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Hydroxamic Acids Block Replication of Hepatitis C Virus

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Abstract

Intrigued by the role of protein acetylation in hepatitis C virus (HCV) replication, we tested known histone deacetylase (HDAC) inhibitors and a focused library of structurally simple hydroxamic acids for inhibition of a HCV sub-genomic replicon. While known HDAC inhibitors with varied inhibitory profiles proved to be either relatively toxic or ineffective, structure-activity relationship (SAR) studies on cinnamic hydroxamic acid and benzo[*b*]thiophen-2-hydroxamic acid gave rise to compounds **22** and **53**, which showed potent and selective anti-HCV activity and therefore are promising starting points for further structural optimization and mechanistic studies.

Key words

Hepatitis C Virus, HCV, cinnamic hydroxamic acid, hydroxamic acid, histone deacetylase, HDAC, HDAC inhibitor, benzothiophene

INTRODUCTION

Infecting 170 million people in the world and 4 million people in the United States, hepatitis C virus (HCV) is a major cause of liver diseases including chronic hepatitis, cirrhosis and hepatocellular carcinoma.[1] For many years, the standard of care (SOC) was pegylated interferon plus ribavirin, which suffered from lack of efficacy and severe side-effects.[2] Recently approved NS3/4A protease inhibitors (**1-3**, Figure 1) have demonstrated enhanced efficacy, but they are undermined by rapid emergence of drug-resistance.[3] Sofosbuvir (**4**), an approved inhibitor of the NS5B RNA dependent RNA polymerase, is less prone to resistance and is active against the major HCV genotypes. These and new generations of direct-acting antivirals (DAA) in various combinations have been aggressively pursued by the pharmaceutical industry to yield all-oral regimens,[4] leading to the most recent approval of the first all-oral pill Harvoni (a combination of ledipasvir and sofosbuvir) by the U.S. Food and Drug Administration (FDA) to treat chronic HCV genotype 1 infection.[5]

In addition to DAA, there has been a continuing interest in exploring therapeutics that target host cellular factors, a strategy that is generally less prone to emergence of resistance and is likely to be active across different HCV genotypes.[6] Inhibitors of cyclophilins are being investigated in advanced clinical trials, supporting the notion that host cellular factors are promising anti-HCV targets.

HCV has evolved to exploit or subvert the host cellular machinery for its replication. Alterations of host functions generally entail reprogramming of gene expression and/or virus-mediated

modification of cellular proteins.[6] Protein acetylation has emerged as a prominent post-translational modification of histones and other cellular proteins. The acetylation levels are controlled by histone acetyltransferases and histone deacetylases (HDACs), the latter of which can be divided into two major groups: zinc-dependent HDACs and NAD-dependent HDACs (sirtuins). HDACs are involved in the regulation of numerous genes and the post-translational modification of a wide range of proteins. More specifically, HDACs are regulators of inflammation and immunity (innate and adaptive),[7] cellular phenomena that are closely involved in HCV infection. Recent proteomic studies have revealed extensive acetylation in mouse and human liver cells.[8] Up-regulation of HDAC activity induced by HCV infection has also been reported.[9] Furthermore, polymorphisms in three HDAC enzymes (HDAC2, 3 and 5) have been shown to be independently associated with sustained virological response in chronic HCV.[10] These observations suggested that HDAC inhibitors could hold promise in blocking HCV replication. Herein we report our investigation of known HDAC inhibitors and a focused library of simple hydroxamic acids based on the cinnamic and fused aromatic core structures using cultured cells containing a sub-genomic HCV replicon. Very recently, benzohydroxamic acids[11] and a pan-HDAC inhibitor suberoylanilide hydroxamic acid (SAHA)[12] have been reported as potential anti-HCV agents. In our current work, we have systematically investigated known HDAC inhibitors including those that are isoform-selective. We have also explored novel hydroxamic acids built upon the cinnamic acid and benzo[*b*]thiophene core structures. More significantly, we have performed extensive structure-activity relationship (SAR) studies to pinpoint key structural features that elicit excellent anti-HCV activity and selectivity.

RESULTS AND DISCUSSION

Evaluation of commonly used HDAC inhibitors. To investigate whether HDAC inhibitors can block HCV replication, we tested commonly used HDAC inhibitors for inhibition of a HCV sub-genomic replicon in Huh7 cells. 2'-C-Methyl adenosine (2mA)[13], a known inhibitor of HCV NS5B polymerase, was routinely used as a positive control in our screening for anti-HCV agents. SAHA (**5**, Figure 2), an FDA-approved HDAC inhibitor, showed a sub-micromolar EC_{50} value (Table 1), which is in agreement with its anti-HCV activity in a recent report.[12] However, SAHA showed significant cytotoxicity at a low molar concentration, leading to a low therapeutic index (TI) as defined as by CC_{50}/EC_{50}. We also tested structurally similar SMAHA (**6**),[14] a dual inhibitor of HDAC and IMPDH, the latter of which has been explored as an anti-cancer target. SMAHA exhibited EC_{50} and CC_{50} values similar to those of SAHA, apparently due to their structural similarity. We proceeded to evaluate PXD101 (**7**, Figure 2), a HDAC inhibitor that has recently been approved by the FDA for the treatment of relapsed or refractory peripheral T-cell lymphoma,[15] and LBH589 (**8**),[16] which has been advanced into oncology clinical trials. Compound **7** (Table 1) was more active than SAHA; however, it also exhibited elevated cytotoxicity, resulting in a TI value almost identical to that of SAHA. Compound **8** proved to be extremely toxic to the replicon cells as indicated by its low nanomolar CC_{50} value.

Evaluation of these HDAC inhibitors suggested that HDAC inhibitors were effective in blocking HCV replication; however, high cytotoxicity would likely limit their application in treating HCV infection. Since compounds **5-8** are all pan-HDAC inhibitors that target all of HDAC1-3, 6, 8 and 10, we decided to explore whether selective HDAC inhibitors are less toxic. To this end, we tested MS-275 (**9**, Figure 2),[17] PCI-34051 (**10**),[18] and Tubastatin A (**11**),[19] which selectively

inhibit HDAC1/2, HDAC8, and HDAC6, respectively. In comparison with SAHA, compound **9** displayed a reduction in both anti-HCV replication and toxicity, leading to a TI value almost identical to that of SAHA (Table 1). Inhibitor **10** exhibited an inhibitory profile very similar to that of **9**. For selective HDAC6 inhibitor Tubastatin A, it was active at 10 μM but cell viability was severely compromised at that concentration. In order to explore the role of HDAC class IIa enzymes (HDAC4, 5, 7 and 9) that are marginally inhibited by compounds **5-11**, we tested TMP269 (**12**, Figure 2),[20] a selective class IIa inhibitor recently reported. Compound **12** turned out to be ineffective at 10 μM (Table 1). Collectively, our evaluation revealed a very narrow TI for known HDAC inhibitors whether they are pan- or selective inhibitors while a selective class IIa inhibitor was ineffective. Therefore, it may be challenging to develop HDAC inhibitors as potential anti-HCV agents.

Evaluation of compounds based on cinnamic hydroxamic acids. In parallel to our testing of known HDAC inhibitors, we also explored cinnamic hydroxamic acid, a core structure that is well represented in HDAC inhibitors and inhibitors of neural toxins.[21] To guide our structural optimization, we performed our SAR studies based on the inhibition of replication of a sub-genomic HCV replicon (EC_{50}) in conjunction with cellular cytotoxicity (CC_{50}). First, we probed the effect of substitutions on position 3 of the unsaturated linker region (Table 2). The parent compound cinnamic hydroxamic acid (**13**) possessed a promising submicromolar EC_{50} against HCV replication and a TI value of 45. Introducing a methyl group (**14**) reduced the anti-replication activity and a more bulky phenyl group (**15**) further decreased the activity, indicating that substitution at position 3 has a detrimental effect. Next, we investigated the influence of electron-withdrawing or –donating groups at the *para* position. As exemplified by inhibitors **16**

and **17**, an electron-donating group, such as methyl or methoxy, greatly diminished the anti-HCV activity, indicating that those groups are not tolerated at the *para* position. Surprisingly, an electron-withdrawing Cl group attached at the *para* position (**18**) also reduced anti-replicon activity. Nevertheless, a stronger electron-withdrawing NO_2 group (**19**) slightly improved the anti-replication activity when compared with the parent compound **13**. Compounds donned with a methylsulfone (**20**) or cyano (**21**) group showed excellent activity together with low reduction in viability at 10 μM. However, at 1 μM their activities were greatly diminished. As a result, no EC_{50} or CC_{50} values was determined. Gratifyingly, compound **22**, in which a strong electron-withdrawing CF_3 group was introduced, exhibited an EC_{50} value of 140 nM. More importantly, it showed limited toxicity and possessed a TI value of 178, a marked improvement over that of the parent compound **13**. Furthermore, it displayed better anti-HCV activity when compared with recently reported benzohydroxamic acids.[11]

With compound **22** in hand, we proceeded to probe the importance of its structural features (Table 3). Saturation of the olefin (**23**) led to a drastic reduction of the anti-HCV activity while removing the olefin moiety (**24**) resulted in nearly 4-fold decrease in activity. Noticeably, compound **24** structurally resembles benzoic hydroxamic acids that have been reported to inhibit HCV replication.[11] To assess the importance of the hydroxamic acid functionality, we prepared compounds **25-27**,[22] in which the hydroxamic acid was replaced with the corresponding methyl ester, carboxylic acid, and primary amide, respectively. Those three compounds showed minimal inhibitory activity against HCV, highlighting the importance of the hydroxamic acid functionality. We also attempted to replace the hydroxamic acid with a benzamide chelator as seen in **9**. Compound **28**[23] showed high activity but compromised cellular viability,

characteristics that are consistent with those of compound **9**. Our SAR study of compounds **23-28** clearly indicates that the unsaturated hydroxamic acid as seen in compound **22** played a crucial role in its anti-HCV activity.

Having determined that α, β-unsaturated hydroxamic acid is a key feature for anti-HCV activity, we continued to investigate substituents on the aromatic ring. (Table 4). First, a CF_3 group was attached at the *meta* (**29**) or *ortho* (**30**) position; however, both modifications turned out to be detrimental to the anti-HCV activity. Second, a CF_3 group was fixed at the *para* position and additional groups were introduced. With an additional fluoro group at the *ortho* position, compound **31** showed an anti-HCV activity very similar to that of compound **22** but compound **31** exhibited a higher toxicity and therefore a relatively low TI. Incorporation of one more fluoro group as shown in compound **32** led to further attenuation of activity and a concomitant reduction of TI. When a chloro group was appended at the *ortho* position (compound **33**), its anti-HCV activity was severely compromised, suggesting that a chloro group is not tolerated at this position. Intriguingly, compound **34**, in which a chloro group was placed at both of the *para* and *ortho* positions, exhibited excellent anti-HCV activity and a TI value comparable to that of compound **31**. Interestingly, compound **34** has been previously investigated for its ability to block the action of neurotoxin.[21b] Third, we also explored whether the phenyl ring could be replaced with a pyridine ring that contains a nitrogen atom at the *ortho* (**35**), *meta* (**36**) or *para* (**37**) position.[24] Compounds **35** and **36** showed good activity at 1 μM, suggesting that a pyridine ring could be used in conjunction with an unsaturated hydroxamic acid. Consequently, we prepared compounds **38** and **39** in an attempt to combine a pyridine ring and a CF_3 group at the

para position (relative to the hydroxamic acid). Unfortunately, both compounds possessed a low TI value even though they showed excellent anti-HCV activity.

In order to find isosteric replacements for the cinnamic hydroxamic acid core, we also explored fused aromatic rings such as furan, benzo[*b*]thiophene and indole (Table 5). First, furan-2-hydroxamic acid (**40**) and benzo[*b*]thiophene-2-hydroxamic acid (**41**) showed low micromolar anti-HCV activities comparable to that of cinnamic hydroxamic acid **13** even though they all possessed low TI values. Second, we tested indole-derived hydroxamic acids in which the functionality was placed on either position 2 or 3 (**42-47**). With a free NH, compound **42** displayed a weak activity. Blocking the NH with phenyl (**43**) did not improve the activity; however, introducing a benzyl group (**44**) significantly increased the anti-HCV activity although it still remained relatively low. Identical structural modifications were performed on indole-3-hydroxamic acid to give compounds **45-47**. Compounds **45** and **46** exhibited negligible activities. In compound **47** benzylation led to a marked boost in activity, unfortunately likely due to its high cytotoxicity. Taken together, our evaluation of fused aromatic hydroxamic acids suggested that benzo[*b*]thiophene-2-hydroxamic acid is a viable substitute for the cinnamic hydroxamic acid scaffold.

Consequently, we focused our efforts on benzo[*b*]thiophene derivatives as shown in Table 6. Initially, we evaluated the effect of an electron-withdrawing group at position 5 or 6 of benzo[*b*]thiophene. A chloro group at position 5 (**48**) slightly improved the activity. In contrast, a nitro group at either position 5 (**49**) or 6 (**50**) significantly decreased the anti-HCV activity with a concomitant lowering of TI. We also tested the effect of a CF_3 group, which imparted a superior

antiviral ability in the cinnamic hydroxamic acid series when it was placed at the *para* position. Unfortunately, while compounds **51** and **52** showed very good activities at 10 μM, their activities waned at 1 μM. These observations suggested that the benzo[*b*]thiophene-2-hydroxamic acid series behave differently from the cinnamic hydroxamic acid in terms of SAR trends.

