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Identification of Novel, Selective, and Stable Inhibitors of Class II Histone Deacetylases. Validation Studies of the Inhibition of the Enzymatic Activity of HDAC4 by Small Molecules as a Novel Approach for Cancer Therapy

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5-Aryl-2-(trifluoroacetyl)thiophenes were identified as a new series of class II HDAC inhibitors (HDACi). Further development of this new series led to compounds such as **6h**, a potent inhibitor of HDAC4 and HDAC6 (HDAC4 WT IC_{50} = 310 nM, HDAC6 IC_{50} = 70 nM) that displays 40-fold selectivity over HDAC1 and improved stability in HCT116 cancer cells ($t_{1/2}$ = 11 h). Compounds **6h** and **2** show inhibition of α-tubulin deacetylation in HCT116 cells at 1 μM concentration and antiproliferation effects only at concentrations where inhibition of histone H3 deacetylation is observed.

Introduction

Histone deacetylases (HDACs[a]) are involved in the deacetylation of histones, transcription factors, and other proteins in eukaryotic cells.[1] HDAC inhibition causes hyperacetylation of histones, which leads to a more open chromatin structure, thereby facilitating gene transcription and ultimately leading to cell-growth arrest, differentiation, and apoptosis.[1b] The development of specific HDACi represents a new strategy in cancer therapy.[2]

There are currently 18 known mammalian HDACs, grouped into four classes based on sequence similarity.[3] The class I, II, and IV HDACs are zinc-dependent enzymes, whereas class III HDACs (SIRT1-SIRT7) are structurally unrelated and require NAD^+ for activity.[4] The class I HDACs (HDAC1, 2, 3, and 8) are ~400 residues long and are generally nuclear, whereas the class II HDACs exhibit nucleocytoplasmic shuttling. In turn, class II HDACs are subdivided into class IIa (HDAC4, 5, 7, and 9) and IIb (HDAC6 and 10). Class IIa enzymes are characterized by the presence of an N-terminal extension of ~600 residues with distinct regulatory and functional properties, whereas the class IIb enzymes contain two catalytic domains.[5] Class IV currently includes only HDAC11, which shares greatest sequence similarity to the class I enzymes.[6]

The cellular function of each individual HDAC isoform is not fully understood and neither is the specific role each plays in tumorogenesis. Notwithstanding, the urgency for alternative therapies for cancer has allowed a rapid development of broad spectrum HDACi. For instance, hydroxamic acid-based HDACi including vorinostat (SAHA),[7] belinostat,[8] or panobinostat[9] and nonhydroxamic acid-based HDACi such as the 2-aminophenylamides MS-275[10] and MGCD-0103[11] or the dithiol FK228[12] are currently in the clinic. Most of them hit a subset of both class I and class II HDACs or, as in the case of the 2-aminophenylamides, show selectivity versus class I HDACs. All of them elicit similar adverse effects, mainly fatigue, nausea, vomiting, and diarrhea, which become dose-limiting in clinical trials. Elucidation of the function of each HDAC subtype would allow the identification of second generation inhibitors to be developed, which could target individual HDAC isoforms and thereby achieve a wider therapeutic index. Consequently, there remains a widely recognized need to identify selective HDACi.[13]

In an effort to develop a second generation HDACi with enhanced efficacy and a larger therapeutic window, we were interested in targeting a more restricted set of HDAC isoforms. RNAi knock-down experiments were performed against all 11 class I and II HDACs in three different cancer cell lines (HeLa, HCT116, and A549). In these experiments, it was observed that ablation of HDAC4 expression led to cell growth inhibition in all three human tumor cell lines but no detectable effects in human fibroblast or myelopoietic progenitors. Moreover, knockdown of HDAC4 in HeLa cells produced mitotic arrest followed by caspase-dependent apoptotic cell death.[14] In parallel, given the problems associated with the isolation and purification of class II HDACs from mammalian cells, two enzymatic assays for the measurement of the inhibitory activity of small molecules against HDAC4 were developed in our laboratory.[15] These two assays use HDAC4 expressed in bacteria and the enzymatic efficiency of this protein was enhanced either by an H−Y mutation in the active site to give a *constitutively active mutant* (CAM) or by use of the wild-type (WT) enzyme with an "unnatural" trifluoroacetyl-lysine substrate.[15] As the natural substrate of HDAC4 is unknown, there is currently no cellular target engagement marker for HDAC4. However, α-tubulin is

