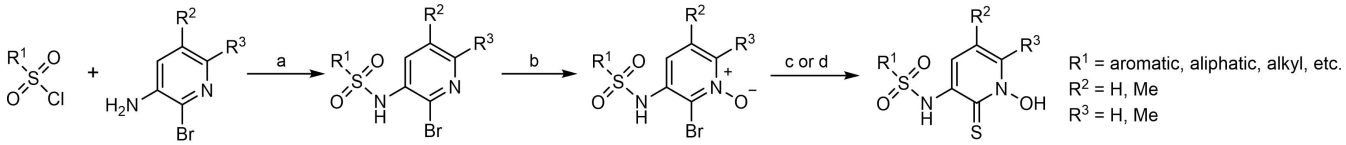


Figure 5. Model of **8** bound to the Zn^{2+} within the active site of IDE. a) Superposition of **8** and **2**, which was used to create b) a model of **8** bound to the Zn^{2+} in the IDE active site. c) A 2-D representation of the model of **8** binding, with Zn^{2+} in orange, coordination bonds as pink dashes, interactions as green arrows, and solvent exposed regions as blue spheres.

Sulfonamide HOPTO derivative synthesis and inhibitory activity

To facilitate the use of these sulfonamide derivatized HOPTOs as tool compounds, a readily adaptable and simple 3-step synthetic route amenable towards rapid derivatization was developed, as detailed in Scheme 1. Combining a sulfonyl chloride with a suitable amine heterocycle starting material in a microwave reactor yielded the desired sulfonamide product. It was found that using excess amine relative to sulfonyl chloride aided in decreasing the amount of undesired disulfonamide side products generated. This synthetic step is highly adaptable to a broad scope of both amine and sulfonyl chloride partners, as evidenced by the scope of sulfonamide HOPTOs prepared in this study (Figure 6). The subsequent step of oxidation using mCPBA achieves selective oxidation at the pyridine nitrogen. The final step of thionation was achieved by heating the oxidized product in a solution of freshly prepared saturated NaSH in the presence of KI. This reaction was quenched with aqueous HCl, and subsequent work-up and chromatography followed by recrystallization from *i*PrOH with varying amounts of H_2O yielded final HOPTO products. In the case of compounds **47** and **48** (which are designed as negative controls because they lack one donor group), thionation was instead achieved by heating the halogenated sulfonamide to reflux at 150 °C in DMF in the presence of excess thiourea. Finally, it should be noted that some HOPTO products were isolated as mixtures with the oxidized dimer (through the sulfur atom). This dimer is easily



Scheme 1. Synthesis of select sulfonamide HOPTO derivatives reported in this study. a) sulfonyl chloride, pyridine, microwave irradiation, 120 °C, 15 min, 25–71%; b) mCPBA, CH_2Cl_2, MeOH, 25–35 °C, 16–72 h, 28–64%; c) sat. aq. NaSH, KI, MeOH, 50–100 °C, 4–16 h, 6–58%; d) thiourea, DMF, 150 °C, followed by 6 M HCl, 110 °C, 2 h, 22–40%.than the amide derivatives.

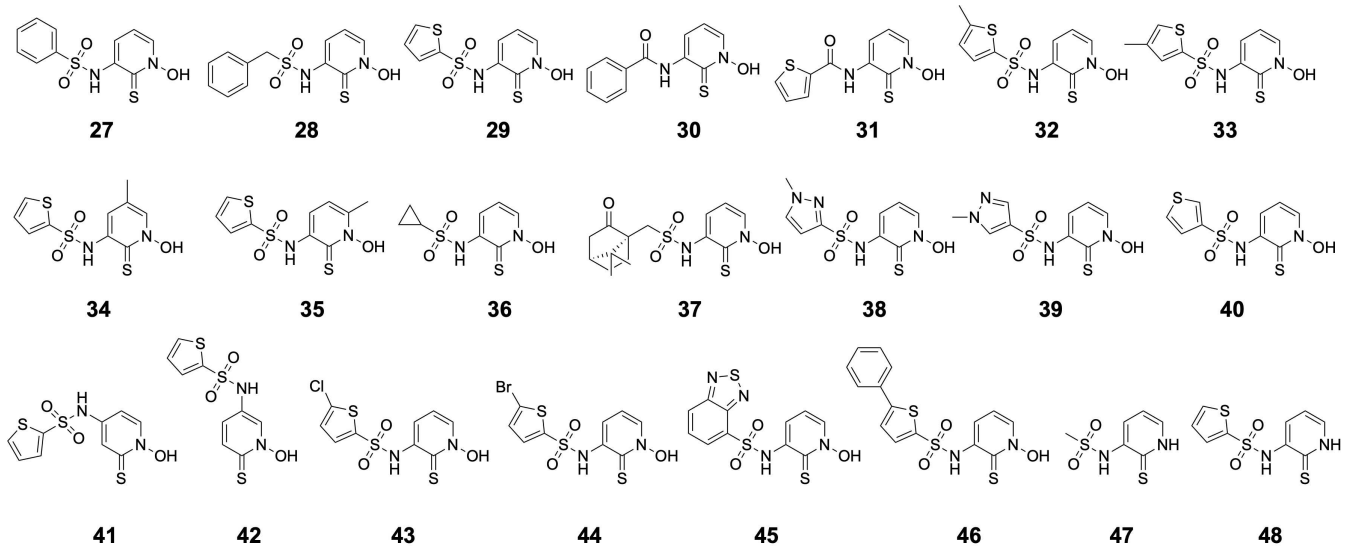


Figure 6. Sulfonamide HOPTO compounds prepared and tested against IDE. Compounds **30** and **31** are amide derivatives, and **47** and **48** are controls for metal binding by lacking the hydroxy moiety that should be essential for metal coordination.

reverted back to the active thione monomer in the presence of DTT as a reductant. Considering IDE requires DTT in the assay buffer to ensure reduction of its 13 Cys residues, DTT in the biochemical assays served the dual function of also ensuring the HOPTO species were in the active thione form.

To further elaborate the lead **8**, it was decided to investigate the structure-activity relationship (SAR) of the sulfonamide moiety to explore other potential interactions within the active site. A preliminary test of phenyl (**27**) and benzyl (**28**) derivatives showed some improvement relative to parent **8**, with **27** having an average K_i value (over all tested substrates) of 22 μM, and **28** having an average K_i value of 35 μM. Utilizing an aromatic isostere replacement of the phenyl ring to a thiophene substituent yielded **29** with a further improved average K_i value of 9 μM (Figure S1 in the Supporting Information).

In addition, to validate the contribution of the sulfonamide to the overall activity of **29**, amide linked compounds **30** and **31** were prepared as analogs of **27** and **29**. As shown in Table 1, **30** had an average K_i value of 57 μM (37 μM not including Substrate V) rendering **30** approximately 2.5 times less active than **27**, and **31** had average K_i values of 55 μM (38 μM not including substrate V) making **31** approximately six times less active than **29**. On the whole, these data suggest that the sulfonamide compounds are more active.

Table 1. Inhibitory activity of HOPTO compounds against IDE substrates.

Cmpd	K_i [μM] Insulin	Gluc.	Amylin	Aβ	V	Ave.	Avg. w/o V
5	39	217	41	108	25	86	101
8	24	114	31	61	19	50	58
27	26	10	26	13	36	22	19
28	57	14	40	19	47	35	33
29	9	3	10	6	15	9	7
30	23	26	63	33	140	57	37
31	27	26	62	36	126	55	38

Sulfonamide HOPTO SAR analysis

In an effort to further improve the activity of the lead **29**, compounds **32**–**46** were synthesized (Figure 6). The methyl derivatives **32**–**35**, were prepared to probe which positions would be amenable to further elaboration. These methyl derivatives exhibited a flat SAR, revealing little difference in activity against IDE. Among a set of derivatives with alkyl sulfonamide substituents, cyclopropane **36** had a comparatively high average K_i value of 45 μM, whereas the bulky camphor **37** was slightly more on par with the thiophene, with an average K_i value of 16 μM. Pyrazole derivatives **38** and **39** were prepared as analogues of the five-member thiophene ring, and again showed somewhat flat SAR with average K_i values of 16 and 23 μM, respectively. To explore alternate variations of the position of the thiophene, the 3-thiophene **40** and alternate position 2-thiophene compounds **41** and **42** were prepared. Of these, **42** was the most interesting, although it had a higher average K_i value of 43 μM, it was the most selective in this study towards sparing amylin degradation (Table 2, Figure S1), making **42** of potential interest as a tool compound in this regard.

To examine more lipophilic substituents, compounds **43**–**46** were synthesized. The halogenated derivatives **43** and **44** showed improvement relative to the parent thiophene, with respective average K_i values of 11 and 7 μM (Figure S1). Between the halogenated derivatives, the brominated thiophene **44** displayed good broad-spectrum activity, inhibiting the degradation of all four physiological substrates (i.e., excluding substrate V) with relatively similar, low micromolar activity (Table 2). Intriguingly, the chlorinated thiophene **43** exhibited a relatively insulin-sparing inhibition profile, for

Table 2. Inhibitory activity of thiophene sulfonamide derivatives against IDE substrates.