Aiming to identify a new position to append an electron-withdrawing group, we attached a phenyl sulfone at position 3 (**53**, Table 6), which resulted in a slightly enhanced activity when compared with the parent compound **41**. More importantly, compound **53** gained a significantly improved TI, suggesting that it may represent a new scaffold suitable for further structural manipulations. First, in order to probe whether the electron-withdrawing ability of the phenyl sulfone contributed to the anti-HCV activity, the corresponding sulfoxide **54** and sulfide **55**, which possess attenuated electron-withdrawing capacities, were prepared and tested. Sulfoxide **54** was two-fold less active than compound **53** while sulfide **55** lost antiviral activity, highlighting the key role of the sulfone functionality. Next, in order to confirm the importance of the hydroxamic acid, we tested the corresponding methyl ester (**56**) and carboxylic acid (**57**), which as anticipated showed essentially no activity at 10 μM. In summary, in addition to the cinnamic hydroxamic acid, we have identified benzo[*b*]thiophene-2-hydroxamic acid as a new scaffold with anti-HCV activity even though the position and the nature of electron-withdrawing groups may vary for optimal antiviral ability. Nonetheless, the structural similarity and the requisite hydroxamic acid functionality strongly suggest that both scaffolds block HCV replication through a common mechanism of action.

Secondary assay to confirm the antiviral activity of leads. To ensure that our identified lead compounds inhibited HCV replicon production or maintenance, representative inhibitors were examined for their ability to reduce replicon RNA by reverse transcription and quantitative PCR (RT-qPCR). The relative HCV replicon RNA level in compound-treated cells was compared to that in DMSO-treated cells using HCV-specific NS5B primers/probes and GAPDH control primers/probes. Compounds **48** and **53** reduced replicon RNA 75-80% at 2 μM and the positive control inhibitor 2mA[13] treatment (0.5 μM) reduced the replicon RNA level approximately 75% (Figure 3). We chose those compound concentrations because they were not toxic to cells and were likely to show significant reduction in replicon RNA levels. We conclude that our hits inhibit HCV replicon RNA production or maintenance as expected.

Preliminary examination of potential targets. We attempted to uncover clues about potential targets of our newly discovered hydroxamic acids. Since the hydroxamic acid functionality in compound **53** is crucial for its activity, compound **53** was evaluated for inhibition of zinc-dependent enzymes that are expected to be sensitive to hydroxamic acid-based molecules. First, it was tested against a full panel of 11 human HDACs (Table S1, Supporting Information). Compound **53** exhibited very weak inhibition of all HDACs except for HDAC8, which was inhibited at a low micromolar level. Compound **53** therefore is unlikely to target any of the HDACs tested with the possible exception of HDAC8. To explore the possibility that HDAC8 inhibition might be responsible for the anti-HCV replicon effect of **53**, we tested a potent and selective HDAC8 inhibitor, compound **10** (Figure 2) in the HCV replicon assay. Compound **10** failed to show any activity, therefore HDAC8 is also unlikely to be a molecular target of compound **53**. Second, compound **53** was tested against a panel of human matrix

metalloproteinases (MMPs) (Table S1, Supporting Information) because MMP inhibitors generally feature a hydroxamic acid functionality.[25] Compound **53** displayed submicromolar IC_{50} values against MMP9, MMP12 and MMP14, which suggests that inhibition of MMP might contribute to compound **53**'s anti-viral activity. Third, compound **53** exhibited low inhibition of tumor necrosis factor-α converting enzyme (TACE), a zinc-dependent enzyme that can also be blocked by hydroxamic acids.[26] Finally, compound **53** did not inhibit HCV NS3/4A or NS5B, two prominent anti-HCV viral targets that have been aggressively explored.

Our preliminary studies suggest that compound **53** does not target common viral target NS33/4A or NS5. Neither does it inhibit HDACs or TACE, both of which are zinc-dependent enzymes. Nevertheless, we cannot rule out the possibility that compound **53** exerts at least part of its anti-viral activity by blocking selected members of MMPs.

Chemistry. We used several synthetic methods to prepare hydroxamic acids. For the syntheses of cinnamic hydroxamic acids **13-15**, **19** and **22**, the corresponding carboxylic acids **59a-e** were activated with either oxalyl chloride/DMF or 1,1'-carbonyldiimidazole (CDI)[27] followed by treatment with a hydroxylamine solution (Scheme 1). Carboxylic acids **59b**[28] and **59c**,[29] which are not commercially available, were readily obtained from ethyl esters **58b**[28] and **58c**,[29] respectively. For the synthesis of hydroxamic acids **16-18**, **21** and **23**, we adopted another synthetic method, in which carboxylic acids **60a-e** were first converted into methyl esters **61a-e**. Treatment of these methyl esters with hydroxylamine hydrochloride in the presence of methanoic NaOMe afforded the desired hydroxamic acids.

The syntheses of hydroxamic acids **20**, **29-33**, and their pyridine analogs **38-39** began with properly substituted bromides or iodides **62a** and **62c-h** (Scheme 2), which underwent Heck reactions to give *tert*-butyl esters **63a** and **63c-h**. After removal of the *tert*-butyl groups under acidic conditions, the resulting carboxylic acids as well as commercially available acid **63b** were converted into the THP-protected hydroxamates **64a-h**, which upon an acidic treatment gave hydroxamic acids **20**, **29-33** and **38-39**. In addition, hydroxamic acids **34-37** were prepared according to literature procedures.[21b, 24, 30]

The syntheses of fused aromatic hydroxamic acids **40-42** and **45** were accomplished through activation of the corresponding acids **65a-d** and subsequent treatment with a hydroxylamine solution (Scheme 3). To prepare the benzylated hydroxamic acids **44** and **47**, benzylation was performed on carboxylic acids **65c-d** to give compounds **66c-d**,[31] which were then converted into hydroxamic acids **44** and **47**. In contrast to the benzylated counterparts, introduction of a phenyl group was achieved on methyl esters **67a-b** via a copper-catalyzed coupling reaction,[32] resulting in compounds **68a-b**.[33] After hydrolysis, the resulting acids **69a-b**[34] were transformed into hydroxamic acids **43** and **46** (Scheme 4).

As shown in Scheme 5, benzo[*b*]thiophene hydroxamic acids **48-52**, which contain a substituent at either position 5 or 6, were prepared from methyl esters **70a-e** after being treated with hydroxylamine. Benzo[*b*]thiophene analogs that are substituted at position 3 were all derived from chloride **71**, which was first displaced by thiophenol to give sulfide **72**. Oxidations of sulfide **72** to the corresponding sulfoxide **73** and sulfone **56** were accomplished with H_2O_2/TMSCl and oxone, respectively. These three methyl esters were individually converted

into hydroxamic acids **53-55**. In order to confirm the importance of the hydroxamic acid functionality, sulfone **56** was also hydrolyzed to give its carboxylic acid **57**.

CONCLUSIONS

Prompted by the prevalence and importance of protein acetylation in liver cells, we evaluated known HDAC inhibitors and structurally simple hydroxamic acids in human liver cells containing a HCV sub-genomic replicon. HDAC inhibitors, regardless of their selectivity profile, failed to show anti-HCV activity with a desired TI value. Nevertheless, our investigation of simple hydroxamic acids led to those based on the cinnamic acid and benzo[*b*]thiophene core structures. Further SAR studies produced compounds **22** and **53**, which possessed excellent anti-viral activity and selectivity. Our SAR studies also revealed that electron-withdrawing CF_3 and phenylsulfone groups are responsible for the enhanced activity and selectivity of compounds **22** and **53**, respectively, observations that will help guide our design of improved HCV inhibitors.

We also demonstrated that compound **53** reduced replicon RNA as judged by RT-qPCR, supporting our conclusion that it is a *bona fide* inhibitor of HCV replication. Our preliminary investigation showed that compound **53** did not block NS3/4A or NS5B, two of the commonly pursued viral targets. Neither does it appear to exert its anti-HCV activity through inhibiting zinc-dependent enzymes HDACs or TACE. While the exact molecular target of compound **53** remains to be elucidated, inhibition of selected members of MMPs might contribute to its activity. Our work represents the first systematic investigation of known HDAC inhibitors and

novel hydroxamic acids for their potential anti-HCV application. We are currently performing activity-based protein profiling[35] to identify the molecular targets of compounds **22** and **53**.

EXPERIMENTAL SECTION

Chemical Synthesis. All commercial reagents were used as provided unless otherwise indicated. Belinostat (**7**), panobinostat (**8**), entinostat (**9**), **10**, and tubastatin A HCl were obtained from Selleckchem. Compound **12** was purchased from Cellagen Technology. An anhydrous solvent dispensing system (J. C. Meyer) using 2 packed columns of neutral alumina was used for drying THF, Et_2O, and CH_2Cl_2, while 2 packed columns of molecular sieves were used to dry DMF. Solvents were dispensed under argon. Flash chromatography was performed with Ultra Pure silica gel (Silicycle) or with RediSep® R_f silica gel columns on a Teledyne ISCO CombiFlash® R_f system using the solvents as indicated. Nuclear magnetic resonance spectra were recorded on a Varian 600 MHz with Me_4Si or signals from residual solvent as the internal standard for ^1H. Chemical shifts are reported in ppm, and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), brs (broad singlet), and dd (double doublet). Values given for coupling constants are first order. High resolution mass spectra were recorded on an Agilent TOF II TOF/MS instrument equipped with either an ESI or APCI interface. Analysis of sample purity was performed on an Agilent 1200 Infinity series HPLC system with a Phenomenex Gemini C18 column (5 μ, 4.6×250 mm). HPLC conditions were the following: solvent A = water, solvent B = MeCN or MeOH; flow rate = 2.0 mL/min. Compounds were eluted with a gradient of from 10% to 100% MeCN/water or from 10 to 100% MeOH/water in 15 min. Purity was determined by the absorbance at 254 nm. All tested compounds have a purity of ≥ 95%.

***N*-Hydroxycinnamamide (13).**[27] A mixture of cinnamic acid (**59a**, 148 mg, 1.0 mmol) and CDI (243 mg, 1.5 mmol) in THF (2 mL) was stirred at rt for 80 min. $NH_2OH \cdot HCl$ (138 mg, 2.0 mmol) was then added and the reaction mixture was allowed to stir overnight. The solvent was evaporated under vacuum and the resulting residue was purified by flash column chromatography to give hydroxamic acid **13** as a pale solid (46 mg, 28%). ^{1}H NMR (CD_3OD, 600 MHz) δ 7.52-7.57 (m, 3H), 7.34-7.36 (m, 3H), 6.47 (d, J = 9.6 Hz, 1H). HRMS (ESI^-) calcd for $C_9H_8NO_2$ 162.0555 $(M-H)^-$, found 162.0556.

(*E*)-*N*-Hydroxy-3-phenylbut-2-enamide (14). To a solution of carboxylic acid **59b**[28] (131 mg, 0.81 mmol) and two drops of DMF in CH_2Cl_2 (1.5 mL) was added oxalyl chloride (304 mg, 2.4 mmol), and the reaction mixture was allowed to stir at 0 ºC for 1 h. After the solvent being removed under vacuum, the residue was dissolved in Et_2O (1 mL) and the resulting solution was added to a solution of NH_2OH, which was prepared from $NH_2OH \cdot HCl$ (1200 mg, 17.3 mmol) and Na_2CO_3 (1200 mg, 11.3 mmol) in Et_2O (8 mL) and H_2O (1.5 mL). The reaction mixture was allowed to stir at rt overnight and the solvent was evaporated under vacuum. The residue was purified by flash column chromatography to give hydroxamic acid **14** as a pale solid (18 mg, 21%). ^{1}H NMR (CD_3OD, 600 MHz) δ 7.50 (d, J = 7.2 Hz, 2H), 7.34-7.41 (m, 3H), 6.10 (s, 1H), 2.50 (s, 3H). HRMS (ESI^-) calcd for $C_{10}H_{10}NO_2$ 176.0712 $(M-H)^-$, found 176.0718.

***N*-Hydroxy-3,3-diphenylacrylamide (15).**[36] In a manner similar to that was described for the preparation of compound **14**, carboxylic acid **59c**[29] (65 mg, 0.29 mmol) was treated with oxalyl chloride (110 mg, 0.87 mmol) followed by a solution of NH_2OH to give hydroxamic acid **15** as a

pale solid (57 mg, 83%).[1]H NMR (DMSO-d_6, 600 MHz) δ 7.38-7.31 (m, 6H), 7.22-7.18 (m, 2H), 7.16-7.12 (m, 2H), 6.27 (s, 1H). HRMS (ESI^-) calcd for $C_{15}H_{12}NO_2$ 238.0868 $(M-H)^-$, found 238.0867.

(*E*)-*N*-Hydroxy-3-(*p*-tolyl)acrylamide (16).[21b, 37] To a solution of carboxylic acid **60a** (162 mg, 1.0 mmol) in MeOH (4 mL) was added thionyl chloride (179 mg, 1.5 mmol), and the reaction mixture was allowed to stir at rt for 2 h. After the solvent being removed under vacuum, $NH_2OH·HCl$ (278 mg, 4.0 mmol) and MeOH (15 mL) was added. To the above suspension was added 25% wt NaOMe in MeOH (1.65 mL, 7.2 mmol). The reaction mixture was allowed to stir at rt for 20 h and concentrated. The residue was suspended in H_2O (20 mL) and the mixture was acidified with 1N HCl to pH = 8. The solid formed was filtered, washed with H_2O, suction-dried, and dried in vacuo to give hydroxamic acid **16** as a pale solid (115 mg, 65%). [1]H NMR (DMSO-d_6, 600 MHz) δ 7.58-7.52 (m, 3H), 7.22 (d, J = 8.4 Hz, 2H), 6.46 (d, J = 16.2 Hz, 1H), 2.32 (s, 3H). HRMS (ESI^+) calcd for $C_{10}H_{12}NO_2$ $(M+H)^+$ 178.0868, found 178.0866.