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[a] Abbreviations: HDAC, histone deacetylase; NAD^+, nicotinamide adenine dinucleotide; WT, wild type; CAM, constitutively active mutant; TFMK, trifluoromethylketone.

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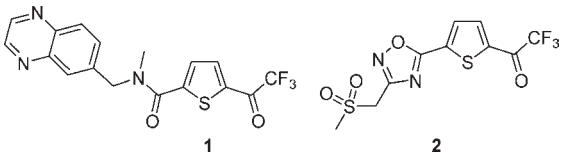


Figure 1. Class II HDAC inhibitors **1** and **2**.

deacetylated by HDAC6[16] and given that the compounds we have developed inhibit both HDAC4 and 6, inhibition of HDAC6 can be used as a surrogate marker to measure inhibition of HDAC4 in cells. With these tools, we initiated a target validation program to identify potent and selective class II HDACi, with the aim to show if inhibition of the deacetylase activity of HDAC4 by a small molecule would recapitulate the growth inhibitory phenotype observed in the RNAi experiments.

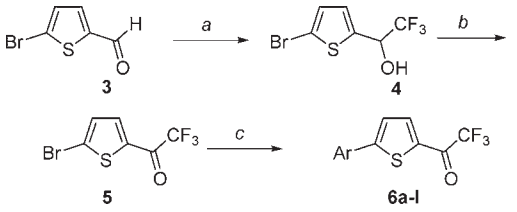
As previously reported, a series of 5-(trifluoroacetyl)-thiophene-2-carboxamides was identified as a novel class of potent and selective class II HDACi.[17] These molecules, exemplified by compound **1** (Figure 1), are active site binders and typically display around 10-fold selectivity for class II HDACs (HDAC4, 6) over their class I HDACs (HDAC1, 3) counterparts. Unfortunately, most of these derivatives lacked cell-based activity as they failed to show inhibition of either histone H3 or α-tubulin deacetylation in HCT116 cells, the cellular substrates of HDAC1 and 6, respectively.[18] The lack of cellular activity displayed by these inhibitors was demonstrated to be due to the instability of the trifluoromethylketone (TFMK) moiety, which is readily reduced in cells to the corresponding inactive alcohol, with half-lives depending on the nature of the amide substituents and to be in the range of 20 min to 2 h.

The modest selectivity and the instability in cells prompted us to seek alternatives structures, aiming to obtain more potent, selective, and stable HDAC4 inhibitors. One approach was based on structural modifications that could address the metabolic instability issue by reducing molecular recognition by the intracellular carbonyl reductases, thus decreasing the subsceptibility of the TFMK group toward reduction. In a recent paper, we reported the identification of compounds where the amide bond was replaced by heteroaromatic bioisosteres such as 1,2,4- and 1,3,4-oxadiazoles and 1,3-thiazoles such as **2**.[19] Herein, we report a novel series of 5-aryl-2-(trifluoroacetyl)thiophenes that are potent and selective class II HDACi and display improved stability in HCT116 cancer cells. Both classes of inhibitor have been used to evaluate the antiproliferative effect of the inhibition of the deacetylase activity HDAC4 by small molecules.