Cmpd	K_i [μM] Insulin	Gluc.	Amylin	Aβ	V	Ave.	Ave. w/o V
32	11	6	13	8	27	13	10
33	23	5	12	6	22	14	11
34	18	6	13	7	18	12	11
35	21	5	14	7	27	15	12
36	51	20	35	22	95	45	32
37	7	7	19	11	46	18	11
38	15	6	15	8	36	16	11
39	21	7	19	13	54	23	15
40	16	8	14	9	26	15	12
41	8	11	27	13	29	17	15
42	27	20	64	20	84	43	33
43	30	2	7	3	13	11	11
44	9	2	7	3	14	7	5
45	26	5	11	10	31	16	13
46	55	2	28	13	10	23	25
47	>200	172	>200	>200	61	n.a.	n.a.
48	>200	62	84	102	77	n.a.	n.a.

example, inhibiting the degradation of glucagon ~15 times more potently than insulin (Table 2, Figure S1). As a preliminary test of inhibitor selectivity, compounds **43** and **44** were screened at a concentration of 100 μM against human carbonic anhydrase II (hCAII); no inhibition of the Zn-dependent hCAII metalloenzyme was observed. The thiadiazole **45** and 2-phenyl thiophene **46** showed no improvement relative to **29** in terms of average K_i values, but notably **46** inhibited the degradation of glucagon 27 and 14 times more potently than insulin and amylin, respectively (Table 2, Figure S1).

Validation of the metal-binding mechanism

To validate the mechanism of action as metal binding at the active site Zn^{2+} ion, a set of non-metal-binding controls were prepared (Figure 6). Control compounds **47** and **48** were prepared as respective methyl and thiophene sulfonamide derivatives lacking the oxygen donor atom of the HOPTO. These controls represent a deletion of a crucial portion of the donor atoms required for the bidentate metal coordination of the 1,2-HOPTO ligand, and neither should be capable of strong metal coordination. As expected, **47** displayed essentially no inhibitory activity (except against the short, fluorogenic peptide, Substrate V, Table 2), while compound **48** displayed greatly reduced activity across all IDE substrates (Table 2). The residual activity for compound **48** is likely due to the strong interactions of the thiophene ring and some metal-binding ability by the remaining thione group from the MBP; however, overall these data are supporting of a metal-binding mode of action by the lead compounds in this study.

Conclusion

The goal of this work was to develop novel, easy-to-synthesize, metal-binding inhibitors of IDE that could be useful as biochemical tools and potential starting points for future therapeutic development. IDE relies on an active-site Zn^{2+} ion for catalytic activity, and controls the levels of several biologically important peptide substrates, making IDE inhibition of high importance towards conditions such as T2DM as well as AD. Screening an MBP library of ~350 unique compounds led to the identification of the HOPTO scaffold as a novel zinc-binding moiety having good activity against IDE. Subsequent testing of a HOPTO sublibrary resulted in the identification of 3-sulfonamide-1,2-HOPTO as a novel IDE inhibitor, with an average K_i value of 50 μM against the tested IDE substrates, and derivatization of the sulfonamide resulted in thiophene sulfonamide **29**, with an average K_i of 9 μM against the IDE substrates. Further SAR studies yielded halogen derivatives **43** and **44**, of which the bromine **44** had broad-spectrum activity, with average K_i value of 5 μM against the physiological IDE substrates. In addition, compounds **43** and **46** showed a promising degree of selectivity between insulin and other diabetes-related IDE substrates, glucagon and amylin. Compounds **29**, **43**, **44** and **46** possess many features not present in most existing IDE inhibitors, including a small size (e.g., MW 288-367) and other druglike properties (Table S3), suggesting they may represent useful pharmacophores for future drug development. Overall, these compounds represent a new class of metal-binding IDE inhibitors that, due to their facile syntheses, can be readily made available to serve as useful tools for investigating the role of IDE and the various substrates it regulates.

Experimental Section

Synthesis

Unless otherwise noted, all reagents and solvents were purchased from commercial suppliers and used with no additional purification. Microwave reactions were performed using a CEM Discover series S-class microwave reactor in pressure-sealed vessels. Silica gel column chromatography was performed using a CombiFlash Rf^+ Teledyne ISCO system, using hexane, ethyl acetate, CH_2Cl_2 or MeOH as eluents. Separations were monitored via a Teledyne ISCO RF^+ Purlon ESI-MS detector with 1 Da resolution. ^1H NMR spectra were obtained using Varian 400-MHz and 500-MHz spectrometers at the Department of Chemistry and Biochemistry at UC San Diego. ^1H NMR data is reported in parts per million relative to the residual non-deuterated solvent signals, and spin multiplicities are given as s (singlet), *br* s (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), dt (doublet of triplets), q (quartet), and m (multiplet). When available, coupling constants (J) are reported in hertz (Hz) (Figure S2–S22). Standard resolution mass spectrometry was performed at either the UC San Diego Molecular Mass Spectrometry Facility or on the previously described Teledyne ISCO RF^+ Purlon ESI-MS detector. High resolution mass spectrometry (HRMS) was measured with an Agilent 6230 time-of-flight (TOF) mass spectrometer using a jet stream electrospray ionization source (ESI).

The synthesis of **5**–**28** was previously reported in Adamek et al.[37] While **29** was previously reported in Adamek et al., this key compound was prepared again for this work. The purity of compounds **29**–**48** was evaluated either by analytical HPLC or elemental (combustion) analysis (Table S1); all assayed compounds were >90% purity, with the exception of compounds **33** and **34**,

which were >84% pure by HPLC. Experimental ^{1}H and ^{13}C NMR for final compounds are reported in Figures S2–S22).

Generation of sulfonyl chloride

5-Phenylthiophene-2-sulfonyl chloride (49): To a solution of DMF (0.87 mL, 11.2 mmol) cooled to 0 °C while under a nitrogen atmosphere, sulfuryl chloride (913 μL, 11.2 mmol) was added dropwise, and the reaction was stirred for 15 min, until the mixture formed a white solid. Then 2-phenylthiophene (1.50 g, 9.36 mmol) was added in one portion, and the solids were heated as a melt at 100 °C for 45 min, noting that upon initial addition of the white thiophene, the solid mixture turned yellow, and upon heating, melt was observed to occur at 55 °C. Upon reaching 100 °C, the reaction mixture had turned from clear yellow to clear green. After heating at 100 °C for 45 min, upon reaction completion, the melt was cooled to 25 °C, diluted with ethyl acetate, and ice water was added. With a chilled separatory funnel, the product was extracted into organic using 2×15 mL ethyl acetate, and the combined organic was washed with brine and dried over magnesium sulfate. The solids were filtered off and discarded, and the resulting filtrate was concentrated under reduced pressure to yield a blue liquid. The product was then purified by column chromatography, running an isocratic in gradient 100% hexanes. Like fractions of the desired product were combined and concentrated under reduced pressure, until there was a remaining volume of ~5 mL hexanes, containing a solid precipitate of the desired product. The precipitate was collected via vacuum filtration and washed with hexanes to obtain **49** (1.25 g, 4.81 mmol, 51%) as a powdery solid that was pale yellow with a greenish tint in color. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): 7.60 (d, J=7.6, 2H), 7.39 (t, J=7.6, 2H), 7.31–7.27 (m, 2H), 7.11 (d, J=2.8, 1H).

Sulfonamide coupling

General sulfonamide coupling protocol: Unless otherwise noted, the following protocol was used for sulfonamide coupling. To a heat-gun dried microwave vessel equipped with stir bar and charged with a solution of starting amine (1.3 equiv) in 2 mL dry pyridine, sulfonyl chloride (1.0 eq) was added in one portion. The mixture was placed in a microwave reactor and irradiated at 120 °C for 15 min. Upon cooling, the reaction mixture was diluted with 15 mL ethyl acetate, and washed with 2×15 mL 4 M HCl. The aqueous was back-extracted with an additional 15 mL ethyl acetate, and the combined organic was washed with brine, dried over magnesium sulfate, and the solids were filtered off and discarded. The filtrate was concentrated under reduced pressure, and the resulting crude was purified by column chromatography, running gradient from 100% hexanes to 100% ethyl acetate. The desired sulfonamide product typically eluted in 50% ethyl acetate in hexanes. Like fractions were combined and concentrated under reduced pressure to obtain the desired sulfonamide product that was used directly in the subsequent step. As previously noted, some coupling reactions resulted in an inseparable mix of sulfonamide and disulfonamide products. These mixtures were carried over directly into the next step with no additional purification.

***N*-(2-Bromopyridin-3-yl)thiophene-2-sulfonamide (29a)**: Following the general method for sulfonamide coupling, from 2-bromopyridin-3-amine (1.50 g, 8.67 mmol) and thiophene-2-sulfonyl chloride (1.27 g, 6.94 mmol), the desired product **29a** (1.53 g, 4.80 mmol, 55%) was obtained as a peach colored solid. ^{1}H NMR (400 MHz, $[D_6]$DMSO): δ 10.41 (br s, 1H), 8.25 (d, J=4.4, 1H), 7.97 (d, J=5.2, 1H), 7.70 (d, J=7.6, 1H), 7.49–7.44 (m, 2H), 7.16 (t, J=4.4, 1H); ESI-MS(−): m/z 317.18 $[M-H]^-$.