(*E*)-*N*-Hydroxy-3-(4-methoxyphenyl)acrylamide (17).[21b, 36-37] In a manner similar to that was described for the preparation of compound **16**, carboxylic acid **60b** (178 mg, 1.0 mmol) was treated with thionyl chloride in MeOH followed by $NH_2OH·HCl$ and 25% wt NaOMe in MeOH to give hydroxamic acid **17** as a pale solid (108 mg, 56%). [1]H NMR (DMSO-d_6, 600 MHz) δ 7.63 (d, J = 9.0 Hz, 2H), 7.54 (d, J = 16.2 Hz, 1H), 6.96 (d, J = 8.4 Hz, 2H), 6.37 (d, J = 16.2 Hz, 1H), 3.79 (s, 3H). HRMS (ESI^+) calcd for $C_{10}H_{12}NO_3$ $(M+H)^+$ 194.0817, found 194.0826.

(*E*)-3-(4-Chlorophenyl)-*N*-hydroxyacrylamide (18).[21b, 36-37] In a manner similar to that was described for the preparation of compound **16**, carboxylic acid **60c** (183 mg, 1.0 mmol) was treated with thionyl chloride in MeOH followed by $NH_2OH \cdot HCl$ and 25% wt NaOMe in MeOH to give hydroxamic acid **18** as a light brown solid (89 mg, 45%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.72 (d, J = 8.4 Hz, 2H), 7.58 (d, J = 15.6 Hz, 2H), 7.47 (d, J = 9.0 Hz, 1H), 6.55 (d, J = 15.6 Hz, 1H). HRMS (ESI$^+$) calcd for $C_9H_9ClNO_2$ $(M+H)^+$ 198.0322, found 198.0322.

(*E*)-*N*-Hydroxy-3-(4-nitrophenyl)acrylamide (19).[21b, 36-37] In a manner similar to that was described for the preparation of compound **13**, carboxylic acid **59d** (193 mg, 1.0 mmol) was treated with CDI (243 mg, 1.5 mmol) and then $NH_2OH \cdot HCl$ (138 mg, 2.0 mmol) to give hydroxamic acid **19** as a yellow solid (146 mg, 70%). ^{1}H NMR (CD_3OD, 600 MHz) δ 8.27 (d, J = 7.2 Hz, 2H), 7.80 (d, J = 7.2 Hz, 2H), 7.66 (d, J = 15.0 Hz, 1H), 6.65 (dd, J = 16.2, 1.8 Hz, 1H). HRMS (ESI$^-$) calcd for $C_9H_7N_2O_4$ 207.0406 $(M-H)^-$, found 207.0405.

(*E*)-*tert*-Butyl 3-(4-(methylsulfonyl)phenyl)acrylate (63a). The reaction vessel was charged with 4-bromophenyl methyl sulfone **62a** (1 mmol), *t*-butyl acrylate (3 mmol), $Pd(OAc)_2$ (5% mmol), Xphos ligand (10% mmol), and NEt_3 (2.0 mmol) in DMF (5 mL). The reaction mixture was heated at 140 ºC for 20 h. The reaction mixture was cooled to rt, diluted with EtOAc (20 mL), and washed with water. The organic phase was dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was purified by silica gel column using EtOAc/hexanes to afford *trans*-cinnamic acid *t*-butyl ester **63a** as a white solid (500 mg, 88%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.95 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 8.4 Hz, 2H), 7.60 (d, J = 16.2 Hz, 1H), 6.49 (d,

J = 16.2 Hz, 1H), 3.07 (s, 3H), 1.55 (s, 9H). HRMS (APCI$^-$) calcd for $C_{10}H_9O_4S$ (M-*t*-Bu)$^-$ 225.0222, found 225.0219.

(*E*)-*tert*-Butyl 3-(2-(trifluoromethyl)phenyl)acrylate (63c).[38] In a manner similar to that was described for the preparation of compound **63a**, iodide **62c** (136 mg, 0.5 mmol) underwent a Heck reaction to give *trans*-cinnamic acid *t*-butyl ester **63c** as a white solid (120 mg, 88%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.97 (d, *J* = 15.0 Hz, 1H), 7.69 (dd, *J* = 6.6, 6.6 Hz, 2H), 7.54 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.45 (dd, *J* = 7.5, 7.5 Hz, 1H), 6.34 (d, *J* = 15.6 Hz, 1H), 1.54 (s, 9H). HRMS (APCI$^-$) calcd for $C_{10}H_6F_3O_2$ (M-*t*-Bu)$^-$ 215.0320, found 215.0322

(*E*)-*tert*-Butyl 3-(2-fluoro-4-(trifluoromethyl)phenyl)acrylate (63d). In a manner similar to that was described for the preparation of compound **63a**, bromide **62d** (243 mg, 1.0 mmol) underwent a Heck reaction to give *trans*-cinnamic acid *t*-butyl ester **63d** as a white solid (170 mg, 59%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.70 (d, *J* = 15.6 Hz, 1H), 7.64 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 10.2 Hz, 1H), 6.54 (d, *J* = 16.2 Hz, 1H), 1.55 (s, 9H). HRMS (APCI$^-$) calcd for $C_{10}H_5F_4O_2$ (M-*t*-Bu)$^-$ 233.0226, found 233.0226.

(*E*)-*tert*-Butyl 3-(2,6-difluoro-4-(trifluoromethyl)phenyl)acrylate (63e). In a manner similar to that was described for the preparation of compound **63a**, bromide **62e** (261 mg, 1.0 mmol) underwent a Heck reaction to give *trans*-cinnamic acid *t*-butyl ester **63e** as a white solid (200 mg, 65%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.64 (d, *J* = 16.2 Hz, 1H), 7.23 (s, 1H), 7.21 (s, 1H), 6.74 (d, *J* = 16.2 Hz, 1H), 1.55 (s, 9H). HRMS (APCI$^-$) calcd for $C_{10}H_4F_5O_2$ (M-*t*-Bu)$^-$ 251.0131, found 251.0127.

(*E*)-*tert*-Butyl 3-(2-chloro-4-(trifluoromethyl)phenyl)acrylate (63f). In a manner similar to that was described for the preparation of compound **63a**, iodide **62f** (153 mg, 0.5 mmol) underwent a Heck reaction to give *trans*-cinnamic acid *t*-butyl ester **63f** as a white solid (100 mg, 65%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.97 (d, *J* = 16.2 Hz, 1H), 7.71 (d, *J* = 8.4 Hz, 1H), 7.68 (s, 1H), 7.52 (d, *J* = 7.8 Hz, 1H), 6.43 (d, *J* = 16.2 Hz, 1H), 1.55 (s, 9H). HRMS (APCI$^-$) calcd for $C_{10}H_5ClF_3O_2$ (M-*t*-Bu)$^-$ 248.9930, found 248.9931.

(*E*)-*tert*-Butyl 3-(5-(trifluoromethyl)pyridin-2-yl)acrylate (63g). In a manner similar to that was described for the preparation of compound **63a**, bromide **62g** (113 mg, 0.5 mmol) underwent a Heck reaction to give *t*-butyl ester **63g** as a white solid (70 mg, 50%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.83 (s, 1H), 7.97 (d, *J* = 7.8 Hz, 1H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.59 (d, *J* = 16.2 Hz, 1H), 6.52 (d, *J* = 16.2 Hz, 1H), 1.55 (s, 9H). HRMS (ESI$^+$) calcd for $C_{13}H_{15}F_3NO_2$ (M+H)$^+$ 274.1055, found 274.1050.

(*E*)-*tert*-Butyl 3-(6-(trifluoromethyl)pyridin-3-yl)acrylate (63h). In a manner similar to that was described for the preparation of compound **63a**, bromide **62h** (261 mg, 1.0 mmol) underwent a Heck reaction to give *t*-butyl ester **63h** as a white solid (150 mg, 55%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.83 (s, 1H), 7.97 (d, *J* = 7.2 Hz, 1H), 7.70 (d, *J* = 8.4 Hz, 1H), 7.59 (d, *J* = 15.6 Hz, 1H), 6.52 (d, *J* = 16.8 Hz, 1H), 1.55 (s, 9H). HRMS (ESI$^+$) calcd for $C_{13}H_{15}F_3NO_2$ (M+H)$^+$ 274.1055, found 274.1057.

(*E*)-*N*-(Tetrahydropyran-2-yloxy)-3-(4-methylsulfonyl)phenyl)acrylamide (64a). A solution of *t*-Butyl ester **63a** (450 mg, 1.59 mmol) in 6 mL of TFA/DCM (2:1) was allowed to stir at rt for 1 h. After removal of the organic solvent, *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (1.0 mmol) and EDC (1.0 mmol) were added. The reaction mixture was allowed to stir at rt overnight and was then washed with water and brine. After removal of the solvent, the residue was purified by silica gel column using EtOAc/hexanes to afford **64a** as a colorless oil (400mg, 70%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.90 (d, *J* = 6.6 Hz, 2H), 7.80-7.60 (m, 3H), 6.65 (d, *J* = 13.8 Hz, 1H), 5.10 (brs, 1H), 4.08-3.98 (m, 1H), 3.68-3.62 (m, 1H), 3.08 (s, 3H), 1.92-1.78 (m, 3H), 1.70-1.54 (m, 3H). HRMS (ESI$^-$) calcd for $C_{15}H_{18}NO_5S$ $(M-H)^-$ 324.0906, found 324.0910.

(*E*)-*N*-(Tetrahydropyran-2-yloxy)-3-(3-(trifluoromethyl)phenyl)acrylamide (64b). A solution of acid **63b** (108 mg, 0.5 mmol), *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (64 mg, 0.55 mmol), and EDC (105 mg, 0.55 mmol) in DCM (10 mL) was allowed to stir at rt overnight and the mixture was washed with water and brine. After removal of the solvent, the residue was purified by silica gel column using EtOAc/hexanes to afford **64b** as a colorless oil (100 mg, 63%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.80-7.72 (m, 2H), 7.67 (d, *J* = 7.2 Hz, 1H), 7.62 (d, *J* = 7.8 Hz, 1H), 7.51 (dd, *J* = 7.5, 7.5 Hz, 1H), 6.58 (d, *J* = 13.8 Hz, 1H), 5.03 (brs, 1H), 3.98 (t, *J* = 9.3 Hz, 1H), 3.70-3.64 (m, 1H), 1.92-1.82 (m, 3H), 1.70-1.58 (m, 3H). HRMS (ESI$^-$) calcd for $C_{15}H_{15}F_3NO_3$ 314.1004 $(M-H)^-$, found 314.1009.

(*E*)-*N*-(Tetrahydropyran-2-yloxy)-3-(2-(trifluoromethyl)phenyl)acrylamide (64c). In a manner similar to that was described for the preparation of compound **64a**, *t*-butyl ester **63c** (120 mg, 0.44 mmol) was converted into the corresponding acid followed by an EDC-mediated

coupling to afford **64c** as a white solid (80 mg, 58%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.73 (brs, 1H), 8.08 (d, *J* = 15.6 Hz, 1H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.66 (d, *J* = 7.2 Hz, 1H), 7.55 (dd, *J* = 7.2, 7.2 Hz, 1H), 7.46 (dd, *J* = 7.5, 7.5 Hz, 1H), 6.50-6.20 (m, 1H), 5.03 (brs, 1H), 4.04-3.94 (m, 1H), 3.70-3.63 (m, 1H), 1.90-1.80 (m, 3H), 1.70-1.56 (m, 3H). HRMS (ESI$^-$) calcd for $C_{15}H_{15}F_3NO_3$ 314.1004 $(M-H)^-$, found 314.1008.

(*E*)-*N*-(Tetrahydropyran-2-yloxy)-3-(2-fluoro-4-(trifluoromethyl)phenyl)acrylamide (64d). In a manner similar to that was described for the preparation of compound **64a**, *t*-butyl ester **63d** (160 mg, 0.55 mmol) was converted into the corresponding acid followed by an EDC-mediated coupling to afford **64d** as a white solid (100 mg, 55%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.59 (brs, 1H), 7.79 (d, *J* = 13.8 Hz, 1H), 7.62 (s, 1H), 7.44-7.30 (m, 2H), 6.72-6.58 (m, 1H), 5.08 (brs, 1H), 4.06-3.96 (m, 1H), 3.70-3.64 (m, 1H), 1.90-1.80 (m, 3H), 1.70-1.58 (m, 3H). HRMS (ESI$^-$) calcd for $C_{15}H_{14}F_4NO_3$ 332.0910 $(M-H)^-$, found 332.0916.

(*E*)-*N*-(Tetrahydropyran-2-yloxy)-3-(2,6-difluoro-4-(trifluoromethyl)phenyl)acrylamide (64e). In a manner similar to that was described for the preparation of compound **64a**, *t*-butyl ester **63e** (170 mg, 0.55 mmol) was converted into the corresponding acid followed by an EDC-mediated coupling to afford **64e** as a colorless oil (110 mg, 57%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.89 (brs, 1H), 7.80 (d, *J* = 15.6 Hz, 1H), 7.22 (s, 1H), 7.21 (s, 1H), 6.82-6.70 (m, 1H), 5.05 (brs, 1H), 4.04-3.92 (m, 1H), 3.70-3.60 (m, 1H), 1.94-1.76 (m, 3H), 1.70-1.58 (m, 3H). HRMS (ESI$^-$) calcd for $C_{15}H_{13}F_5NO_3$ 350.0816 $(M-H)^-$, found 350.0813.

(*E*)-*N*-(Tetrahydropyran-2-yloxy)-3-(2-chloro-4-(trifluoromethyl)phenyl)acrylamide (64f). In a manner similar to that was described for the preparation of compound **64a**, *t*-butyl ester **63f** (60 mg, 0.22 mmol) was converted into the corresponding acid followed by an EDC-mediated coupling to afford **64f** as a white solid (45 mg, 65%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.05 (brs, 1H), 8.07 (d, *J* = 14.4 Hz, 1H), 7.70-7.60 (m, 2H), 7.52-7.46 (m, 1H), 6.57-6.40 (m, 1H), 5.05 (brs, 1H), 4.4-3.95 (m, 1H), 3.70-3.64 (m, 1H), 1.92-1.79 (m, 3H), 1.70-1.58 (m, 3H). HRMS (ESI$^-$) calcd for $C_{15}H_{14}ClF_3NO_3$ 348.0614 $(M-H)^-$, found 348.0609.