Chemistry

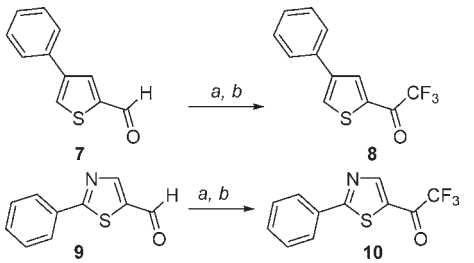
5-Aryl substituted 2-(trifluoroacetyl)thiophenes were prepared by the method shown in Scheme 1. Reaction of 5-bromothiophene-2-carbaldehyde **3** with Ruppert's reagent in the presence of cesium fluoride afforded the secondary alcohol **4**.[20] Subsequent oxidation of (**4**) with Dess−Martin periodinane produced the trifluomethylketone **5**.[21] Reaction of the key intermediate **5** with arylboronic acids under standard Suzuki cross-coupling conditions gave the desired 5-aryl-2-trifluoroacetylthiophenes **6a**−**l**. Similar methodology as for the addition of the trifluoromethyl group and subsequent oxidation of the secondary alcohol intermediate was applied to the synthesis of 5-phenyl-2-trifluoroacetylthiophene **8** and

Scheme 1. Synthesis of 2,2,2-Trifluoro-1-(5-arylthiophen-2-yl)-ethanones **6a**−**l**



[a] Reagents and conditions: (a) $TMSCF_3$, CsF, DME, room temp (97%); (b) Dess−Martin periodinane, CH_2Cl_2, room temp (70%); (c) $ArB(OH)_2$, Na_2CO_3, $Pd(Ph_3P)_4$, DMF, 90 °C, o/n.

Scheme 2. Synthesis of 2,2,2-Trifluoro-1-(4-phenylthiophen-2-yl)-ethanone **8** and 2,2,2-Trifluoro-1-(2-aryl-1,3-thiazol-5-yl)-ethanone **10**



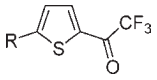
[a] Reagents and conditions: (a) $TMSCF_3$, CsF, DME, room temp; (b) Dess−Martin periodinane, CH_2Cl_2 or DMF, room temp.

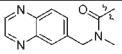
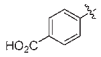
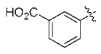
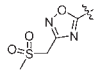
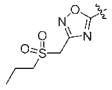
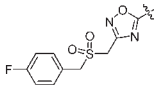
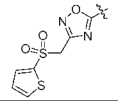
2-phenyl-5-trifluoroacetyl-1,3-thiazole **10** from the corresponding commercially available carbaldehydes **7** and **9** as illustrated in Scheme 2.

Results and Discussion

Analysis of the X-ray structure of HDAC4 with a 5-(trifluoroacetyl)thiophene-2-carboxamide inhibitor showed that the central thiophene-2-carboxamide portion of the inhibitor binds in the lipophilic channel connecting the zinc binding site to the surface of the enzyme.[22] We hypothesized that replacement of the carboxamide moiety by a lipophilic substituent could be tolerated and may potentially give additional binding interactions with the enzyme. First, we explored the replacement of the carboxamide linker by aromatic six-membered rings. Introduction of a phenyl ring in position 5 of the thiophene gave a modestly active compound, **6a**, that displayed low micromolar activity in both HDAC4 WT and HDAC4 CAM assays with around 10-fold selectivity against HDAC1. Compound **6a** also showed submicromolar potency against HDAC6. On the other hand, when the aromatic substituent was moved to position 4, as in compound **8**, a significant loss of potency was observed against HDAC4 and 6. With the aim to improve activity of **6a** against class II HDACs, substituents were added to the phenyl ring. Introduction of a methoxy group in the para position as in **6b** led to an increase in activity against HDAC4 WT, maintaining high selectivity against HDAC1. However, **6b** was much less active against HDAC6. When the methoxy group was migrated to positions meta, **6c**, or ortho, **6d**, a significant loss of activity against HDAC4 WT was observed. This fact can be explained by a possible clash of the substituent with the channel that connects the active site with the surface of the enzyme.[22] When we introduced an electron-withdrawing group such as cyano in the para position of the phenyl ring