***N*-(2-Bromopyridin-3-yl)benzamide (30a)**: To a 0 °C solution of 2-bromopyridin-3-amine (0.600 g, 3.47 mmol) and pyridine (0.56 mL, 6.9 mmol) in 10 mL CH_2Cl_2, a mixture of benzoyl chloride (269 μL, 2.31 mmol) in 5 mL CH_2Cl_2 was added dropwise. The reaction mixture was allowed to slowly warm to 25 °C for 16 h, and was then diluted with an additional 15 mL CH_2Cl_2, and was washed with 20 mL 4 M HCl. The aqueous was back-extracted with 10 mL CH_2Cl_2, and the combined organic phase was washed with brine, dried over magnesium sulfate, and the solids were filtered off and discarded. The filtrate was concentrated under reduced pressure, and the resulting crude was purified by column chromatography, running gradient from 100% hexanes to 100% ethyl acetate. The desired product eluted in 50% ethyl acetate in hexanes. Like fractions were combined and concentrated under reduced pressure to obtain **30a** (0.549 g, 1.98 mmol, 86%) as a clear oil that solidified upon standing. ^{1}H NMR (400 MHz, $[D_6]$DMSO): δ 10.10 (br s, 1H), 8.30 (dd, J_1=4.4, J_2=1.2, 1H), 8.01 (d, J=7.2, 3H), 7.65–7.47 (m, 4H); ESI-MS(+): m/z 277.12 $[M+H]^+$.

***N*-(2-Bromopyridin-3-yl)thiophene-2-carboxamide (31a)**: Following the same protocol used in **30a**, from 2-bromopyridin-3-amine (0.600 g, 3.47 mmol), pyridine (0.56 mL, 6.9 mmol), and thiophene-2-carbonyl chloride (247 μL, 2.31 mmol), the desired **31a** (0.536 g, 1.89 mmol, 82%) was obtained as a clear oil that solidified upon standing. ^{1}H NMR (400 MHz, $[D_6]$DMSO): δ 10.23 (br s, 1H), 8.30 (d, J=4.8, 1H), 8.02 (d, J=3.6, 1H), 7.96 (d, J=7.6, 1H), 7.90 (d, J=5.2, 1H), 7.51 (dd, J_1=7.6, J_2=0.8, 1H), 7.25 (t, J=5.2, 1H); ESI-MS(+): m/z 283.11 $[M+H]^+$.

***N*-(2-Bromopyridin-3-yl)-5-methylthiophene-2-sulfonamide (32a)**: Following the general method for sulfonamide coupling, from 2-bromopyridin-3-amine (0.600 g, 3.47 mmol) and 5-methylthiophene-2-sulfonyl chloride (608 μL, 4.51 mmol), the desired product **32a** (0.380 g, 1.14 mmol, 33%) was obtained as a light yellow oil that solidified upon standing. ^{1}H NMR (400 MHz, $[D_6]$DMSO): δ 10.31 (br s, 1H), 8.24 (dd, J_1=4.8, J_2=1.6, 1H), 7.68 (dd, J_1=7.6, J_2=1.6, 1H), 7.45 (dd, J_1=8.0, J_2=4.8, 1H), 7.30 (d, J=3.6, 1H), 6.87 (dd, J_1=4.0, J_2=1.2, 1H), 2.48 (s, 3H); ESI-MS(+): m/z 333.10 $[M+H]^+$.

***N*-(2-Bromopyridin-3-yl)-4-methylthiophene-2-sulfonamide (33a)**: Following the general method for sulfonamide coupling, from 2-bromopyridin-3-amine (0.484 g, 2.80 mmol) and 4-methylthiophene-2-sulfonyl chloride (0.500 g, 2.54 mmol), the desired product **33a** (0.560 g, 1.68 mmol, 66%) was obtained as a slightly impure white solid that was used directly in the next step without any additional purification. ^{1}H NMR (400 MHz, $[D_6]$DMSO): δ 10.39 (br s, 1H), 8.24 (dd, J_1=4.4, J_2=1.2, 1H), 7.67 (dd, J_1=8.0, J_2=1.2, 1H), 7.55–7.54 (m, 2H), 7.45 (dd, J_1=7.6, J_2=4.4, 1H), 7.35 (s, 1H), 2.19 (s, 3H); ESI-MS(−): m/z 333.15 $[M-H]^-$.

***N*-(2-Bromo-5-methylpyridin-3-yl)thiophene-2-sulfonamide (34a)**: Following the general method for sulfonamide coupling, from 2-bromo-5-methylpyridin-3-amine (0.500 g, 2.67 mmol) and thiophene-2-sulfonyl chloride (0.635 g, 3.48 mmol), the desired product **34a** (0.513 g, 1.20 mmol, 43%) was obtained as a white solid contaminated with approximately 25% disulfonamide product. The product was used directly in the next step without any additional purification. ^{1}H NMR (400 MHz, $[D_6]$DMSO): δ 10.31 (br s, 1H), 8.10 (s, 1H), 7.96 (d, J=5.2, 1H), 7.52 (s, 1H), 7.48 (d, J=3.6, 1H), 7.16 (t, J=4.8, 1H), 2.25 (s, 3H); ESI-MS(−): m/z 331.29 $[M-H]^-$.

***N*-(2-Bromo-6-methylpyridin-3-yl)thiophene-2-sulfonamide (35a)**: Following the general method for sulfonamide coupling, from 2-bromo-6-methylpyridin-3-amine (0.500 g, 2.67 mmol) and thiophene-2-sulfonyl chloride (0.635 g, 3.48 mmol), the desired product **35a** (0.746 g, 1.90 mmol, 71%) was obtained as a white solid contaminated with approximately 15% disulfonamide prod-

uct. The product was used directly in the next step without any additional purification. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.25 (br s, 1H), 7.96 (d, J=4.8, 1H), 7.53 (d, J=8.0, 1H), 7.45 (d, J=3.6, 1H), 7.30 (d, J=7.6, 1H), 7.15 (t, J=4.8, 1H), 2.41 (s, 3H); ESI-MS(−): *m/z* 331.26 $[M-H]^-$.

***N*-(2-Bromopyridin-3-yl)cyclopropanesulfonamide (36 a)**: Following the general method for sulfonamide coupling, from 2-bromopyridin-3-amine (0.750 g, 4.34 mmol) and cyclopropanesulfonyl chloride (662 μL, 6.50 mmol), the desired product **36 a** (0.850 g, 3.07 mmol, 71%) was obtained as a light yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 9.70 (br s, 1H), 8.26 (dt, J_1=4.4, J_2=1.6, 1H), 7.84 (dt, J_1=8.0, J_2=1.6, 1H), 7.46 (qd, J_1=4.4, J_2=1.6, 1H), 2.77–2.70 (m, 1H), 1.01–0.96 (m, 2H), 0.90–0.86 (m, 2H); ESI-MS(+): *m/z* 279.12 $[M+H]^+$.

***N*-(2-Chloropyridin-3-yl)-1-((1*R*,4*S*)-7,7-dimethyl-2-oxobicyclo [2.2.1]heptan-1-yl)methanesulfonamide (37 a)**: Following the general method for sulfonamide coupling, from 2-chloropyridin-3-amine (0.350 g, 2.02 mmol) and ((1R,4S)-7,7-dimethyl-2-oxobicyclo [2.2.1]heptan-1-yl)methanesulfonyl chloride (0.650 g, 2.59 mmol), the desired product **37 a** (0.382 g, 1.11 mmol, 43%) was obtained as a pale yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 9.79 (br s, 1H), 8.25 (d, J=4.8, 1H), 7.95 (d, J=8.0, 1H), 7.47 (dd, J_1=8.0, J_2=4.8, 1H), 3.50 (d, J=14.8, 1H), 3.15 (d, J=14.8, 1H), 2.38–2.29 (m, 2H), 2.06 (t, J=4.4, 1H), 1.97–1.91 (m, 2H), 1.59–1.52 (m, 1H), 1.43–1.37 (m, 1H), 1.01 (s, 3H), 0.79 (s, 3H); ESI-MS(+): *m/z* 343.31 $[M+H]^+$.

***N*-(2-Bromopyridin-3-yl)-1-methyl-1*H*-pyrazole-3-sulfonamide (38 a)**: Following the general method for sulfonamide coupling, from 2-bromopyridin-3-amine (0.500 g, 2.89 mmol) and 1-methyl-1H-pyrazole-3-sulfonyl chloride (0.475 g, 2.63 mmol), the desired product **38 a** (0.354 g, 1.12 mmol, 43%) was obtained as a pale tan oil that solidified upon standing. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.18 (br s, 1H), 8.21 (dd, J_1=4.8, J_2=1.6, 1H), 7.88 (d, J=2.0, 1H), 7.72 (dd, J_1=8.0, J_2=1.6, 1H), 7.42 (dd, J_1=8.0, J_2=4.8, 1H), 6.57 (d, J=2.4, 1H), 3.91 (s, 3H); ESI-MS(+): *m/z* 317.25 $[M+H]^+$.

***N*-(2-bromopyridin-3-yl)-1-methyl-1*H*-pyrazole-4-sulfonamide (39 a)**: Following the general method for sulfonamide coupling, from 2-bromopyridin-3-amine (0.500 g, 2.89 mmol) and 1-methyl-1H-pyrazole-4-sulfonyl chloride (0.626 g, 3.47 mmol), the desired product **39 a** (0.611 g, 1.93 mmol, 67%) was obtained as a clear oil that solidified upon standing to a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 9.97 (br s, 1H), 8.23 (s, 1H), 8.21 (dd, J_1=4.8, J_2=1.6, 1H), 7.69 (s, 1H), 7.67 (d, J=1.6, 1H), 7.42 (dd, J_1=8.0, J_2=4.8, 1H), 3.85 (s, 3H); ESI-MS(−): *m/z* 315.20 $[M-H]^-$.