(*E*)-*N*-(Tetrahydropyran-2-yloxy)-3-(5-(trifluoromethyl)pyridin-2-yl)acrylamide (64g). In a manner similar to that was described for the preparation of compound **64a**, *t*-butyl ester **63g** (70 mg, 0.26 mmol) was converted into the corresponding acid followed by an EDC-mediated coupling to afford **64g** as a colorless oil (40 mg, 48%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.86 (s, 1H), 8.58 (brs, 1H), 7.05 (d, *J* = 7.8 Hz, 1H), 7.74 (d, *J* = 14.4 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.14-7.00 (m, 1H), 5.05 (brs, 1H), 4.02-3.94 (m, 1H), 3.72-3.65 (m, 1H), 1.90-1.81 (m, 3H), 1.70-1.58 (m, 3H). HRMS (ESI$^+$) calcd for $C_{14}H_{16}F_3N_2O_3$ 317.1113 $(M+H)^+$, found 317.1106.

(*E*)-*N*-(Tetrahydropyran-2-yloxy)-3-(6-(trifluoromethyl)pyridin-3-yl)acrylamide (64h). In a manner similar to that was described for the preparation of compound **64a**, *t*-butyl ester **63h** (150 mg, 0.55 mmol) was converted into the corresponding acid followed by an EDC-mediated coupling to afford **64h** as a colorless oil (90 mg, 52%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.86 (s, 1H), 8.02-7.92 (m, 1H), 7.76 (d, *J* = 16.2 Hz, 1H), 7.71 (d, *J* = 8.4 Hz, 1H), 6.62-6.57 (m, 1H), 5.04 (brs, 1H), 4.02-3.94 (m, 1H), 3.72-3.64 (m, 1H), 1.90-1.81 (m, 3H), 1.70-1.58 (m, 3H). HRMS (ESI$^+$) calcd for $C_{14}H_{16}F_3N_2O_3$ 317.1113 $(M+H)^+$, found 317.1117.

(*E*)-*N*-Hydroxy-3-(4-(methylsulfonyl)phenyl)acrylamide (20). To a solution of **64a** (300 mg, 0.92 mmol) in DCM (5 mL) was added HCl in Et_2O (3 mL, 2M). The reaction mixture was stirred at rt for 2 h. The white solid precipitate was filtrated and washed with DCM to give hydroxamic acid **20** as white solid (86.2 mg, 39%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.94 (d, J = 7.2 Hz, 2H), 7.81 (d, J = 7.8 Hz, 2H), 7.53 (d, J = 15.6 Hz, 1H), 6.66 (d, J = 15.6 Hz, 1H), 3.23 (s, 3H). HRMS (ESI$^+$) calcd for $C_{10}H_{12}NO_4S$ $(M+H)^+$ 242.0487, found 242.0481.

(*E*)-3-(4-Cyanophenyl)-*N*-hydroxyacrylamide (21).[21b] In a manner similar to that was described for the preparation of compound **16**, carboxylic acid **60d** (100 mg, 0.58 mmol) was treated with thionyl chloride in MeOH followed by $NH_2OH{\cdot}HCl$ and 25% wt NaOMe in MeOH to give hydroxamic acid **21** as a white solid (43 mg, 40%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.86 (d, J = 8.4 Hz, 2H), 7.50 (d, J = 7.8 Hz, 2H), 7.50 (d, J = 16.2 Hz, 1H), 6.63 (d, J = 16.2 Hz, 1H). HRMS (ESI$^-$) calcd for $C_{10}H_7N_2O_2$ $(M-H)^-$ 187.0508, found 187.0501.

(*E*)-*N*-Hydroxy-3-(4-trifluoromethylphenyl)acrylamide (22).[37] In a manner similar to that was described for the preparation of compound **13**, carboxylic acid **59e** (216 mg, 1.0 mmol) was treated with CDI (243 mg, 1.5 mmol) and then $NH_2OH{\cdot}HCl$ (138 mg, 2.0 mmol) to give hydroxamic acid **22** as a white solid (68 mg, 29%). ^{1}H NMR (CD_3OD, 600 MHz) δ 7.75 (d, J = 8.4 Hz, 2H), 7.70 (d, J = 8.4 Hz, 2H), 7.63 (d, J = 15.6 Hz, 1H), 6.60 (d, J = 15.6 Hz, 1H). HRMS (ESI$^-$) calcd for $C_{10}H_7F_3NO_2$ 230.0429 $(M-H)^-$, found 230.0428.

***N*-Hydroxy-3-(4-(trifluoromethyl)phenyl)propanamide (23).** In a manner similar to that was described for the preparation of compound **16**, carboxylic acid **60e** (109 mg, 0.50 mmol) was treated with thionyl chloride in MeOH followed by $NH_2OH \cdot HCl$ and 25% wt NaOMe in MeOH to give hydroxamic acid **23** as a white solid (17 mg, 15%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.63 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 7.8 Hz, 2H), 3.31 (brs, 2H), 2.90 (t, J = 7.8 Hz, 2H), 2.57 (t, J = 8.1 Hz, 2H). HRMS (ESI$^-$) calcd for $C_{10}H_9F_3NO_2$ $(M-H)^-$ 232.0585, found 232.0581.

(*E*)-*N*-Hydroxy-3-(3-(trifluoromethyl)phenyl)acrylamide (29). In a manner similar to that was described for the preparation of compound **20**, a solution of compound **64b** (50 mg, 0.16 mmol) in DCM (1 mL) was treated with HCl in Et_2O (1 mL, 2M) for 2 h to give hydroxamic acid **29** as a white solid (23 mg, 63%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.79 (s, 1H), 9.12 (brs, 1H), 7.92 (s, 1H), 7.88 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 8.4 Hz, 1H), 7.65 (dd, J = 8.1, 8.1 Hz, 1H), 7.55 (d, J = 16.2 Hz, 1H), 6.60 (d, J = 15.6 Hz, 1H). HRMS (ESI$^+$) calcd for $C_{10}H_7F_3NO_2$ $(M+H)^+$ 230.0429, found 230.0424.

(*E*)-*N*-Hydroxy-3-(2-(trifluoromethyl)phenyl)acrylamide (30).). In a manner similar to that was described for the preparation of compound **20**, a solution of compound **64c** (60 mg, 0.19 mmol) in DCM (1 mL) was treated with HCl in Et_2O (1 mL, 2M) for 2 h to give hydroxamic acid **30** as a white solid (40 mg, 91%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.91 (s, 1H), 9.16 (s, 1H), 7.83-7.77 (m, 2H), 7.76-7.56 (m, 2H), 7.59 (dd, J = 7.5, 7.5 Hz, 1H), 6.52 (d, J = 15.0 Hz, 1H). HRMS (ESI$^-$) calcd for $C_{10}H_7F_3NO_2$ $(M-H)^-$ 230.0429, found 230.0426.

(*E*)-3-(2-Fluoro-4-(trifluoromethyl)phenyl)-*N*-hydroxyacrylamide (31). In a manner similar to that was described for the preparation of compound **20**, a solution of compound **64d** (60 mg, 0.18 mmol) in DCM (1 mL) was treated with HCl in Et_2O (1 mL, 2M) for 2 h to give hydroxamic acid **31** as a white solid (12 mg, 27%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.89 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.77 (d, *J* = 11.4 Hz, 1H), 7.64 (d, *J* = 8.4 Hz, 1H), 7.52 (d, *J* = 15.6 Hz, 1H), 6.69 (d, *J* = 15.6 Hz, 1H). HRMS (ESI$^+$) calcd for $C_{10}H_6F_4NO_2$ $(M+H)^+$ 248.0335, found 248.0338.

(*E*)-3-(2,6-Difluoro-4-(trifluoromethyl)phenyl)-*N*-hydroxyacrylamide (32). In a manner similar to that was described for the preparation of compound **20**, a solution of compound **64e** (60 mg, 0.17 mmol) in DCM (1 mL) was treated with HCl in Et_2O (1 mL, 2M) for 2 h to give hydroxamic acid **32** as a white solid (13 mg, 29%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 11.05 (s, 1H), 9.27 (s, 1H), 7.74 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 16.2 Hz, 1H), 6.80 (d, *J* = 16.2 Hz, 1H). HRMS (ESI$^+$) calcd for $C_{10}H_5F_5NO_2$ $(M+H)^+$ 266.0240, found 266.0246.

(*E*)-3-(2-Chloro-4-(trifluoromethyl)phenyl)-*N*-hydroxyacrylamide (33). In a manner similar to that was described for the preparation of compound **20**, a solution of compound **64f** (50 mg, 0.14 mmol) in DCM (1 mL) was treated with HCl in Et_2O (1 mL, 2M) for 2 h to give hydroxamic acid **31** as a white solid (33 mg, 90%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.95 (s, 1H), 7.92 (d, *J* = 8.4 Hz, 1H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.72 (d, *J* = 15.6 Hz, 1H), 6.64 (d, *J* = 16.2 Hz, 1H). HRMS (ESI$^-$) calcd for $C_{10}H_7ClF_3NO_2$ $(M-H)^-$ 264.0039, found 264.0032.

(*E*)-*N*-Hydroxy-3-(5-(trifluoromethyl)pyridin-2-yl)acrylamide hydrochloride (38). In a manner similar to that was described for the preparation of compound **20**, a solution of compound **64g** (40 mg, 0.13 mmol) in DCM (1 mL) was treated with HCl in Et_2O (1 mL, 2M) for 2 h to give hydroxamic acid **38** as a white solid (6 mg, 18%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.98 (s, 1H), 8.26 (d, J = 7.8 Hz, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.58 (d, J = 15.0 Hz, 1H), 7.07 (d, J = 15.0 Hz, 1H). HRMS (ESI$^-$) calcd for $C_9H_6F_3N_2O_2$ $(M-H)^-$ 231.0381, found 231.0389.

(*E*)-*N*-Hydroxy-3-(6-(trifluoromethyl)pyridin-3-yl)acrylamide hydrochloride (39). In a manner similar to that was described for the preparation of compound **20**, a solution of compound **64h** (45 mg, 0.14 mmol) in DCM (1 mL) was treated with HCl in Et_2O (1 mL, 2M) for 2 h to give hydroxamic acid **39** as white solid (8 mg, 22%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.93 (s, 1H), 9.19 (brs, 1H), 8.96 (s, 1H), 8.26 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 7.8 Hz, 1H), 7.58 (d, J = 16.2 Hz, 1H), 6.71 (d, J = 15.6 Hz, 1H). HRMS (ESI$^-$) calcd for $C_9H_6F_3N_2O_2$ $(M-H)^-$ 231.0381, found 231.0391.

***N*-Hydroxybenzofuran-2-carboxamide (40).** In a manner similar to that was described for the preparation of compound **13**, carboxylic acid **65a** (100 mg, 0.62 mmol) was treated with CDI (150 mg, 0.93 mmol) and then $NH_2OH{\cdot}HCl$ (85 mg, 1.2 mmol) to give hydroxamic acid **40** as a pale solid (71 mg, 65%). ^{1}H NMR (CD_3OD, 600 MHz) δ 7.71 (d, J = 7.8 Hz, 1H), 7.58 (d, J = 7.8 Hz, 1H), 7.44-7.47 (m, 2H), 7.32 (t, J = 7.2 Hz, 1H). HRMS (ESI$^-$) calcd for $C_9H_6NO_3$ 176.0348 $(M-H)^-$, found 176.0348.

***N*-Hydroxybenzo[*b*]thiophene-2-carboxamide (41).**[39] In a manner similar to that was described for the preparation of compound **13**, carboxylic acid **65b** (100 mg, 0.56 mmol) was treated with CDI (137 mg, 0.84 mmol) and then $NH_2OH \cdot HCl$ (78 mg, 1.1 mmol) to give hydroxamic acid **41** as a pale solid (63 mg, 58%). ^{1}H NMR (CD_3OD, 600 MHz) δ 7.93 (d, J = 8.4 Hz, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.85 (s, 1H), 7.42-7.47 (m, 2H). HRMS (ESI^-) calcd for $C_9H_6NO_2S$ 192.0119 $(M-H)^-$, found 192.0120.

***N*-Hydroxy-1*H*-indole-2-carboxamide (42).**[40] In a manner similar to that was described for the preparation of compound **14**, carboxylic acid **65c** (322 mg, 2.0 mmol) was treated with oxalyl chloride (279 mg, 2.2 mmol) followed by a solution of NH_2OH to give hydroxamic acid **42** as a tan solid (184 mg, 52%). ^{1}H NMR (CD_3OD, 600 MHz) δ 7.57-7.61 (m, 1H), 7.43-7.46 (m, 1H), 7.19-7.23 (m, 1H), 7.04-7.08 (m, 1H), 6.97 (s, 1H). HRMS (ESI^-) calcd for $C_9H_7N_2O_2$ 175.0508 $(M-H)^-$, found 175.0511.

***N*-Hydroxy-1*H*-indole-3-carboxamide (45).**[41] In a manner similar to that was described for the preparation of compound **13**, carboxylic acid **65d** (161 mg, 1.0 mmol) was treated with CDI (243 mg, 1.5 mmol) and then $NH_2OH \cdot HCl$ (138 mg, 2.0 mmol) to give hydroxamic acid **45** as a tan solid (154 mg, 88%). ^{1}H NMR (CD_3OD, 600 MHz) δ 8.05 (d, J = 7.8 Hz, 1H), 7.81 (s, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.19 (t, J = 7.2 Hz, 1H), 7.15 (t, J = 7.2 Hz, 1H). HRMS (ESI^-) calcd for $C_9H_7N_2O_2$ 175.0508 $(M-H)^-$, found 175.0505.