Table 1. Enzymatic Activity of Compounds **1**, **2**, **6a**−**l**, **8**, and **10**−**13**

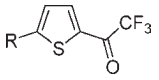


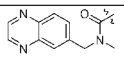
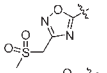
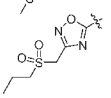
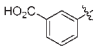
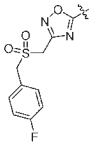
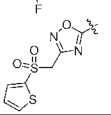
Compd	R	HDAC1 IC_{50} (nM)[a]	HDAC4 CAM IC_{50} (nM)[a]	HDAC4 WT IC_{50} (nM)[a]	HDAC6 IC_{50} (nM)[a]
1		580±25 (5)	87±12 (5)	98±5.7 (5)	89±11 (5)
6a		>20,000	2,800±810	2,800±490	170±22
8		>10,000	>10,000	3,200±85	1,900±310
6b		>1,000	>1,000	110±7.0	>1,000
6c		>10,000	>5,000	3,800±420	3,100±730
6d		>10,000	3,500±210	>10,000	6,300±73
6e		2,700±70	14±2.1	160±6.0	41±6.0
6f		>10,000	12±1.0	72±2.5	29±2.9
6g		2,500±84	60±0.5	74±0.7	27±7.8
6h		12,800±1,200 (5)	96±5.7 (5)	310±23 (5)	70±3.5 (5)
6i		>1,000	>1,000	540±8.0	>1,000
6j		910±100	54±7.7	160±9.0	15±0.7
6k		>10,000	6,200±130	>10,000	>5,000
6l		>10,000	1,400±250	>10,000	37±1.7
10		3,700±430	190±15	130±10	210±16
2		2,700±300 (5)	780±48 (5)	24±2.6 (5)	230±23 (5)
11		2,200±100	520±85	30±2.9	240±52
12		6,600±43	680±57	60±8.5	700±56
13		3,900±350	1,000±48	35±2.3	520±99

[a] IC_{50} values are the mean ± standard error of three independent determinations unless indicated in brackets.

as in compound **6e**, a dramatic improvement in the inhibitory activity against all the HDAC isoforms was observed, and in particular class II HDACs. We have recently shown that trifluoroacetyl thiophene derivatives are active site binders with the hydrated trifluoromethylketone chelating the active site zinc in a bidentate manner.[22] Introduction of electron-withdrawing groups in the substituents of the thiophene ring would favor the displacement of the trifluoromethylketone equilibra to the hydrated form, leading to an increase of activity in the enzymatic assays. An improvement in both activity and selectivity over class I HDAC was also observed in the 4-acetyl analogue **6f**, which displayed double digit nanomolar activity against HDAC4 and 6 with more than 100-fold selectivity against HDAC1. Moreover, introduction of a carboxylic acid as in **6g** afforded potent class II HDAC inhibitors although a minor loss in class I HDAC selectivity was observed. A slight improvement of selectivity over class I HDAC was observed when the carboxylic group was placed in

Table 2. Stability of 2-Aryl-5-(trifluoroacetyl)thiophenes in the S9 Subcellular Fraction of HCT116 Cells and in Whole HCT116 Cells



Compd	R	S9 fraction Stability $t_{1/2}$ (min)	HCT116 Stability $t_{1/2}$ (h)	Compd	R	S9 fraction Stability $t_{1/2}$ (min)	HCT116 Stability $t_{1/2}$ (h)
1		15	3.5	**10**		15	N.D.[a]
6e		>120	8	**2**		>120	13
6f		>120	N.D.[a]	**11**		45	12
6h		N.D.[a]	11	**12**		>120	5.5
6j		10	N.D.[a]	**13**		50	6

[a] N.D.: not determined.