***N*-(2-Bromopyridin-3-yl)thiophene-3-sulfonamide (40 a)**: Following the general method for sulfonamide coupling, from 2-bromopyridin-3-amine (0.600 g, 3.47 mmol) and thiophene-3-sulfonyl chloride (823 mg, 4.51 mmol), the desired product **40 a** (0.583 g, 1.83 mmol, 53%) was obtained as a light brown oil that solidified upon standing. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.17 (br s, 1H), 8.22–8.21 (m, 1H), 8.12–8.11 (m, 1H), 7.76–7.74 (m, 1H), 7.64–7.61 (m, 1H), 7.44–7.40 (m, 1H), 7.28–7.26 (m, 1H); ESI-MS(−): *m/z* 317.19 $[M-H]^-$.

***N*-(2-Bromopyridin-4-yl)thiophene-2-sulfonamide (41 a)**: Following the general method for sulfonamide coupling, from 2-bromopyridin-4-amine (0.750 g, 4.34 mmol) and thiophene-2-sulfonyl chloride (1.19 g, 6.50 mmol), the desired product **41 a** (1.37 g, 4.29 mmol, 55%) was obtained as a pale yellow oil that solidified upon standing to a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 11.57 (br s, 1H), 8.19 (d, J=6.0, 1H), 8.01 (dd, J_1=4.8, J_2=1.2, 1H), 7.79 (dd, J_1=3.6, J_2=1.2, 1H), 7.24 (d, J=2.0, 1H), 7.20–7.16 (m, 2H); ESI-MS(+): *m/z* 321.13 $[M+H]^+$.

***N*-(6-Bromopyridin-3-yl)thiophene-2-sulfonamide (42 a)**: Following the general method for sulfonamide coupling, from 6-bromopyridin-3-amine (0.750 g, 4.34 mmol) and thiophene-2-sulfonyl chloride (1.19 g, 6.50 mmol), the desired product **42 a** (1.38 g, 4.29 mmol) was obtained in theoretical yield as a pale yellow solid contaminated with approximately 50% disulfonamide product. The product was used directly in the next step without any additional purification. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.87 (br s, 1H), 8.26 (dd, J_1=4.8, J_2=1.2, 1H), 8.12 (d, J=2.8, 1H), 7.95 (dd, J_1=5.2, J_2=1.2, 1H), 7.79 (dd, J_1=4.0, J_2=1.6, 1H), 7.62–7.57 (m, 2H), 7.30 (t, J=5.2, 1H); ESI-MS(+): *m/z* 321.15 $[M+H]^+$.

***N*-(2-Bromopyridin-3-yl)-5-chlorothiophene-2-sulfonamide (43 a)**: Following the general method for sulfonamide coupling, from 2-bromopyridin-3-amine (3.00 g, 17.3 mmol) and 5-chlorothiophene-2-sulfonyl chloride (1.86 mL, 13.9 mmol), the desired product **43 a** (2.27 g, 6.41 mmol, 37%) was obtained as a light tan solid contaminated with approximately 10% disulfonamide product. The product was used directly in the next step without any additional purification. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.65 (br s, 1H), 8.28 (dd, J_1=4.4, J_2=1.2, 1H), 7.71 (dd, J_1=8.0, J_2=1.6, 1H), 7.48 (dd, J_1=8.0, J_2=4.8, 1H), 7.37 (d, J=4.0, 1H), 7.25 (d, J=4.0, 1H); ESI-MS(−): *m/z* 353.09 $[M-H]^-$.

5-Bromo-N-(2-bromopyridin-3-yl)thiophene-2-sulfonamide (44 a): Following the general method for sulfonamide coupling, from 2-bromopyridin-3-amine (1.00 g, 5.78 mmol) and 5-bromothiophene-2-sulfonyl chloride (1.21 g, 4.62 mmol), the desired product **44 a** (1.41 g, 3.54 mmol, 61%) was obtained as a pale yellow solid contaminated with approximately 20% disulfonamide product. The product was used directly in the next step without any additional purification. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.64 (br s, 1H), 8.28 (d, J=4.4, 1H), 7.72–7.68 (m, 2H), 7.51–7.46 (m, 2H); ESI-MS(−): *m/z* 397.03 $[M-H]^-$.

***N*-(2-bromopyridin-3-yl)benzo[c][1,2,5]thiadiazole-4-sulfonamide (45 a)**: Following the general method for sulfonamide coupling, from 2-bromopyridin-3-amine (0.350 g, 2.02 mmol) and benzo [c][1,2,5]thiadiazole-4-sulfonyl chloride (0.380 g, 1.62 mmol), the desired product **45 a** (0.382 g, 1.03 mmol, 64%) was obtained as a cream colored solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.49 (br s, 1H), 8.41 (d, J=8.8, 1H), 8.21 (dd, J_1=4.8, J_2=1.6, 1H), 8.13 (d, J=6.8, 1H), 7.84–7.76 (m, 2H), 7.43 (dd, J_1=8.0, J_2=4.8, 1H); ESI-MS(+): *m/z* 373.16 $[M+H]^+$.

N-(2-bromopyridin-3-yl)-5-phenylthiophene-2-sulfonamide (46 a): Following the general method for sulfonamide coupling, from 2-bromopyridin-3-amine (1.08 g, 6.23 mmol) and **49** (1.24 g, 4.79 mmol), the desired product **46 a** (1.13 g, 2.85 mmol, 60%) was obtained as a fluffy bright white solid. ^{1}H NMR (400 MHz, [D$_6$] DMSO): δ 10.53 (br s, 1H), 8.28–8.26 (m, 1H), 7.75–7.70 (m, 3H), 7.56 (d, J=2.4, 1H), 7.48–7.39 (m, 5H); ESI-MS(−): *m/z* 393.23 $[M-H]^-$.

***N*-(2-bromopyridin-3-yl)methanesulfonamide (47 a)**: Following the general method for sulfonamide coupling, from 2-bromopyridin-3-amine (1.08 g, 6.23 mmol) and methanesulfonyl chloride (645 μL, 8.33 mmol), the desired product **47 a** (0.510 g, 2.05 mmol, 25%) was obtained as a pale yellow liquid contaminated with approximately 50% disulfonamide product. The product was used directly in the next step without any additional purification. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 9.66 (br s, 1H), 8.23 (d, J=8.0, 1H), 7.83 (d, J=7.6, 1H), 7.48–7.45 (m, 1H), 3.11 (s, 3H); ESI-MS(−): *m/z* 249 $[M-H]^-$.

Oxidation

General oxidation coupling protocol: Unless otherwise noted, the following protocol was used. To a solution of sulfonamide in 20 mL 1:1 CH_2Cl_2/MeOH, a solution of mCPBA (5 equiv) in 10 mL 1:1

CH_2Cl_2/MeOH was added in one portion. The reaction mixture was then heated at 35 °C for 72 h. Upon completion, the reaction mixture was concentrated under reduced pressure and the resulting crude was purified by column chromatography, running gradient first from 100% hexanes to 100% ethyl acetate, and then from 100% CH_2Cl_2 to 15% MeOH in CH_2Cl_2. The desired products typically eluted in 8–10% MeOH in CH_2Cl_2. Like fractions were combined and concentrated under reduced pressure to obtain the desired oxidized product that was used directly in the subsequent step

2-Bromo-3-(thiophene-2-sulfonamido)pyridine 1-oxide (29 b): Following the general method for oxidation, from **29 a** (1.50 g, 4.70 mmol) and mCPBA (6.32 g, 77.0% Wt, 28.2 mmol), at 25 °C for 16 h, the desired product **29 b** (0.742 g, 2.21 mmol, 55%) was obtained as a light yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.61 (br s, 1H), 8.35 (d, J=6.8, 1H), 7.99 (d, J=4.8, 1H), 7.55 (d, J=3.6, 1H), 7.39 (t, J=6.8, 1H), 7.21–7.16 (m, 2H); ESI-MS(−): *m/z* 333.11 $[M-H]^-$.

3-Benzamido-2-bromopyridine 1-oxide (30 b): Following the general method for oxidation, from **30 a** (0.540 g, 1.95 mmol) and mCPBA (2.18 g, 77.0% Wt, 9.74 mmol), at 35 °C for 16 h, the desired product **30 b** (0.169 g, 0.577 mmol, 30%) was obtained as an off-white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.34 (br s, 1H), 8.42 (d, J=6.4, 1H), 7.98 (d, J=7.2, 1H), 7.64 (t, J=7.2, 1H), 7.58–7.52 (m, 2H), 7.46 (t, J=6.4, 1H); ESI-MS(+): *m/z* 293.05 $[M+H]^+$.

2-Bromo-3-(thiophene-2-carboxamido)pyridine 1-oxide (31 b): Following the general method for oxidation, from **31 a** (0.530 g, 1.87 mmol) and mCPBA (2.10 g, 77.0% Wt, 9.36 mmol), at 35 °C for 16 h, the desired product **31 b** (0.358 g, 1.20 mmol, 64%) was obtained as a yellow oil that solidified upon standing. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.38 (br s, 1H), 8.42 (dd, J_1=6.0, J_2=1.6, 1H), 8.01 (dd, J_1=2.8, J_2=0.8, 1H), 7.92 (dd, J_1=4.8, J_2=0.8, 1H), 7.51–7.46 (m, 2H), 7.25 (dt, J_1=3.6, J_2=1.2, 1H); ESI-MS(+): *m/z* 299.02 $[M+H]^+$.

2-Bromo-3-((5-methylthiophene)-2-sulfonamido)pyridine 1-oxide (32 b): Following the general method for oxidation, from **32 a** (0.380 g, 1.28 mmol) and mCPBA (1.28 g, 77.0% Wt, 5.70 mmol), at 25 °C for 67 h, the desired product **32 b** (0.202 g, 0.578 mmol, 51%) was obtained as an off-white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.52 (br s, 1H), 8.35 (d, J=6.4, 1H), 7.40–7.37 (m, 2H), 7.21 (d, J=8.0, 1H), 6.88 (d, J=3.2, 1H), 2.48 (s, 3H); ESI-MS(−): *m/z* 347.16 $[M-H]^-$.