1-Benzyl-1*H*-indole-2-carboxylic acid (66c).[31a] A mixture of indole-2-carboxylic acid (**65c**, 805 mg, 5.0 mmol) and NaH (800 mg, 60% dispersion, 8.0 mmol) in DMF (24 mL) at was stirred at

0 ºC for 1h, and benzyl bromide (940 mg, 5.5 mmol) was added. The reaction mixture was allowed to stir at rt overnight, and a solution of 10% NH_4Cl (5mL) and H_2O (10 mL) was carefully added. The resulting mixture was extracted with EtOAc (15×3 mL) and the combined organic layer was washed with brine and evaporated under vacuum. The residue was purified by flash column chromatography to give carboxylic acid **66c** as a yellowish solid (176 mg, 17%). ^{1}H NMR (CD_3OD, 600 MHz) δ 7.70 (d, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 1H), 7.39 (s, 1H), 7.29 (t, *J* = 7.8 Hz, 1H), 7.24 (t, *J* = 6.6 Hz, 2H), 7.19 (q, *J* = 7.2 Hz, 1H), 7.14 (t, *J* = 7.8 Hz, 1H), 7.04 (d, *J* = 7.8 Hz, 2H), 5.91 (s, 2H). HRMS (ESI$^-$) calcd for $C_{16}H_{12}NO_2$ 250.0868 $(M-H)^-$, found 250.0865.

1-Benzyl-1*H*-indole-3-carboxylic acid (66d).[31b] In a manner similar to that was described for the preparation of compound **66c**, indole-3-carboxylic acid (**65d**, 895 mg, 5.56 mmol) was alkylated to give carboxylic acid **66d** as a pinkish solid (1.07 g, 76%). ^{1}H NMR (CD_3OD, 600 MHz) δ 8.11-8.13 (m, 1H), 8.04 (s, 1H), 7.40-7.42 (m, 1H), 7.34 (t, *J* = 7.2 Hz, 2H), 7.29 (t, *J* =7.2 Hz, 1H), 7.21-7.23 (m, 4H), 5.46 (s, 2H). HRMS (ESI$^-$) calcd for $C_{16}H_{12}NO_2$ 250.0868 $(M-H)^-$, found 250.0861.

1-Benzyl-*N*-hydroxy-1*H*-indole-2-carboxamide (44). In a manner similar to that was described for the preparation of compound **14**, carboxylic acid **66c** (100 mg, 0.40 mmol) was treated with oxalyl chloride (56 mg, 0.44 mmol) followed by a solution of NH_2OH to give hydroxamic acid **44** as a pale solid (36 mg, 34%). ^{1}H NMR (CD_3OD, 600 MHz) δ 7.64 (d, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 8.4 Hz, 1H), 7.21-7.25 (m, 3H), 7.17-7.19 (t, *J* = 7.2 Hz, 1H), 7.12 (t, *J* = 7.2 Hz, 1H),

7.06 (d, J = 7.8 Hz, 2H), 6.99 (s,1H), 5.82 (s, 2H). HRMS (ESI$^-$) calcd for $C_{16}H_{13}N_2O_2$ 265.0977 $(M-H)^-$, found 265.0975.

1-Benzyl-*N*-hydroxy-1*H*-indole-3-carboxamide (47). In a manner similar to that was described for the preparation of compound **14**, carboxylic acid **66d** (261 mg, 1.0 mmol) was treated with oxalyl chloride (150 mg, 1.1 mmol) followed by a solution of NH_2OH to give hydroxamic acid **47** as a pale solid (112 mg, 40%). ^{1}H NMR (CD_3OD, 600 MHz) δ 9.89 (s, 1H), 8.20-8.22 (m, 1H), 8.15 (s, 1H), 7.43-7.44 (m, 1H), 7.33 (t, J = 7.2 Hz, 2H), 7.26-7.30 (m, 3H), 7.24 (d, J = 7.2 Hz, 2H), 5.46 (s, 2H). HRMS (ESI$^+$) calcd for $C_{16}H_{15}N_2O_2$ 267.1134 $(M+H)^+$, found 267.1133.

Methyl 1-phenyl-1*H*-indole-2-carboxylate (68a).[33a] A mixture of methyl ester **67a** (70 mg, 0.40 mmol), bromobenzene (75 mg, 0.48 mmol), CuI (3.8 mg, 0.020 mmol), *trans-N,N'*-dimethylcyclohexane-1,2-diamine (11.2 mg, 0.08 mmol), and K_3PO_4 (178 mg, 0.84 mmol) in toluene (2 mL) in a sealed tube was heated at 120 °C for 22 h. The solvent was evaporated under vacuum and the residue was purified by flash column chromatography to give compound **68a** as a white solid (89 mg, 89%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.73 (d, J = 7.8 Hz, 1H), 7.51-7.53 (m, 2H), 7.46-7.48 (m, 1H), 7.44 (s, 1H), 7.34 (d, J = 7.8 Hz, 2H), 7.25-7.28 (m, 1H), 7.19 (t, J = 6.6 Hz, 1H), 7.10 (d, J = 7.8 Hz, 1H), 3.78 (s, 3H). HRMS (ESI$^+$) calcd for $C_{16}H_{14}NO_2$ 252.1025 $(M+H)^+$, found 252.1022.

Methyl 1-phenyl-1*H*-indole-3-carboxylate (68b).[33b] In a manner similar to that was described for the preparation of compound **68a**, methyl ester **67b** (700 mg, 4.0 mmol) and bromobenzene (753 mg, 4.8 mmol) underwent a C-N coupling to give compound **68b** as a yellowish syrup (202

mg, 20%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.26 (d, *J* = 7.8 Hz, 1H), 8.02 (s, 1H), 7.54-7.56 (m, 2H), 7.49-7.51 (m, 3H), 7.44 (t, *J* = 7.2 Hz, 1H), 7.32 (t, *J* = 7.2 Hz, 1H), 7.28 (t, *J* = 7.2 Hz, 1H), 3.94 (s, 3H). HRMS (ESI^+) calcd for $C_{16}H_{14}NO_2$ 252.1025 $(M+H)^+$, found 252.1029.

1-Phenyl-1*H*-indole-2-carboxylic acid (69a).[34] A solution of methyl ester **68a** (175 mg, 0.70 mmol) in 2N NaOH (5.0 mL), MeOH (2.5 mL) and THF (2.5 mL) was allowed to stir overnight, and the organic solvents were removed under vacuum. The residue was extracted with EtOAc and the organic layer was concentrated. The resulting residue was purified by flash column chromatography to give carboxylic acid **69a** as a pale solid (139 mg, 84%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.73 (d, *J* = 8.4 Hz, 1H), 7.55 (s, 1H), 7.51 (t, *J* = 7.2 Hz, 2H), 7.47 (t, *J* = 7.2 Hz, 1H), 7.33 (d, *J* = 7.2 Hz, 2H), 7.29 (t, *J* = 7.8Hz, 1H), 7.19 (t, *J* = 7.8 Hz, 1H), 7.09 (d, *J* = 8.4 Hz, 1H). HRMS (ESI^-) calcd for $C_{15}H_{10}NO_2$ 236.0712$(M-H)^-$, found 236.0713.

1-Phenyl-1*H*-indole-3-carboxylic acid (69b). In a manner similar to that was described for the preparation of compound **69a**, methyl ester **68b** (257 mg, 1.0 mmol) was hydrolyzed to give carboxylic acid **69b** as a white solid (155 mg, 64%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.33 (d, *J* = 7.8 Hz, 1H), 8.14 (s, 1H), 7.56-7.59 (m, 2H), 7.51-7.54 (m, 3H), 7.47 (t, *J* = 7.2 Hz, 1H), 7.36 (t, *J* = 7.8 Hz, 1H), 7.31 (t, *J* = 7.8 Hz, 1H). HRMS (ESI^-) calcd for $C_{15}H_{10}NO_2$ 236.0712 $(M-H)^-$, found 236.0711.

***N*-Hydroxy-1-phenyl-1*H*-indole-2-carboxamide (43).** In a manner similar to that was described for the preparation of compound **14**, carboxylic acid **69a** (40 mg, 0.17 mmol) was treated with oxalyl chloride (145 mg, 1.1 mmol) followed by a solution of NH_2OH to give

hydroxamic acid **43** as a pale solid (34 mg, 81%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.66 (d, *J* = 7.8 Hz, 1H), 7.34-7.42 (m, 3H), 7.30 (d, *J* = 7.2 Hz, 2H), 7.25 (d, *J* = 7.8 Hz, 1H), 7.19 (t, *J* = 7.2 Hz, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 7.06 (s, 1H). HRMS (ESI$^-$) calcd for $C_{15}H_{11}N_2O_2$ 251.0821 $(M-H)^-$, found 251.0823.

***N*-Hydroxy-1-phenyl-1*H*-indole-3-carboxamide (46).** In a manner similar to that was described for the preparation of compound **14**, carboxylic acid **69b** (47 mg, 0.20 mmol) was treated with oxalyl chloride (76 mg, 0.60 mmol) followed by a solution of NH_2OH to give hydroxamic acid **46** as a tan solid (28 mg, 56%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.18 (d, *J* = 7.2 Hz, 1H), 7.99 (s, 1H), 7.62-7.64 (m, 2H), 7.60 (d, *J* = 5.2 Hz, 2H), 7.52 (t, *J* = 9.0 Hz, 2H), 7.24-7.32 (m, 2H). HRMS (ESI$^-$) calcd for $C_{15}H_{11}N_2O_2$ 251.0821 $(M-H)^-$, found 251.0816.

5-Chloro-*N*-hydroxybenzo[*b*]thiophene-2-carboxamide (48). To a suspension of methyl ester **70a** (227 mg, 1.00 mmol) in anhydrous MeOH (15 mL) were added $NH_2OH{\cdot}HCl$ (265 mg, 3.81 mmol) and then 25% wt NaOMe in MeOH (1.60 mL, 6.99 mmol). The reaction mixture was allowed to stir at rt for 20 h and concentrated. The residue was suspended in H_2O (20 mL) and the mixture was acidified with 1N HCl to pH = 8. The solid formed was filtered, washed with H_2O, suction-dried, and dried in vacuo to give hydroxamic acid **48** as a pale solid (173 mg, 76%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.19 (brs, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.99 (s, 1H), 7.80 (s, 1H), 7.43 (d, *J* = 7.2 Hz, 1H). HRMS (ESI$^-$) calcd for $C_9H_5ClNO_2S$ $(M-H)^-$ 225.9730, found 225.9735.

***N*-Hydroxy-5-nitrobenzo[*b*]thiophene-2-carboxamide (49).** In a manner similar to that was described for the preparation of compound **48**, methyl ester **70b** (237 mg, 1.00 mmol) was treated with $NH_2OH \cdot HCl$ (264 mg, 3.80 mmol) and then NaOMe solution (1.60 mL, 6.99 mmol) to give hydroxamic acid **49** as a yellow solid (220 mg, 92%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 11.60 (brs, 1H), 9.44 (brs, 1H), 8.89 (s, 1H), 8.31 (d, J = 9.6 Hz, 1H), 8.24 (dd, J = 9.6, 1.8 Hz, 1H), 8.14 (s, 1H). HRMS (ESI$^-$) calcd for $C_9H_5N_2O_4S$ $(M-H)^-$ 236.9970, found 236.9968.

***N*-Hydroxy-6-nitrobenzo[*b*]thiophene-2-carboxamide (50).** In a manner similar to that was described for the preparation of compound **48**, methyl ester **70c** (237 mg, 1.00 mmol) was treated with $NH_2OH \cdot HCl$ (266 mg, 3.83 mmol) and then NaOMe solution (1.60 mL, 6.99 mmol) to give hydroxamic acid **50** as a tan solid (137 mg, 58%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.08 (s, 1H), 8.21 (d, J = 7.8 Hz, 1H), 8.13 (d, J = 9.0 Hz, 1H), 8.00 (s, 1H). HRMS (ESI$^-$) calcd for $C_9H_5N_2O_4S$ $(M-H)^-$ 236.9970, found 236.9977.

***N*-Hydroxy-5-(trifluoromethyl)benzo[*b*]thiophene-2-carboxamide (51).** In a manner similar to that was described for the preparation of compound **48**, methyl ester **70d** (130 mg, 0.5 mmol) was treated with $NH_2OH \cdot HCl$ (140 mg, 2.0 mmol) and then NaOMe solution (0.84 mL, 3.6 mmol) to give hydroxamic acid **51** as a white solid (40 mg, 31%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 11.64 (s, 1H), 9.36 (s, 1H), 8.39 (s, 1H), 8.29 (d, J = 8.4 Hz, 1H), 8.06 (s, 1H), 7.75 (d, J = 9.0 Hz, 1H). HRMS (ESI$^-$) calcd for $C_{10}H_5F_3NO_2S$ $(M-H)^-$ 259.9993, found 259.9998.

***N*-Hydroxy-6-(trifluoromethyl)benzo[*b*]thiophene-2-carboxamide (52).** In a manner similar to that was described for the preparation of compound **48**, methyl ester **70e** (130 mg, 0.5 mmol)

was treated with $NH_2OH \cdot HCl$ (140 mg, 2.0 mmol) and then NaOMe solution (0.84 mL, 3.6 mmol) to give hydroxamic acid **52** as a white solid (55 mg,42%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.60 (s, 1H), 8.24-8.19 (m, 2H), 7.76 (d, J = 8.4 Hz, 1H). HRMS (ESI$^-$) calcd for $C_{10}H_5F_3NO_2S$ $(M-H)^-$ 259.9993, found 259.9996.