the meta position, and compound **6h** displayed 40-fold selectivity for HDAC4 WT, and 180-fold for HDAC6, against HDAC1. Next, we explored the introduction of heteroaromatic systems as substitutents of the thiophene core. The 2-pyridyl analogue **6i** was essentially inactive in the HDAC4 CAM assay and against HDAC6. However, the electron-deficient quinoxaline-6-yl analogue **6j** showed a significant improvement in activity against class II HDACs but displayed also submicromolar activity against HDAC1. On the other hand, the electron-rich indol-5-yl analogue **6k** was essentially inactive against HDAC1 and 4, similarly, a loss of activity was observed in the 2,3-dihydro-1,4-benzodioxin-6-yl analogue **6l**, although this compound showed good HDAC6 activity. Replacement of the thiophene core by 1,3-thiazole was also examined, as in compound **10**, and this structural modification led to an improvement of activity against all the isoforms tested. Encouragingly, **10** displayed IC_{50} = 130 and 210 nM against HDAC4 WT and HDAC6 and around 25-fold selectivity over HDAC1.

Having identified potent and selective class II HDAC inhibitors, we sought to identify the most stable derivatives for use in validation studies in cells. Accordingly, the inhibitors were profiled for stability in the S9 subcellular fraction of HCT116 cells and also in HCT116 cells.[18] As a reference compound, we used our initial lead from the carboxamide series **1**, which displayed a short half-life in the S9 fraction ($t_{1/2}$ = 15 min) and in HCT116 cells ($t_{1/2}$ = 3.5 h). Interestingly, introduction of the aromatic ring with an electron-withdrawing group in position para, as in compounds **6e** and **6f**, led to a stabilization of the molecules, showing half-lives of more than 2 h in the S9 fraction assay and 8 h in HCT116 cells. This result could be rationalized by the electron-withdrawing group favoring the displacement of the trifluoromethylketone equilibra to the hydrated form, making it less proned to the reduction. A further stabilization in cells was displayed by compound **6h** ($t_{1/2}$ = 11 h) that bears a carboxylate group in the meta position. However, compound **6j**, which possesses a heterocyclic system, was unstable and not investigated further. Similarly, the 1,3-thiazole analogue **10**, which displayed good potency against class II HDACs, showed a short half-life ($t_{1/2}$ = 15 min), which prevented its use in cells. In parallel, we also tested some analogues bearing a 1,2,4-oxadiazole ring linked to the thiophene core (Table 1).[19] Interestingly, compound **2** showed a remarkable stability in the S9 subcellular fraction ($t_{1/2}$ > 120 min) and also in cells ($t_{1/2}$ = 13 h). The stability of these compounds could be affected by modification of the sulphone substituent. Elongation of the alkyl chain as in compound **11** resulted in a modest reduction in $t_{1/2}$, while the addition of a phenyl ring as in benzyl sulfone **12** led to a further decrease of the half-life in cells with $t_{1/2}$ = 5.5 h. Given the fact that the validation studies for HDAC4 would be carried out in HCT116 cells, **2** and **6h** appeared to be suitable probe compounds to evaluate the phenotype of HDAC4 inhibition by a small molecule in HCT116 cancer cells (Table 2).

Although compounds **2** and **6h** had longer half-lives than other analogues, these were still shorter than the duration of the HCT116 cell proliferation assay and the histone H3 and α-tubulin deacetylation assays in HCT116 cells, 72 and 24 h, respectively. To ensure that sufficient quantities of our inhibitors were present for the duration of the experiments, the medium was changed and fresh HDACi was added every 12 h.