2-Bromo-3-((4-methylthiophene)-2-sulfonamido)pyridine 1-oxide (33 b): Following the general method for oxidation, from **33 a** (0.560 g, 1.68 mmol) and mCPBA (1.88 g, 77.0% Wt, 8.40 mmol), at 25 °C for 72 h, the desired product **33 b** (0.192 g, 0.550 mmol, 33%) was obtained as a yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.56 (br s, 1H), 8.35 (d, J=6.4, 1H), 7.58 (s, 1H), 7.42 (s, 1H), 7.38 (t, J=6.4, 1H), 7.19 (d, J=8.4, 1H), 2.20 (s, 3H); ESI-MS(−): *m/z* 349.10 $[M-H]^-$.

2-Bromo-5-methyl-3-(thiophene-2-sulfonamido)pyridine 1-oxide (34 b): Following the general method for oxidation, from **34 a** (0.500 g, 1.13 mmol) and mCPBA (1.68 g, 77.0% Wt, 7.50 mmol), at 30 °C for 16 h, the desired product **34 b** (0.101 g, 0.289 mmol, 26%) was obtained as a pale yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.53 (br s, 1H), 8.29 (s, 1H), 7.98 (d, J=5.2, 1H), 7.55 (d, J=4.0, 1H), 7.16 (t, J=4.0, 1H), 7.01 (s, 1H), 2.20 (s, 3H); ESI-MS(+): *m/z* 349.11 $[M+H]^+$.

2-Bromo-6-methyl-3-(thiophene-2-sulfonamido)pyridine 1-oxide (35 b): Following the general method for oxidation, from **35 a** (0.730 g, 1.86 mmol) and mCPBA (5.87 g, 77.0% Wt, 2.45 mmol), at 30 °C for 20 h, the desired product **35 b** (0.216 g, 0.289 mmol, 33%) was obtained as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.50 (br s, 1H), 7.98 (d, J=4.8, 1H), 7.52 (d, J=3.6, 1H), 7.46 (d, J=8.4, 1H), 7.16 (t, J=8.8, 1H), 7.11 (d, J=8.4, 1H), 2.37 (s, 3H); ESI-MS(+): *m/z* 349.14 $[M+H]^+$.

2-Bromo-3-(cyclopropanesulfonamido)pyridine 1-oxide (36 b): Following the general method for oxidation, from **36 a** (0.890 g, 3.13 mmol) and mCPBA (3.60 g, 77.0% Wt, 16.1 mmol), at 30 °C for 16 h, the desired product **36 b** (0.470 g, 0.160 mmol, 50%) was obtained as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 9.87 (br s, 1H), 8.36 (dd, J_1=5.2, J_2=2.4, 1H), 7.42–7.37 (m, 2H), 2.81–2.75 (m, 1H), 1.03–0.98 (m, 2H), 0.97–0.91 (m, 2H); ESI-MS(+): *m/z* 295.14 $[M+H]^+$.

2-Chloro-3-((((1R,4S)-7,7-dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl)methyl)sulfonamido)pyridine 1-oxide (37 b): Following the general method for oxidation, from **37 a** (0.380 g, 1.11 mmol) and mCPBA (1.24 g, 77.0% Wt, 5.54 mmol), at 40 °C for 4 d, the desired product **37 b** (0.241 g, 0.672 mmol, 61%) was obtained as a yellow oil that solidified upon standing. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 7.98 (d, J=6.4, 1H), 7.42 (d, J=8.4, 1H), 7.19 (t, J=8.0, 1H), 3.37 (d, J=5.6, 1H), 2.95 (d, J=14.8, 1H), 2.52–2.46 (m, 1H), 2.33–2.27 (m, 1H), 2.01 (t, J=4.0, 1H), 1.90–1.86 (m, 2H), 1.53–1.46 (m, 1H), 1.39–1.33 (m, 1H), 1.00 (s, 3H), 0.76 (s, 3H); ESI-MS(−): *m/z* 357.31 $[M-H]^-$.

2-Bromo-3-((1-methyl-1*H*-pyrazole)-3-sulfonamido)pyridine 1-oxide (38 b): Following the general method for oxidation, from **38 a** (0.350 g, 1.10 mmol) and mCPBA (1.24 g, 77.0% Wt, 5.52 mmol), at 30 °C for 72 h, the desired product **38 b** (0.166 g, 0.498 mmol, 45%) was obtained as a yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.40 (br s, 1H), 8.33 (d, J=6.4, 1H), 7.90 (s, 1H), 7.36 (d, J=1.2, 1H), 7.25 (d, J=8.4, 1H), 6.61 (s, 1H), 3.91 (s, 3H); ESI-MS(+): *m/z* 333.14 $[M+H]^+$.

2-Bromo-3-((1-methyl-1*H*-pyrazole)-3-sulfonamido)pyridine 1-oxide (39 b): Following the general method for oxidation, from **39 a** (0.600 g, 1.89 mmol) and mCPBA (2.12 g, 77.0% Wt, 9.46 mmol), at 30 °C for 16 h, the desired product **39 b** (0.212 g, 0.636 mmol, 34%) was obtained as an off-white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.18 (br s, 1H), 8.31 (dd, J_1=6.4, J_2=1.2, 1H), 8.28 (s, 1H), 7.74 (s, 1H), 7.36 (t, J=6.8, 1H), 7.20 (dd, J_1=8.4, J_2=0.8, 1H), 3.85 (s, 3H); ESI-MS(+): *m/z* 331.17 $[M+H]^+$.

2-Bromo-3-(thiophene-3-sulfonamido)pyridine 1-oxide (40 b): Following the general method for oxidation, from **40 a** (0.580 g, 1.82 mmol) and mCPBA (2.04 g, 77.0% Wt, 9.09 mmol), at 25 °C for 67 h, the desired product **40 b** (0.256 g, 0.763 mmol, 42%) was obtained as an off-white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.61 (br s, 1H), 8.35 (d, J=6.8, 1H), 7.99 (d, J=4.8, 1H), 7.55 (d, J=3.6, 1H), 7.39 (t, J=6.8, 1H), 7.21–7.16 (m, 2H); ESI-MS(−): *m/z* 333.22 $[M-H]^-$.

2-Bromo-4-(thiophene-2-sulfonamido)pyridine 1-oxide (41 b): Following the general method for oxidation, from **41 a** (1.00 g, 3.13 mmol) and mCPBA (2.11 g, 77.0% Wt, 9.40 mmol), at 35 °C for 16 h, the desired product **41 b** (0.298 g, 0.889 mmol, 28%) was obtained as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 11.30 (br s, 1H), 8.31 (d, J=7.2, 1H), 7.98 (d, J=4.8, 1H), 7.70 (d, J=3.6, 1H), 7.44 (d, J=2.8, 1H), 7.19–7.14 (m, 2H); ESI-MS(+): *m/z* 335.10 $[M+H]^+$.

2-Bromo-5-(thiophene-2-sulfonamido)pyridine 1-oxide (42 b): Following the general method for oxidation, from **42 a** (1.00 g, 3.13 mmol) and mCPBA (2.11 g, 77.0% Wt, 9.40 mmol), at 35 °C for 16 h, the desired product **42 b** (0.323 g, 0.964 mmol, 31%) was obtained as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 11.09 (br s, 1H), 8.13 (d, J=2.0, 1H), 7.99 (dd, J_1=4.8, J_2=1.6, 1H), 7.81 (d, J=8.8, 1H), 7.68 (dd, J_1=4.0, J_2=1.2, 1H), 7.17 (dd, J_1=5.2, J_2=4.0, 1H), 7.04 (dd, J_1=8.8, J_2=2.0, 1H); ESI-MS(+): *m/z* 335.08 $[M+H]^+$.

2-Bromo-3-((5-chlorothiophene)-2-sulfonamido)pyridine 1-oxide (43 b): Following the general method for oxidation, from **43 a** (1.80 g, 1.86 mmol) and mCPBA (6.84 g, 77.0% Wt, 5.09 mmol), at 35 °C for 48 h, the desired product **43 b** (0.876 g, 2.37 mmol, 47%) was obtained as a yellow-tan solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.85 (br s, 1H), 8.39 (dd, J_1 = 6.4, J_2 = 0.8, 1H), 7.44 (d, J = 4.0, 1H), 7.40 (t, J = 6.4, 1H), 7.25 (d, J = 4.0, 1H), 7.22 (dd, J_1 = 8.4, J_2 = 0.8, 1H); ESI-MS(−): *m/z* 369.12 $[M-H]^-$.

2-Bromo-3-((5-chlorothiophene)-2-sulfonamido)pyridine 1-oxide (44 b): Following the general method for oxidation, from **44 a** (1.40 g, 3.52 mmol) and mCPBA (3.94 g, 77.0% Wt, 17.6 mmol), at 35 °C for 6 d, the desired product **44 b** (0.497 g, 1.20 mmol, 34%) was obtained as a tan solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.81 (br s, 1H), 8.37 (d, J = 6.4, 1H), 7.42–7.39 (m, 2H), 7.34 (d, J = 4.0, 1H), 7.21 (d, J = 8.4, 1H); ESI-MS(−): *m/z* 413.02 $[M-H]^-$.