Methyl 3-(phenylthio)benzo[*b*]thiophene-2-carboxylate (72). A mixture of chloride **71** (679 mg, 3.00 mmol), K_2CO_3 (839 mg, 6.07 mmol) and thiophenol (0.37 mL, 3.6 mmol) in DMF (20 mL) was heated at 80 ºC for 6 h. After cooling to rt, the reaction mixture was poured into ice-cold water (250 mL) and the mixture was extracted with EtOAc (200 mL). The organic layer was washed with H_2O (100 mL), brine (100 mL) and dried over Na_2SO_4. After filtration, the filtrate was concentrated and the residue was purified by flash column chromatography to give compound **72** as a white solid (247 mg, 27%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.14 (d, J = 8.4 Hz, 1H), 7.73 (d, J = 8.4 Hz, 1H), 7.57 (dd, J = 7.5, 7.5 Hz, 1H), 7.43 (dd, J = 7.5, 7.5 Hz, 1H), 7.29-7.25 (m, 2H), 7.21-7.15 (m, 3H), 3.87 (s, 3H). HRMS (ESI$^+$) calcd for $C_{16}H_{13}O_2S_2$ $(M+H)^+$ 301.0357, found 301.0362.

Methyl 3-(phenylsulfonyl)benzo[*b*]thiophene-2-carboxylate (56). A mixture of sulfide **72** (187 mg, 0.622 mmol), Oxone (969 mg, 1.58 mmol) and $NaHCO_3$ (135 mg, 1.61 mmol) in acetone (15 mL) and H_2O (15 mL) was allowed to stir at rt for 20 h. After the organic solvent was removed, the mixture was poured into H_2O (40 mL). The solid was filtered, washed with H_2O, suction-dried, and dried in vacuo to give sulfone **56** as a white solid (169 mg, 82%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.78 (d, J = 7.8 Hz, 1H), 7.90 (d, J = 7.2 Hz, 2H), 7.83 (d, J = 8.4

Hz, 1H), 7.50-7.42 (m, 3H), 7.42-7.36 (m, 2H), 4.02 (s, 3H). HRMS (ESI^+) calcd for $C_{16}H_{13}O_4S_2$ $(M+H)^+$ 333.0255, found 333.0254.

3-(Phenylsulfonyl)benzo[*b*]thiophene-2-carboxylic acid (57). A solution of methyl ester **56** (34 mg, 0.10 mmol) in 1N NaOH (1.0 mL), MeOH (0.5 mL) and THF (0.5 mL) was allowed to stir overnight, and the organic solvents were removed under vacuum. The aqueous solution was acidified with 1N HCl to pH = 2. The white precipitate was collected, washed with H_2O, suction-dried, and dried in vacuo to give carboxylic acid **57** as a white solid (27 mg, 83%). ^1H NMR (CD_3OD, 600 MHz) δ 8.42 (d, *J* = 8.4 Hz, 1H), 7.91 (d, *J* = 7.8 Hz, 2H), 7.83 (d, *J* = 7.2 Hz, 1H), 7.46 (dd, *J* = 7.5, 7.5 Hz, 2H), 7.40 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.36 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.27 (dd, *J* = 7.8, 7.8 Hz, 1H). HRMS (ESI^-) calcd for $C_{15}H_9O_4S_2$ $(M-H)^-$ 316.9942, found 316.9948.

Methyl 3-(phenylsulfinyl)benzo[*b*]thiophene-2-carboxylate (73). To a solution of methyl ester **72** (265 mg, 0.88 mmol) in CH_3CN (10 mL) was added 30% H_2O_2 (0.18 mL, 1.76 mmol) and then TMSCl (0.11 mL, 0.88 mmol) dropwise. The reaction mixture was stirred for 5 min and the organic solvents were removed under vacuum. The resulting residue was purified by flash column chromatography to give **73** as a white solid (230 mg, 83%). ^1H NMR (DMSO-d_6, 600 MHz) δ 8.59 (d, *J* = 8.4 Hz, 1H), 8.14 (d, *J* = 8.4 Hz, 1H), 7.83 (d, *J* = 7.8 Hz, 2H), 7.58-7.52 (m, 3H), 7.50-7.43 (m, 2H), 3.98 (s, 3H). HRMS (ESI^+) calcd for $C_{16}H_{13}O_3S_2$ $(M+H)^+$ 317.0306, found 317.0304.

***N*-Hydroxy-3-(phenylsulfonyl)benzo[*b*]thiophene-2-carboxamide (53).** In a manner similar to that was described for the preparation of compound **48**, methyl ester **56** (101 mg, 0.304 mmol) was treated with $NH_2OH \cdot HCl$ (82 mg, 1.2 mmol) and then NaOMe solution (0.50 mL, 2.2 mmol) to give hydroxamic acid **53** as a pale solid (30 mg, 30%). ^{1}H NMR (CD_3OD, 600 MHz) δ 8.33 (d, *J* = 8.4 Hz, 1H), 7.96 (d, *J* = 8.4 Hz, 1H), 7.88 (d, *J* = 7.2 Hz, 2H), 7.51 (dd, *J* = 7.5, 7.5 Hz, 2H), 7.50-7.45 (m, 2H), 7.38 (dd, *J* = 7.5, 7.5 Hz, 1H). HRMS (ESI$^-$) calcd for $C_{15}H_{10}NO_4S_2$ $(M-H)^-$ 332.0051, found 332.0060

***N*-Hydroxy-3-(phenylsulfinyl)benzo[*b*]thiophene-2-carboxamide (54).** In a manner similar to that was described for the preparation of compound **48**, methyl ester **73** (160 mg, 0.51 mmol) was treated with $NH_2OH \cdot HCl$ (140 mg, 2.02 mmol) and then NaOMe solution (0.83 mL, 3.64 mmol) to give hydroxamic acid **54** as a light yellow solid (59 mg, 37%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 11.77 (brs, 1H), 9.67 (brs, 1H), 8.29 (d, *J* = 8.4 Hz, 1H), 8.11 (d, *J* = 7.8 Hz, 1H), 7.87 (d, *J* = 7.8 Hz, 2H), 7.54 (dd, *J* = 7.5, 7.5 Hz, 2H), 7.47 (dd, *J* = 7.8, 15 Hz, 2H), 7.39 (dd, *J* = 7.5, 7.5 Hz, 1H). HRMS (ESI$^+$) calcd for $C_{15}H_{12}NO_3S_2$ $(M+H)^+$ 318.0259, found 318.0256.

***N*-Hydroxy-3-(phenylthio)benzo[*b*]thiophene-2-carboxamide (55).** In a manner similar to that was described for the preparation of compound **48**, methyl ester **72** (156 mg, 0.52 mmol) was treated with $NH_2OH \cdot HCl$ (145 mg, 2.08 mmol) and then NaOMe solution (0.86 mL, 3.74 mmol) to give hydroxamic acid **55** as a light orange solid (37 mg, 24%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 11.27 (brs, 1H), 9.51 (brs, 1H), 8.11 (d, *J* = 8.4 Hz, 1H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.49 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.42 (dd, *J* = 7.2, 7.2 Hz, 1H), 7.27-7.22 (m, 2H), 7.17-7.10 (m, 3H). HRMS (ESI$^+$) calcd for $C_{15}H_{12}NO_2S_2$ $(M+H)^+$ 302.0309, found 302.0305.

HCV replicon assay. HCV genotype 1b Huh-7/HCV1b-Rluc replicon cells (obtained from G. Luo, University of Alabama, Birmingham) expressing luciferase in the context of a self-replicating partial viral genome were maintained as described.[42] To evaluate compounds for anti-replicon activity, 6 X 10^3 replicon cells per well were introduced into 96-well tissue culture dishes. The next day, cell culture medium containing compound dissolved in DMSO or DMSO alone was added to the replicon cells. Three days later, the cells were analyzed for luciferase expression using ViVi-Ren Live Cell Subtrate (Promega) as described.[42] All compounds and controls were performed in triplicate. 2mA was included in single-dose experiments as controls. The 50% effective concentration (EC_{50}), compound concentration that reduced luciferase activity 50%, was determined by comparing luciferase activity for eight serial dilutions and vehicle-treated cells using GraphPad Prism software.

Cell proliferation assay. Potential toxicity of compounds was evaluated using the CellTiter 96 AQ_{ueous} One Solution Cell Proliferation assay (Promega) as previously described.[42] Replicon cells were plated out at 6 X 10^3 cells per well in a clear 96-well tissue culture plate (Corning). The next day, the cells were incubated at 37 ºC/5% CO_2 in culture medium containing compound (dissolved in DMSO), DMSO alone, or nothing added for three days. All compounds and controls were performed in triplicate. CellTiter 96 AQ_{ueous} One Solution Cell Proliferation reagent was added according to manufacturer's instructions and viability measured by spectrometry at 450 nm with a SpectraMax E5 (Molecular Devices). The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of compound that reduced cell

proliferation by 50%. The CC_{50} was determined by comparing absorbance readings from eight serial dilutions of compound and vehicle-treated cells using GraphPad Prism software.

RT-qPCR Assay. Replicon-containing cells (6 X 10^3 per well) were plated in a clear 96-well tissue culture dish (Corning) and exposed to culture medium containing compound dissolved in DMSO or DMSO alone for three days at 37 ºC/5% CO_2. The relative level of replicon RNA for compound-treated cells compared to DMSO-treated cells was measured using the TaqMan® Gene Expression Cells-to-CT system (Applied Biosystems) as per manufacturer's instructions and as previously described.[42b] The primers/probes to measure HCV replicon cDNA were custom synthesized to NS5B (Applied Biosystems)[42b] and the control primers/probe recognized GAPDH cDNA (Applied Biosystems, catalog number 402869). Samples were processed using a Chromo4 real time detector and DNAEngine thermocycler (Bio-Rad, Hercules, CA) with cycling parameters of 50 ºC for 2 min, 95 ºC for ten min, 95 ºC for 15 sec, 60 ºC for 1 min, last two steps repeated 39 more times. Compounds and controls were analyzed in triplicate and parallel wells were examined for either HCV replicon RNA or GAPDH RNA. Relative levels of HCV RNA were calculated using the comparative C_T method.[43] In this method, the level of HCV replicon RNA in compound treated cells, normalized to GAPDH endogenous reference RNA and relative to a calibrator (HCV replicon RNA level in DMSO treated cells), is calculated using the formula $2^{-\Delta\Delta C}{}_T$. C_T is the PCR cycle where the signal crosses a threshold into the exponential phase of amplification. For this method it is important that PCR efficiencies are similar for the primer/probe sets (+/- 10%). We previously determined efficiencies over five-fold serial dilutions (3 logs) to be 100% (R^2 0.999) and 110% (R^2 0.997) for our NS5B primers/probe and GAPDH primers/probe, respectively.[42b] GAPDH is a valid endogenous control gene because the

mean C_T values for GAPDH RNA levels in compound-treated and DMSO-treated cells were very similar with a mean plus standard deviation of 23.4 +/- 0.3 (DMSO C_T = 23.2 +/- 0.3).

Supporting Information Available: The inhibitory activity of compound **53** against human HDAC1-11, human MMP1-3, MMP7-10, MMP12-14, TACE, HCV NS3/4A and HCV NS5B, and the associated assay conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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Abbreviations Used

HCV, Hepatitis C Virus; SOC, standard of care; DAA, direct-acting antivirals; HDAC, histone deacetylases; SAHA, suberoylanilide hydroxamic acid; TI, therapeutic index; RT-qPCR, reverse transcription and quantitative PCR; MMP, matrix metalloproteinase; TACE, tumor necrosis factor-α converting enzyme; CDI, 1,1'-carbonyldiimidazole

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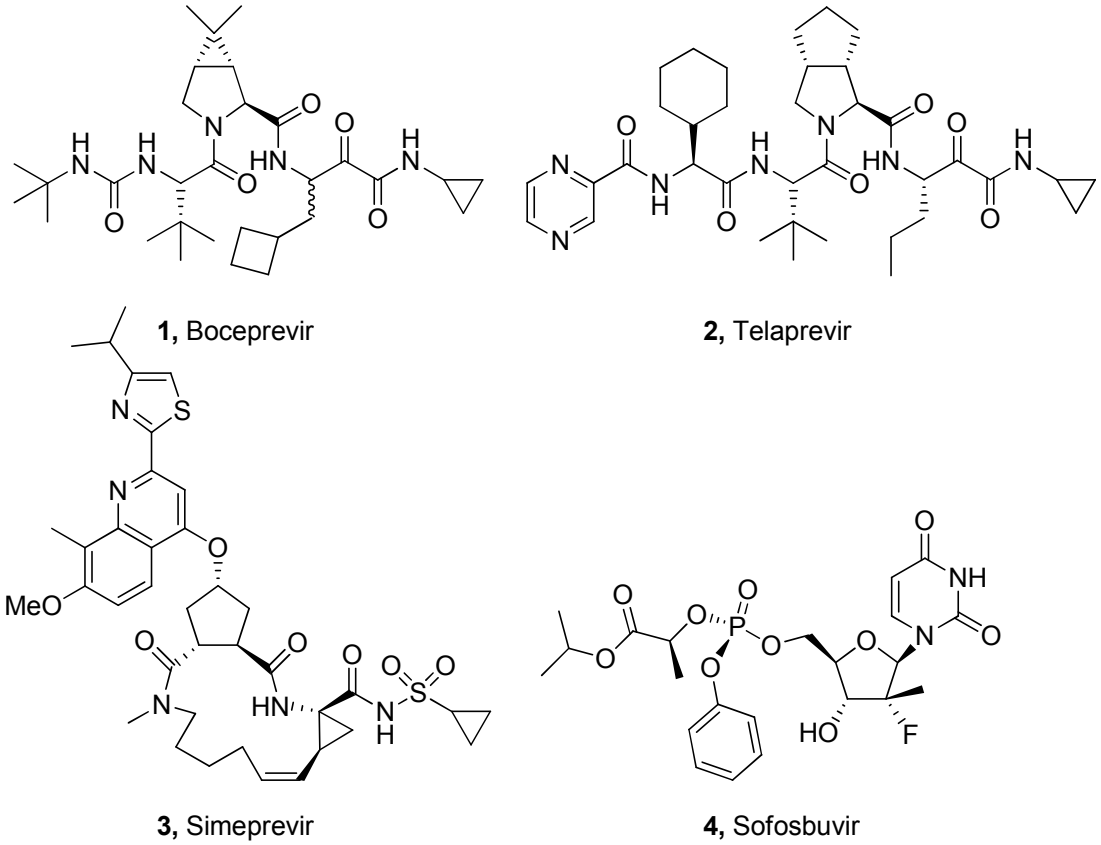


Figure 1. Recently FDA-approved anti-HCV agents.

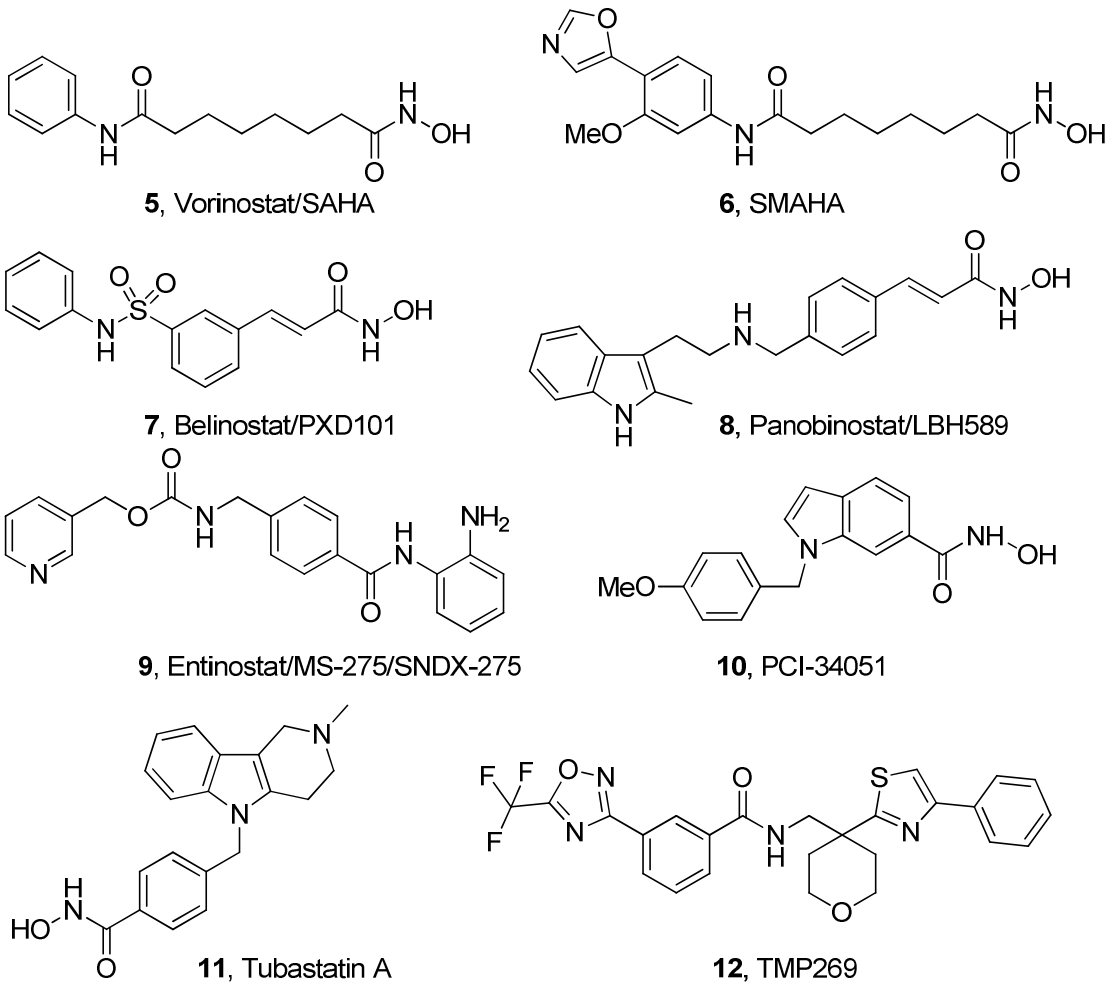


Figure 2. Selected HDAC inhibitors.

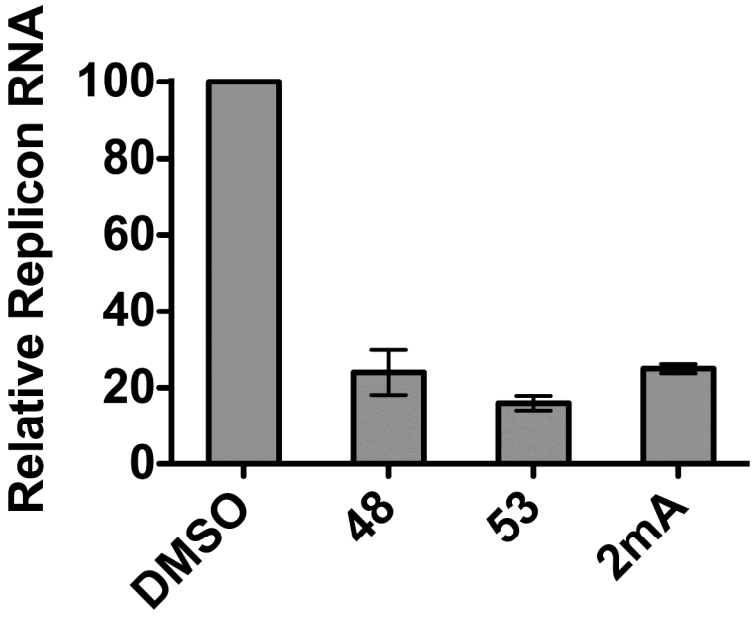
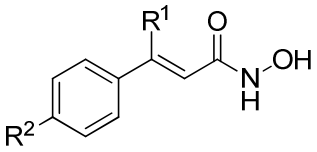


Figure 3. Relative HCV replicon RNA levels determined using RT-qPCR for cells treated with compounds **48** and **53** (2 μM), 2mA (0.5 μM), and DMSO alone.

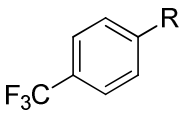
Compound	HDAC Selectivity	% inhibition at 10 μM		EC_{50} (μM)	CC_{50} (μM)	TI
		HCV	viability			
5	pan			0.36 ± 0.20	1.9 ± 0.1	5
6	pan			0.21 ± 0.21	2.6 ± 3.9	12
7	pan			0.12 ± 0.01	0.68 ± 0.27	6
8	pan			ND	0.0035	
9	1/2			1.4 ± 0.1	5.1 ± 4.3	4
10	8			1.8 ± 0.6	11 ± 4	6
11	6	97%	70%			
12	class IIa	22%	21%			
2mA[a]				0.20 ± 0.11	54 ± 15	270

Table 1. Evaluation of selected HDAC inhibitors. TI, therapeutic index, TI = CC_{50}/EC_{50}. ND, not determined. *a*. 2mA was used as a positive control in anti-HCV and cytotoxicity assays, and served as a benchmark for anti-HCV activity. Typical EC_{50} and CC_{50} values were shown. Percentage of inhibition was measured in triplicate and the average reported. Dose-response experiments were conducted in triplicate, and the averages reported. For compounds that were tested independently multiple times, the average of independent results together with standard errors were shown.



Compound	R^1	R^2	% inhibition at 10 μM		EC_{50} (μM)	CC_{50} (μM)	TI
			HCV	viability			
13	H	H			0.86 ± 0.09	39 ± 8	45
14	Me	H			3.2 ± 0.1	59 ± 18	18
15	Ph	H	64%	4%			
16	H	Me	15%	0%			
17	H	OMe	15%	0%			
18	H	Cl			6.0	> 50	> 8
19	H	NO_2			0.45 ± 0.24	29 ± 4	64
20	H	$MeS(O)_2$	93% 30%[a]	0% 0%[a]			
21	H	CN	99% 45%[a]	18% 16%[a]			
22	H	CF_3			0.14 ± 0.04	25 ± 0	178

Table 2. Evaluation of inhibitors based on cinnamic hydroxamic acid. *a*. inhibition at 1 μM. Percentage of inhibition was measured in triplicate and the average reported. Dose-response experiments were conducted in triplicate, and the averages reported. For compounds that were tested independently multiple times, the average of independent results together with standard errors were shown.



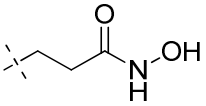
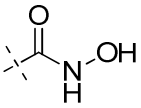
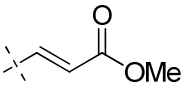
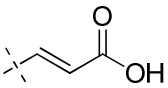
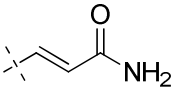
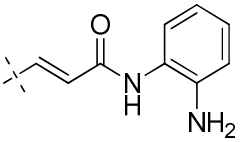
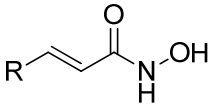
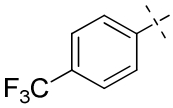
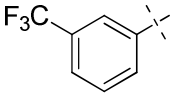
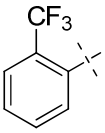
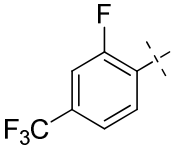
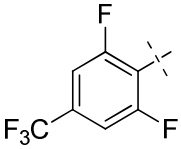
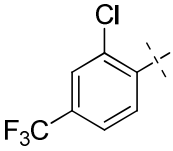
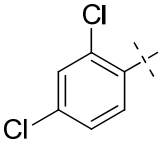
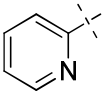
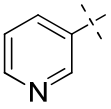
Compound	R	% inhibition at 10 μM		EC_{50} (μM)	CC_{50} (μM)	TI
		HCV	viability			
23		35%	0%			
24				5.4 ± 0.1	> 100	> 18
25		0%	1%			
26		19%	0%			
27		6%	1%			
28		94%	82%			

Table 3. Examination of the unsaturated hydroxamic acid functionality. Percentage of inhibition was measured in triplicate and the average reported. Dose-response experiments were conducted in triplicate, and the averages reported. For compounds that were tested independently multiple times, the average of independent results together with standard errors were shown.



Compound	R	% inhibition at 1 μM		EC_{50} (μM)	CC_{50} (μM)	TI
		HCV	viability			
22				0.14 ± 0.04	25 ± 0	178
29		43%	6%			
30		40%	5%			
31				0.12 ± 0.07	14 ± 1	117
32				0.54	12	22
33				1.1	15	14
34				0.078	8.1	104
35		58%	1%			
36		40%	2%			

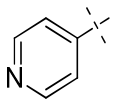
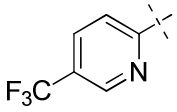
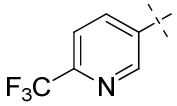
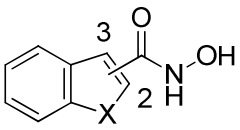
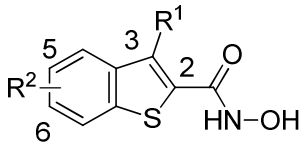
37		20%	0%			
38				0.38	13	34
39				0.40	19	48

Table 4. Examination of aromatic rings. Percentage of inhibition was measured in triplicate and the average reported. Dose-response experiments were conducted in triplicate, and the averages reported. For compounds that were tested independently multiple times, the average of independent results together with standard errors were shown.

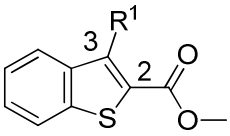


Compound	Position	X	% inhibition at 10 μM		EC_{50} (μM)	CC_{50} (μM)	TI
			HCV	viability			
40	2	O			1.8 ± 1.5	54 ± 10	30
41	2	S			1.0 ± 0.8	28 ± 5	28
42	2	NH	45%	7%			
43	2	NPh	41%	15%			
44	2	NBn	82%	28%			
45	3	NH	0%	ND			
46	3	NPh	11%	ND			
47	3	NBn	99%	92%			

Table 5. Examination of fused aromatic rings. Percentage of inhibition was measured in triplicate and the average reported. Dose-response experiments were conducted in triplicate, and the averages reported. For compounds that were tested independently multiple times, the average of independent results together with standard errors were shown.