3-(Benzo[c][1,2,5]thiadiazole-4-sulfonamido)-2-bromopyridine 1-oxide (45 b): Following the general method for oxidation, from **45 a** (0.350 g, 0.943 mmol) and mCPBA (1.06 g, 77.0% Wt, 4.71 mmol), at 30 °C for 5 d, the desired product **45 b** (0.147 g, 0.380 mmol, 40%) was obtained as a tan solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 8.26 (d, J = 8.8, 1H), 8.08 (d, J = 7.2, 1H), 7.94–7.92 (m, 1H), 7.60 (t, J = 6.8, 1H), 7.17 (d, J = 8.4, 1H), 7.10–7.06 (m, 1H); ESI-MS(+): *m/z* 389.11 $[M+H]^+$.

2-Bromo-3-((5-phenylthiophene)-2-sulfonamido)pyridine 1-oxide (46 b): Following the general method for oxidation, from **46 a** (1.05 g, 2.66 mmol) and mCPBA (2.98 g, 77.0% Wt, 13.3 mmol), at 35 °C for 16 h, the desired product **46 b** (0.590 g, 1.43 mmol, 54%) was obtained as a pale yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.73 (br s, 1H), 8.36 (d, J = 6.4, 1H), 7.71 (d, J = 7.6, 2H), 7.57–7.54 (m, 2H), 7.47–7.39 (m, 4H), 7.25 (d, J = 8.4, 1H); ESI-MS(−): *m/z* 409.17 $[M-H]^-$.

Thionation

General thionation protocol: The starting material and 1.5 equivalents of KI were dissolved in 5 mL DI water with a minimal amount of MeOH to dissolve as needed. Then 5 mL of saturated freshly prepared sodium hydrogen sulfide solution (excess) was added to the solution containing the starting material. The reaction mixture was then heated at 50–100 °C for 3–16 h. Upon reaction completion as indicated by TLC, the reaction mixture was cooled to 0 °C, and slowly quenched with 6 M HCl. ***CAUTION!*** The neutralization of NaSH using acid generates H_2S gas, which is both highly flammable and highly toxic by inhalation; only perform neutralization in a well-vented fume hood. After waiting ~5 min to allow the resultant H_2S gas to evolve and disperse, the resulting mixture was extracted into organic using 3×15 mL ethyl acetate. The combined organic was washed with aqueous 1 M $Na_2S_2O_3$, until the aqueous went from cloudy to clear. Then the organic was washed with brine, dried over magnesium sulfate, and the solids were filtered off and discarded. The filtrate was concentrated under reduced pressure, and the remaining residue was then purified by column chromatography, using a gradient of 100% hexanes to 100% CH_2Cl_2, and then to 15% MeOH in CH_2Cl_2. The desired product typically eluted around 80% CH_2Cl_2 in hexanes. When necessary, the collected product was recrystallized from 7:3 IPA to water. The crystals were collected via vacuum filtration and rinsed with a small amount of cold IPA to obtain the desired final products.

***N*-(1-Hydroxy-2-thioxo-1,2-dihydropyridin-3-yl)thiophene-2-sulfonamide (29)**: Following the above general thionation protocol, from **29 b** (0.500 g, 1.49 mmol) with KI (0.371 g, 2.24 mmol) in 5 mL saturated NaSH solution (excess) at 100 °C for 8 h, **29** (0.160 g, 0.555 mmol, 37%) was obtained as a pale yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 9.50 (br s, 1H), 8.17 (d, J = 6.8, 1H), 7.98 (d, J = 4.8, 1H), 7.72 (d, J = 4.0, 1H), 7.59 (d, J = 8.0, 1H), 7.14 (t, J = 4.8, 1H), 6.88 (d, J = 7.6, 1H); ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ 164.3, 138.6, 137.3, 135.4, 134.3, 134.0, 128.4, 119.4, 112.8; HRMS (ESI-TOF): *m/z* calcd for $[C_9H_7N_2O_3S_3]^-$: 286.9624 $[M-H]^-$; found: 286.9626.

***N*-(1-Hydroxy-2-thioxo-1,2-dihydropyridin-3-yl)benzamide (30)**: Following the above general thionation protocol, from **30 b** (0.160 g, 0.546 mmol) with KI (0.136 g, 0.819 mmol) in 5 mL saturated NaSH solution (excess) at 40 °C for 16 h, **30** (0.065 g, 0.260 mmol, 48%) was obtained as an off white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.55 (br s, 1H), 8.55 (d, J = 7.6, 1H), 8.21 (d, J = 7.2, 1H), 7.96 (d, J = 7.6, 2H), 7.69–7.60 (m, 3H), 6.98 (t, J = 7.6, 1H); ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ 165.2, 163.4, 138.7, 134.1, 133.1, 132.6, 129.7, 127.5, 119.1, 113.4; HRMS (ESI-TOF): *m/z* calcd for $[C_{12}H_9N_2O_2S]^-$: 245.0390 $[M-H]^-$; found: 245.0391.

***N*-(1-Hydroxy-2-thioxo-1,2-dihydropyridin-3-yl)thiophene-2-carboxamide (31)**: Following the above general thionation protocol, from **31 b** (0.350 g, 1.17 mmol) with KI (0.291 g, 1.76 mmol) in 5 mL saturated NaSH solution (excess) at 40 °C for 16 h, **31** (0.130 g, 0.515 mmol, 44%) was obtained as a gray solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.42 (br s, 1H), 8.41 (d, J = 7.6, 1H), 8.21 (d, J = 6.8, 1H), 7.96 (d, J = 4.8, 1H), 7.82 (d, J = 3.2, 1H), 7.27 (t, J = 3.6, 1H), 6.96 (t, J = 6.8, 1H); ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ 163.3, 160.0, 138.7, 138.4, 133.5, 132.6, 130.0, 129.2, 119.3, 113.3; HRMS (ESI-TOF): *m/z* calcd for $[C_{10}H_7N_2O_2S_2]^-$: 250.9954 $[M-H]^-$; found: 250.9957.

***N*-(1-Hydroxy-2-thioxo-1,2-dihydropyridin-3-yl)-5-methylthiophene-2-sulfonamide (32)**: Following the above general thionation protocol, from **32 b** (0.200 g, 0.573 mmol) with KI (0.143 g, 0.859 mmol) in 5 mL saturated NaSH solution (excess) at 100 °C for 8 h, **32** (0.058 g, 0.18 mmol, 33%), was obtained as a pale yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 9.44 (br s, 1H), 8.17 (d, J = 6.8, 1H), 7.58–7.56 (m, 2H), 6.90–6.87 (m, 2H), 2.45 (s, 3H); ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ 164.0, 149.7, 137.4, 135.3, 134.8, 133.9, 127.0, 118.7, 112.9, 15.6; HRMS (ESI-TOF): *m/z* calcd for $[C_{10}H_9N_2O_3S_3]^-$: 300.9781 $[M-H]^-$; found: 300.9780.

***N*-(1-Hydroxy-2-thioxo-1,2-dihydropyridin-3-yl)-4-methylthiophene-2-sulfonamide (33)**: Following the above general thionation protocol, from **33 b** (0.190 g, 0.544 mmol) with KI (0.135 g, 0.816 mmol) in 5 mL saturated NaSH solution (excess) at 100 °C for 5 h, **33** (0.070 g, 0.230 mmol, 43%) was obtained as a pale yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 9.47 (br s, 1H), 8.17 (dd, J_1 = 6.8, J_2 = 1.2, 1H), 7.58–7.56 (m, 3H), 6.89 (t, J = 8.0, 1H), 2.16 (s, 3H); ^{13}C NMR (126 MHz, CD_3OD): δ 161.6, 138.4, 138.3, 137.2, 134.8, 129.4, 128.8, 118.9, 111.9, 13.9; HRMS (ESI-TOF): *m/z* calcd for $[C_{10}H_9N_2O_3S_3]^-$: 300.9781 $[M-H]^-$; found: 300.9778.

***N*-(1-Hydroxy-5-methyl-2-thioxo-1,2-dihydropyridin-3-yl) thiophene-2-sulfonamide (34)**: Following the above general thionation protocol, from **34 b** (0.100 g, 0.286 mmol) with KI (0.071 g, 0.430 mmol) in 3 mL saturated NaSH solution (excess) at 75 °C for 3 h, **34** (0.0057 g, 0.019 mmol, 7%) was obtained as a very pale yellow solid. ^{1}H NMR (400 MHz, [D$_6$]acetone): δ 8.65 (br s, 1H), 8.17 (s, 1H), 7.92 (d, J = 4.8, 1H), 7.77 (d, J = 4.0, 1H), 7.63 (s, 1H), 7.16 (t, J = 4.0, 1H), 2.34 (s, 3H); ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ 165.8, 157.3, 134.7, 128.0, 116.6, 110.2, 67.3, 53.3, 39.8, 20.3. HRMS (ESI-TOF): *m/z* calcd for $[C_{10}H_9N_2O_3S_3]^-$: 300.9781 $[M-H]^-$; found: 300.9779.

***N*-(1-Hydroxy-6-methyl-2-thioxo-1,2-dihydropyridin-3-yl) thiophene-2-sulfonamide (35)**: Following the above general thionation protocol, from **35 b** (0.210 g, 0.601 mmol) with KI (0.150 g, 0.902 mmol) in 3 mL saturated NaSH solution (excess) at 75 °C for 3 h, **35** (0.011 g, 0.036 mmol, 6%) was obtained as a pale yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 12.49 (br s, 1H), 9.35 (br s, 1H), 7.95 (d, J = 4.8, 1H), 7.68 (d, J = 4.0, 1H), 7.55 (d, J = 8.0, 1H),

7.12 (t, $J=4.0$, 1H), 6.85 (d, $J=8.0$, 1H), 2.41 (s, 3H); HRMS (ESI-TOF): *m/z* calcd for $[C_{10}H_9N_2O_3S_3]^-$: 300.9781 $[M-H]^-$; found: 300.9778.

***N*-(1-Hydroxy-2-thioxo-1,2-dihydropyridin-3-yl) cyclopropanesulfonamide (36)**: Following the above general thionation protocol, from **36b** (0.460 g, 0.157 mmol) with KI (0.391 g, 2.35 mmol) in 5 mL saturated NaSH solution (excess) at 100 °C for 8 h, **36** (0.132 g, 0.534 mmol, 34%) was obtained as a pale yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 8.94 (br s, 1H), 8.22 (dd, $J_1=6.8$, $J_2=1.2$, 1H), 7.58 (dd, $J_1=7.6$, $J_2=0.8$, 1H), 6.90 (t, $J=7.6$, 1H), 2.87–2.81 (m, 1H), 1.05–0.95 (m, 4H); ^{13}C NMR (126 MHz, CD_3OD): δ 161.5, 138.1, 128.9, 119.6, 112.1, 29.8, 4.9; HRMS (ESI-TOF): *m/z* calcd for $[C_8H_9N_2O_3S_2]^-$: 245.0060 $[M-H]^-$; found: 245.0061.

1-((*1R,4S*)-7,7-Dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl)-*N*-(1-hydroxy-2-thioxo-1,2-dihydropyridin-3-yl)methanesulfonamide (37): Following the above general thionation protocol, from **37b** (0.240 g, 0.669 mmol) with KI (0.167 g, 1.00 mmol) in 5 mL saturated NaSH solution (excess) at 80 °C for 6 h, **37** (0.011 g, 0.031 mmol, 5%) was obtained as a dark gray solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 11.63 (br s, 1H), 8.45 (br s, 1H), 8.31 (d, $J=6.4$, 1H), 7.81 (d, $J=7.6$, 1H), 7.11 (t, $J=7.2$, 1H), 3.66 (d, $J=15.2$, 1H), 3.30 (d, $J=10.8$, 1H), 2.46–2.34 (m, 2H), 2.15–2.13 (m, 1H), 1.94 (d, $J=18.4$, 2H), 1.82–1.75 (m, 1H), 1.53–1.47 (m, 1H), 1.09 (s, 3H), 0.87 (s, 3H); HRMS (ESI-TOF): *m/z* calcd for $[C_{15}H_{19}N_2O_4S_2]^-$: 355.0792 $[M-H]^-$; found: 355.0789.

***N*-(1-Hydroxy-2-thioxo-1,2-dihydropyridin-3-yl)-1-methyl-1*H*-pyrazole-3-sulfonamide (38)**: Following the above general thionation protocol, from **38b** (0.166 g, 0.498 mmol) with KI (0.124 g, 0.747 mmol) in 5 mL saturated NaSH solution (excess) at 100 °C for 5 h, **38** (0.047 g, 0.17 mmol, 33%) was obtained as a light gray solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 9.35 (br s, 1H), 8.14 (d, $J=6.8$, 1H), 7.88 (d, $J=1.6$, 1H), 7.54 (d, $J=7.6$, 1H), 6.87 (t, $J=7.6$, 1H), 6.78 (d, $J=1.6$, 1H), 3.87 (s, 3H); ^{13}C NMR (126 MHz, CD_3OD): δ 160.8, 148.6, 137.3, 132.7, 128.7, 117.9, 111.9, 107.1, 38.5; HRMS (ESI-TOF): *m/z* calcd for $[C_9H_9N_4O_3S_2]^-$: 285.0122 $[M-H]^-$; found: 285.0122.

***N*-(1-Hydroxy-2-thioxo-1,2-dihydropyridin-3-yl)-1-methyl-1*H*-pyrazole-4-sulfonamide (39)**: Following the above general thionation protocol, from **39b** (0.210 g, 0.630 mmol) with KI (0.157 g, 0.945 mmol) in 5 mL saturated NaSH solution (excess) at 100 °C for 5 h, **39** (0.057 g, 0.20 mmol, 32%) was obtained as a dark gray solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 9.24 (br s, 1H), 8.14 (d, $J=6.8$, 1H), 8.44 (s, 1H), 8.14 (d, $J=6.8$, 1H), 7.85 (s, 1H), 7.53 (d, $J=8.0$, 1H), 6.86 (t, $J=7.6$, 1H), 3.82 (s, 3H); ^{13}C NMR (126 MHz, CD_3OD): δ 161.3, 138.4, 137.4, 133.1, 129.1, 120.6, 118.7, 112.0, 38.2; HRMS (ESI-TOF): *m/z* calcd for $[C_9H_9N_4O_3S_2]^-$: 285.0122 $[M-H]^-$; found: 285.0121.

***N*-(1-Hydroxy-2-thioxo-1,2-dihydropyridin-3-yl)thiophene-3-sulfonamide (40)**: Following the above general thionation protocol, from **40b** (0.250 g, 0.746 mmol) with KI (0.186 g, 1.12 mmol) in 5 mL saturated NaSH solution (excess) at 100 °C for 8 h, **40** (0.053 g, 0.18 mmol, 25%) was obtained as a pale yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 9.36 (br s, 1H), 8.40–8.39 (m, 1H), 8.14 (d, $J=6.8$, 1H), 7.73–7.71 (m, 1H), 7.54 (d, $J=8.0$, 1H), 7.35 (d, $J=5.2$, 1H), 6.84 (t, $J=8.0$, 1H); HRMS (ESI-TOF): *m/z* calcd for $[C_9H_7N_2O_3S_3]^-$: 286.9624 $[M-H]^-$; found: 286.9623.

***N*-(1-Hydroxy-2-thioxo-1,2-dihydropyridin-4-yl)thiophene-2-sulfonamide (41)**: Following the above general thionation protocol, from **41b** (0.290 g, 0.865 mmol) with KI (0.215 g, 2.30 mmol) in 5 mL saturated NaSH solution (excess) at 100 °C for 8 h, **41** (0.083 g, 0.288 mmol, 33%) was obtained as a very pale yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 11.95 (br s, 1H), 11.44 (br s, 1H), 8.25 (dd, $J_1=7.2$, $J_2=3.6$, 1H), 8.04–8.02 (m, 1H), 7.76–7.74 (m, 1H), 7.22–7.18 (m, 2H), 6.64–6.62 (m, 1H); ^{13}C NMR (126 MHz, CD_3OD): δ: 167.9, 143.0, 139.6, 133.5, 133.4, 133.2, 127.3, 116.7, 105.1; HRMS (ESI-TOF): *m/z* calcd for $[C_9H_7N_2O_3S_3]^-$: 286.9624 $[M-H]^-$; found: 286.9627.

***N*-(1-Hydroxy-6-thioxo-1,6-dihydropyridin-3-yl)thiophene-2-sulfonamide (42)**: Following the above general thionation protocol, from **42b** (0.310 g, 0.925 mmol) with KI (0.230 g, 2.39 mmol) in 5 mL saturated NaSH solution (excess) at 100 °C for 8 h, **42** (0.028 g, 0.097 mmol, 10%) was obtained as a pale yellow solid. ^{1}H NMR (400 MHz, [D$_6$]acetone): δ 8.31 (dd, $J_1=2.4$, $J_2=0.4$, 1H), 7.93 (dd, $J_1=4.8$, $J_2=1.2$, 1H), 7.64 (dd, $J_1=3.6$, $J_2=1.2$, 1H), 7.58 (dd, $J_1=9.2$, $J_2=0.4$, 1H), 7.33 (dd, $J_1=9.2$, $J_2=2.4$, 1H), 7.19 (dd, $J_1=5.2$, $J_2=4.0$, 1H); ^{13}C NMR (126 MHz, CD_3OD): δ 164.8, 139.0, 133.1, 132.8, 131.7, 128.6, 127.4, 126.99, 126.2; HRMS (ESI-TOF): *m/z* calcd for $[C_9H_7N_2O_3S_3]^-$: 286.9624 $[M-H]^-$; found: 286.9626.

5-Chloro-*N*-(1-hydroxy-2-thioxo-1,2-dihydropyridin-3-yl) thiophene-2-sulfonamide (43): Following the above general thionation protocol, from **43b** (0.300 g, 0.812 mmol) with KI (0.202 g, 1.22 mmol) in 5 mL saturated NaSH solution (excess) at 65 °C for 16 h, **43** (0.152 g, 0.471 mmol, 58%) was obtained as a pale yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 9.64 (br s, 1H), 8.23 (dd, $J_1=6.8$, $J_2=0.8$, 1H), 7.62–7.57 (m, 2H), 7.23 (d, $J=4.0$, 1H), 6.89 (t, $J=7.2$, 1H); ^{13}C NMR (126 MHz, CD_3OD): δ 162.4, 137.5, 137.4, 136.8, 132.9, 130.1, 127.1, 120.6, 111.7; HRMS (ESI-TOF): *m/z* calcd for $[C_9H_6ClN_2O_3S_3]^-$: 320.9235 $[M-H]^-$; found: 320.9234.