48-55



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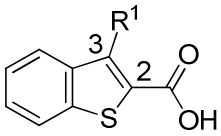

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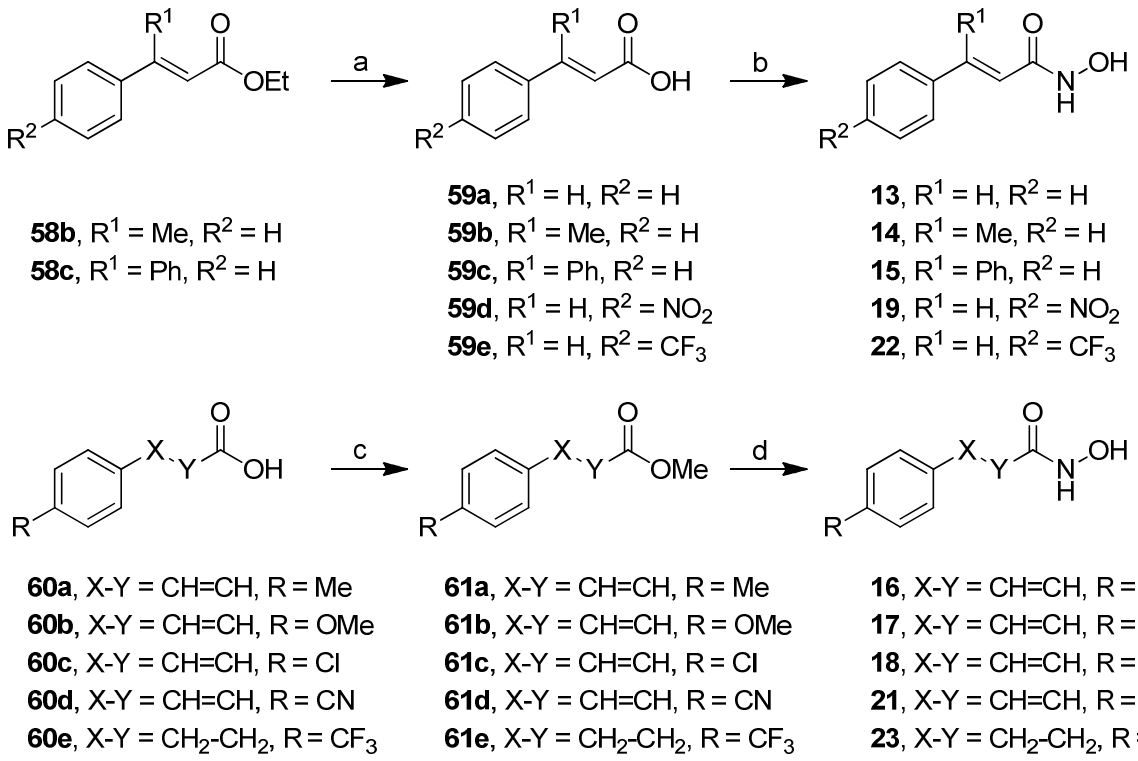
Compound	R^1	R^2	% inhibition at 10 μM		EC_{50} (μM)	CC_{50} (μM)	TI
			HCV	viability			
41	H	H			1.0 ± 0.8	28 ± 5	28
48	H	5-Cl			0.69 ± 0.01	27 ± 1	39
49	H	5-NO_2			2.4 ± 1.9	34 ± 4	14
50	H	6-NO_2			3.7 ± 1.8	54 ± 8	15
51	H	5-CF_3	94% 16%[a]	0% 10%[a]			
52	H	6-CF_3	83% 0%[a]	13% 4%[a]			
53	$PhS(O)_2$	H			0.61 ± 0.40	57 ± 21	93
54	PhS(O)	H			1.4 ± 0.4	> 50	> 36
55	PhS	H	8%	4%			
56	$PhS(O)_2$	--	5%	0%			
57	$PhS(O)_2$	--	19%	2%			

Table 6. Evaluation of benzo[*b*]thiophene derivatives. *a*. inhibition at 1 μM. Percentage of inhibition was measured in triplicate and the average reported. Dose-response experiments were conducted in triplicate, and the averages reported. For compounds that were tested independently multiple times, the average of independent results together with standard errors were shown.

Scheme 1[a]



58b, R^1 = Me, R^2 = H
58c, R^1 = Ph, R^2 = H

59a, R^1 = H, R^2 = H
59b, R^1 = Me, R^2 = H
59c, R^1 = Ph, R^2 = H
59d, R^1 = H, R^2 = NO_2
59e, R^1 = H, R^2 = CF_3

13, R^1 = H, R^2 = H
14, R^1 = Me, R^2 = H
15, R^1 = Ph, R^2 = H
19, R^1 = H, R^2 = NO_2
22, R^1 = H, R^2 = CF_3

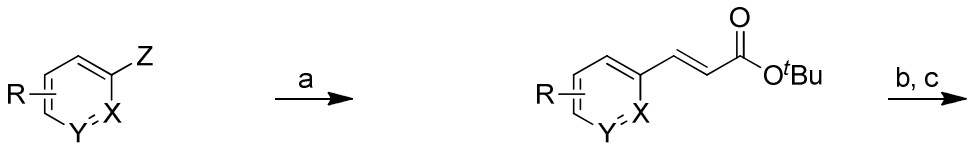
60a, X-Y = CH=CH, R = Me
60b, X-Y = CH=CH, R = OMe
60c, X-Y = CH=CH, R = Cl
60d, X-Y = CH=CH, R = CN
60e, X-Y = CH_2-CH_2, R = CF_3

61a, X-Y = CH=CH, R = Me
61b, X-Y = CH=CH, R = OMe
61c, X-Y = CH=CH, R = Cl
61d, X-Y = CH=CH, R = CN
61e, X-Y = CH_2-CH_2, R = CF_3

16, X-Y = CH=CH, R = Me
17, X-Y = CH=CH, R = OMe
18, X-Y = CH=CH, R = Cl
21, X-Y = CH=CH, R = CN
23, X-Y = CH_2-CH_2, R = CF_3

[a] Reagents and conditions: (a) NaOH, H_2O, THF, MeOH; (b) CDI, CH_2Cl_2; or $(COCl)_2$, DMF, CH_2Cl_2; then NH_2OH solution; (c) $SOCl_2$, MeOH; (d) $NH_2OH \cdot HCl$, NaOMe, MeOH.

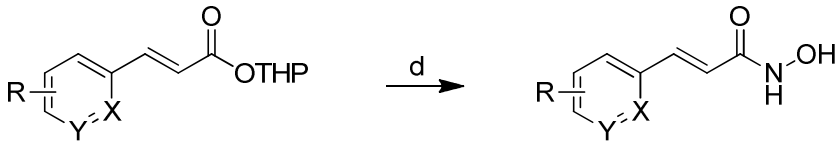
Scheme 2[a]



62a, R = 4-SO_2Me, X = CH, Y = CH, Z = Br

62c, R = 2-CF_3, X = CH, Y = CH, Z = I
62d, R = 2-F, 4-CF_3, X = CH, Y = CH, Z = Br
62e, R = 2,6-2F, 4-CF_3, X = CH, Y = CH, Z = Br
62f, R = 2-Cl, 4-CF_3, X = CH, Y = CH, Z = I
62g, R = 4-CF_3, X = N, Y = CH, Z = Br
62h, R = 4-CF_3, X = CH, Y = N, Z = Br

63a, R = 4-SO_2Me, X = CH, Y = CH
63b, R = 3-CF_3, X = CH, Y = CH, acid
63c, R = 2-CF_3, X = CH, Y = CH
63d, R = 2-F, 4-CF_3, X = CH, Y = CH
63e, R = 2,6-2F, 4-CF_3, X = CH, Y = CH
63f, R = 2-Cl, 4-CF_3, X = CH, Y = CH
63g, R = 4-CF_3, X = N, Y = CH
63h, R = 4-CF_3, X = CH, Y = N

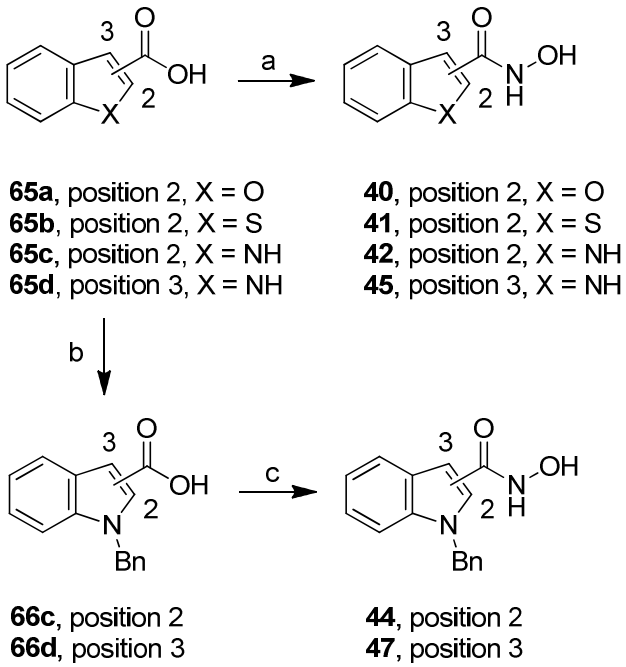


64a, R = 4-SO_2Me, X = CH, Y = CH
64b, R = 3-CF_3, X = CH, Y = CH
64c, R = 2-CF_3, X = CH, Y = CH
64d, R = 2-F, 4-CF_3, X = CH, Y = CH
64e, R = 2,6-2F, 4-CF_3, X = CH, Y = CH
64f, R = 2-Cl, 4-CF_3, X = CH, Y = CH
64g, R = 4-CF_3, X = N, Y = CH
64h, R = 4-CF_3, X = CH, Y = N

20, R = 4-SO_2Me, X = CH, Y = CH
29, R = 3-CF_3, X = CH, Y = CH
30, R = 2-CF_3, X = CH, Y = CH
31, R = 2-F, 4-CF_3, X = CH, Y = CH
32, R = 2,6-2F, 4-CF_3, X = CH, Y = CH
33, R = 2-Cl, 4-CF_3, X = CH, Y = CH
38, R = 4-CF_3, X = N, Y = CH
39, R = 4-CF_3, X = CH, Y = N

[a] Reagents and conditions: (a) *t*-butyl acrylate, $Pd(OAc)_2$, Xphos, Et_3N, DMF, 140 ºC, 12 h; (b) TFA, CH_2Cl_2, 1 h; (c) NH_2OTHP, HBTU, Et_3N, 12 h; (d) HCl, Et_2O, CH_2Cl_2, 2 h.

Scheme 3[a]



65a, position 2, X = O
65b, position 2, X = S
65c, position 2, X = NH
65d, position 3, X = NH

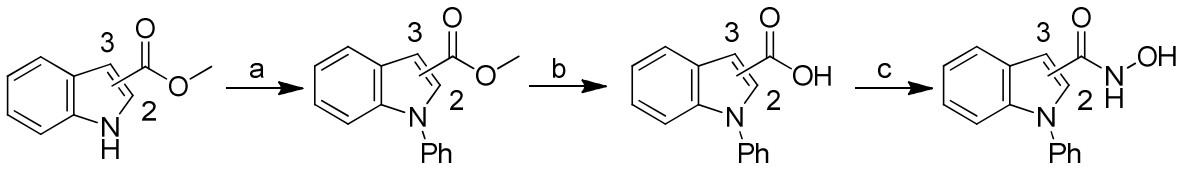
40, position 2, X = O
41, position 2, X = S
42, position 2, X = NH
45, position 3, X = NH

66c, position 2
66d, position 3

44, position 2
47, position 3

[a] Reagents and conditions: (a) CDI, CH_2Cl_2; or $(COCl)_2$, DMF, CH_2Cl_2; then NH_2OH solution; (b) BnBr, NaH, DMF; (c) $(COCl)_2$, DMF, CH_2Cl_2; then NH_2OH solution.

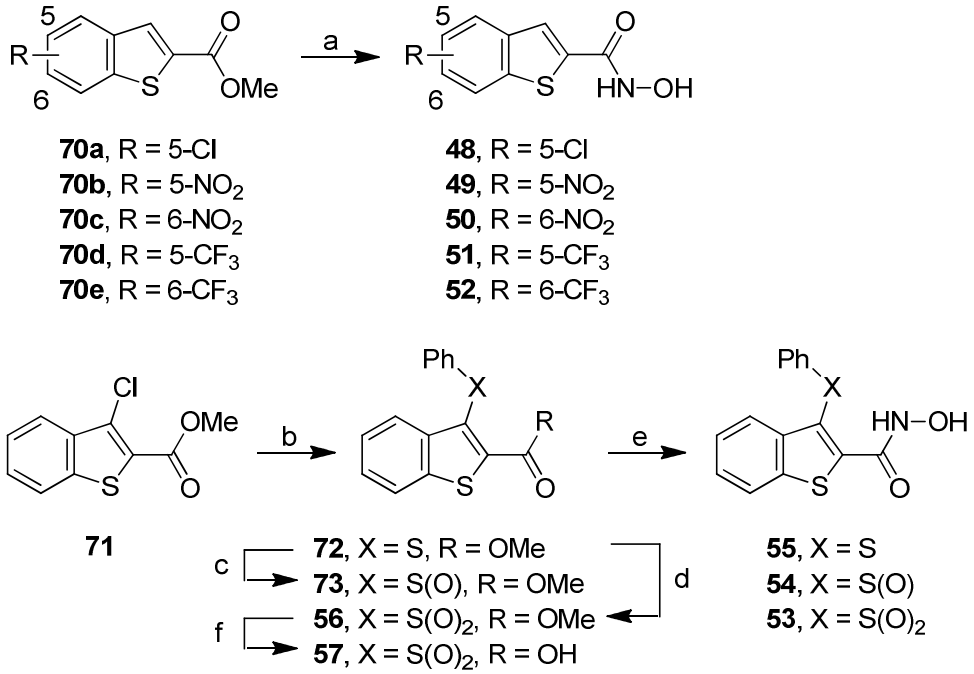
Scheme 4[a]



67a, position 2 **68a**, position 2 **69a**, position 2 **43**, position 2
67b, position 3 **68b**, position 3 **69b**, position 3 **46**, position 3

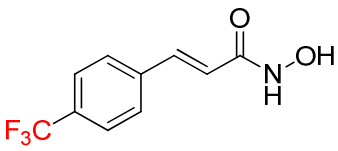
[a] Reagents and conditions: (a) PhBr, CuI, *trans-N,N'*-dimethylcyclohexane-1,2-diamine, K_3PO_4, toluene, 120 ºC; (b) NaOH, H_2O, THF, MeOH; (c) $(COCl)_2$, DMF, CH_2Cl_2; then NH_2OH solution.

Scheme 5[a]



[a] Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, NaOMe, MeOH; (b) PhSH, K_2CO_3, DMF, 80 ºC; (c) H_2O_2, TMSCl, CH_3CN, 5 min; (d) Oxone, $NaHCO_3$, H_2O, acetone; (e) $NH_2OH{\cdot}HCl$, NaOMe, MeOH; (f) NaOH, H_2O, THF, MeOH.

Table of Contents Graphic



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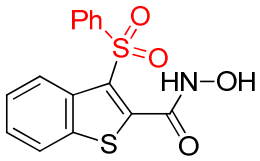
HCV EC_{50} = 0.14 μM
CC_{50}/EC_{50} = 178



53

HCV EC_{50} = 0.61 μM
CC_{50}/EC_{50} = 93