5-Bromo-*N*-(1-hydroxy-2-thioxo-1,2-dihydropyridin-3-yl) thiophene-2-sulfonamide (44): Following the above general thionation protocol, from **44b** (0.300 g, 0.724 mmol) with KI (0.180 g, 1.09 mmol) in 5 mL saturated NaSH solution (excess) at 75 °C for 17 h, **44** (0.050 g, 0.14 mmol, 19%) was obtained as a pale yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 9.62 (br s, 1H), 8.22 (d, $J=6.8$, 1H), 7.59–7.56 (m, 2H), 7.32 (d, $J=3.6$, 1H), 6.89 (t, $J=7.2$, 1H); ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ 164.6, 139.7, 137.0, 134.9, 134.4, 132.1, 121.0, 120.8, 112.8; HRMS (ESI-TOF): *m/z* calcd for $[C_9H_6BrN_2O_3S_3]^-$: 364.8729 $[M-H]^-$; found: 364.8728.

***N*-(1-Hydroxy-2-thioxo-1,2-dihydropyridin-3-yl)benzo[*c*][1,2,5] thiadiazole-4-sulfonamide (45)**: Following the above general thionation protocol, from **45b** (0.150 g, 0.387 mmol) with KI (0.0965 g, 0.581 mmol) in 5 mL saturated NaSH solution (excess) at 75 °C for 17 h, **45** (0.018 g, 0.053 mmol, 14%) was obtained as a deep yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 9.73 (br s, 1H), 8.40 (d, $J=8.8$, 1H), 8.32 (d, $J=7.2$, 1H), 8.06 (d, $J=6.8$, 1H), 7.84 (t, $J=6.8$, 1H), 7.56 (d, $J=8.0$, 1H), 6.78 (t, $J=6.8$, 1H); ^{13}C NMR (126 MHz, CD_3OD): δ 156.7, 153.4, 146.8, 135.0, 129.8, 127.8, 125.8, 125.4, 124.3, 115.7, 110.3; HRMS (ESI-TOF): *m/z* calcd for $[C_{11}H_7N_4O_3S_3]^-$: 338.9686 $[M-H]^-$; found: 338.9683.

***N*-(1-Hydroxy-2-thioxo-1,2-dihydropyridin-3-yl)-5-phenylthiophene-2-sulfonamide (46)**: Following the above general thionation protocol, from **46b** (0.400 g, 0.973 mmol) with KI (0.242 g, 1.46 mmol) in 5 mL saturated NaSH solution (excess) at 60 °C for 16 h, **46** (0.153 g, 0.420 mmol, 43%) was obtained as a light yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 9.58 (br s, 1H), 8.19 (d, $J=7.2$, 1H), 7.74 (d, $J=3.6$, 1H), 7.70–7.66 (m, 2H), 7.62 (d, $J=8.0$, 1H), 7.55 (d, $J=4.0$, 1H), 7.51–7.39 (m, 4H), 6.90 (t, $J=7.6$, 1H); ^{13}C NMR (126 MHz, CD_3OD): δ 161.9, 152.1, 137.1, 137.1, 134.2, 132.3, 129.9, 129.0, 128.9, 125.8, 123.0, 119.2, 111.9; HRMS (ESI-TOF): *m/z* calcd for $[C_{15}H_{11}N_2O_3S_3]^-$: 362.9937 $[M-H]^-$; found: 362.9935.

***N*-(2-Thioxo-1,2-dihydropyridin-3-yl)methanesulfonamide (47)**: To a solution of **47a** (0.500 g, 50% Wt, 0.996 mmol) in 15 mL DMF, thiourea (1.82 g, 23.9 mmol) was added in one portion, and the reaction was placed under argon and heated to reflux at 150 °C for 12 h, after which the reaction mixture had changed color to dark brown. Then the reaction was cooled to 110 °C, and 6 M HCl (15 mL, 90 mmol) was added in one portion; the reaction was heated at 110 °C for an additional 2 h. Then the reaction mixture

was cooled to 25 °C, diluted with 50 mL brine, and extracted into organic using 3×15 mL ethyl acetate. The combined organic was washed with an additional 15 mL brine, dried over magnesium sulfate, and the solids were filtered off and discarded. The filtrate was concentrated under reduced pressure, and the resulting crude was purified by column chromatography, running gradient from 100% hexanes to 100% ethyl acetate, and then from 100% CH_2Cl_2 to 15% MeOH in CH_2Cl_2. The desired product eluted in 85% ethyl acetate in hexanes. Like fractions of the desired product were combined and concentrated under reduced pressure. The resulting solid was recrystallized from 3:1 ethyl acetate to hexanes, and the solid was collected via vacuum filtration and washed with hexanes to obtain **47** (0.081 g, 0.40 mmol, 40%) as a pale yellow solid. ^{1}H NMR (400 MHz, [D_6]DMSO): δ 8.71 (br s, 1H), 7.61–7.58 (m, 2H), 6.90 (t, J=6.8, 1H), 3.16 (s, 3H); HRMS (ESI-TOF): *m/z* calcd for $C_6H_9N_2O_2S_2^+$: 205.0100 $[M+H]^+$; found: 205.0103.

***N*-(2-Thioxo-1,2-dihydropyridin-3-yl)thiophene-2-sulfonamide (48)**: Following the same protocol used in **47**, from **29a** (0.500 g, 1.57 mmol) and thiourea (1.43 g, 18.8 mmol) the desired **48** (0.094 g, 0.35 mmol, 22%) was obtained as a deep yellow solid. ^{1}H NMR (400 MHz, [D_6]DMSO): δ 9.27 (br s, 1H), 7.98 (d, J=4.8, 1H), 7.73 (d, J=0.8, 1H), 7.65 (d, J=7.6, 1H), 7.57 (d, J=6.0, 1H), 7.16–7.15 (m, 1H), 6.88 (d, J=7.6, 1H); ^{13}C NMR (126 MHz, [D_6]DMSO): δ 168.3, 138.6, 136.4, 135.3, 134.3, 133.8, 128.4, 122.6, 114.2; HRMS (ESI-TOF): *m/z* calcd for $C_9H_7N_2O_2S_3^-$: 270.9675 $[M-H]^-$; found: 270.9675.

Activity assays

The degradation of Aβ, glucagon, amylin and insulin was quantified by fluorescence polarization-based assays using fluorescein-labeled and biotinylated peptides, as described.[38–39] For substrate V, fluorescence dequenching was monitored continuously (λ_{ex}= 385 nm; λ_{em}=425 nm). Bacterially expressed, recombinant human IDE,[40] was used in all assays. Reactions were performed in Buffer A (PBS supplemented with 0.05% BSA and 0.5 mM DTT) and, for discontinuous assays, terminated by addition of 10-fold excess avidin relative to the substrate concentration and 2 mM 1,10-phenanthroline. A peptidic inhibitor of IDE, P12-3 A[41] was used as a positive control. All MBP fragments were tested in at least 3 independent experiments, with results normalized to DMSO-only and no-enzyme controls. All activity assays were conducted in 384-well format on a SpectraMAX M5^e multilable plate reader (Molecular Devices). IC_{50} values were obtained from normalized activity data by curve fitting within Prism v8.1 (GraphPad Software Inc.) and converted to K_i values using the Cheng-Prusoff equation and published K_M values for each substrate.[2,38–39]

hCAII assay. The plasmid for recombinant expression of hCAII with a T7 RNA polymerase promoter and ampicillin resistance gene (pACA) was a gift from Thomas R. Ward (Universität Basel, Switzerland). The protein for this activity assays was expressed in BL21 *Escherichia coli* cells and purified as reported previously.[42] Assays were carried out in clear-bottom Costar 96-well plates with a total volume of 100 μL per well. The assay buffer was comprised of 50 mM HEPES pH 8.0. Compounds were added from a 50 mM DMSO stock to a final concentration of 100 μM and incubated with hCAII (40 nM final concentration) for 10 min at room temperature. *p*-Nitrophenyl acetate was used as the substrate (500 μM final concentration), and the absorbance at 405 nm was monitored for 20 min at 1 min intervals. Percent inhibition was determined by comparing the activity of wells containing inhibitors to the activity of those without.

Molecular modeling

The in silico model of **8** bound to the IDE active site was prepared by using the superpose function within the Molecular Operating Environment (MOE) software suite to align the structure of Zn-bound **8** from the structure of [($Tp^{Ph,Me}$)Zn(**8**)][37] with the hydroxamic acid of **2** from PDB ID: 4NXO. The atoms of the thione in **8** were aligned with the carbonyl atoms of the hydroxamate group in **2**, and likewise, the hydroxy atoms of **8** were aligned with the hydroxy atoms of **2**, resulting in a model of **8** bound to the Zn^{2+} within the IDE active site. Upon modeling **8** in the IDE active site, the image was visually rendered using PyMOL.

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Conflict of Interests

S.M.C. is a cofounder of and has an equity interest in Cleave Therapeutics and Forge Therapeutics, companies that might potentially benefit from the research results. S.M.C. also serves on the Scientific Advisory Board for Forge Therapeutics. The terms of this arrangement have been reviewed and approved by the University of California, San Diego in accordance with its conflict of interests policies.

Keywords: fragment-based drug discovery · high-throughput screening · insulin-degrading enzymes · medicinal chemistry · metalloenzymes

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FULL PAPERS

Metal-binding inhibitors of the human metalloenzyme insulin-degrading enzyme (IDE) were discovered by screening a focused library that revealed 3-sulfonamide-1-hydroxypyridine-2-thione as a novel inhibitor of insulin degrading enzyme (IDE). Structure-activity relationship (SAR) studies led to several thiophene-sulfonamide-hydroxypyridinethione compounds with broad-spectrum activity against several IDE substrates, thus demonstrating the potential of these compounds to be used as pharmacological tools for probing IDE's biological functions.