Compound **2** is a potent inhibitor of HDAC4 WT (IC_{50} = 24 nM) that displays IC_{50} = 230 nM on HDAC6 and more than 100-fold selectivity over HDAC1 (IC_{50} = 2700 nM). This compound showed inhibition of α-tubulin deacetylation in HCT116 cells at submicromolar concentrations (EC_{50} = 640 nM) (Table 3), demonstrating that the compound entered cells and was stable enough to inhibit HDAC6 in cells, and by association also HDAC4. Moreover, compound **2** showed inhibition of H3 deacetylation due to HDAC1 inhibition at 14-fold higher concentration, EC_{50} = 9000 nM. However, despite inhibiting HDAC6 and by correlation HDAC4 at submicromolar concentrations, this compound showed antiproliferative activity in HCT116 cells at much higher concentrations, EC_{50} = 12.8 μM. This is similar to the concentration

Table 3. Enzymatic and Cell-Based Activity of Compounds **2** and **6h**

compd	HDAC1 IC_{50} (nM)[a]	HDAC4 CAM IC_{50} (nM)[a]	HDAC4 WT IC_{50} (nM)[a]	HDAC6 IC_{50} (nM)[a]	Tub-Ac EC_{50} (nM)[b]	H3 EC_{50} (nM)[b]	PRO EC_{50} (nM)[b]
2	2,700 ± 300	780 ± 48	24 ± 2.6	230 ± 23	640	9,000	12,800
6h	12,800 ± 1,200	96 ± 5.7	310 ± 23	70 ± 3.5	1000	> 50,000	> 50,000

[a] IC_{50} values are the mean ± standard error of five independent determinations. [b] EC_{50} values are the mean of two independent determinations.

needed to inhibit deacetylation of histone H3, suggesting that inhibition of histone deacetylation, and therefore inhibition of HDAC1, correlates to antiproliferation effects. Similarly, compound **6h** is a potent inhibitor of HDAC4 CAM (IC_{50} = 96 nM) that inhibits HDAC6 at comparable concentrations, IC_{50} = 70 nM, and shows no inhibition of HDAC1 at 10 μM. This compound showed inhibition of α-tubulin deacetylation in HCT116 cells with EC_{50} = 1 μM; on the basis of this it is expected that HDAC4 would also be inhibited in cells at similar concentration. In contrast, no inhibition of histone H3 deacetylation or inhibition of HCT116 cell growth was observed at 50 μM.

These results strongly suggest that inhibition of the deacetylase activity of HDAC4 by small molecules does not cause inhibition of HCT116 cell growth and therefore does not recapitulate the growth inhibitory phenotype observed in the RNAi experiments. These results suggest that inhibition of the catalytic activity of HDAC4 by small molecules is not a viable approach for cancer therapy. Indeed, these results were sufficient to terminate our research around this target. A possible explanation for the apparent contradiction with the silencing experiments with RNA can be based on the fact that, although the role of class IIa HDACs in tissue-specific gene regulation is well-documented,[23] the contribution of the catalytic domain to this activity is not understood. A substantial body of evidence illustrates that class IIa HDACs exert transcriptional repression through protein−protein interactions using the N-terminal domain, and while these protein−protein interactions would be abolished by the RNA interference, the inhibition of the catalytic activity of HDAC4 by a small molecule may have no influence on the protein−protein interaction.[5b,c,24] Similarly, it should be remembered that currently there is no cellular HDAC4 target engagement assay, and although we have developed two in vitro assays, these may not be a true representation of the physiological conditions. It should also be remembered that when comparing small-molecule versus RNAi inhibition, we need to consider the kinetic, mechanistic, and potential off-target differences of each treatment,[25] and these subtle differences may lead to profound discrepancies between the outcome of both techniques.

Conclusions

We have identified a novel series of 5-aryl-2-(trifluoroacetyl)thiophene that are potent class II HDAC inhibitors. Some analogues from this new class of compounds inhibit HDAC4 WT, HDAC4 CAM, and HDAC6 at nanomolar concentrations and display 100-fold selectivity over HDAC1. Introduction of substituents in the aryl ring or replacement of this aryl ring by 1,2,4-oxadiazole led to compounds with improved stability in HCT116 cells. One of these compounds, **6h**, together with a 5-(1,2,4-oxadiazole)-2-(trifluoroacetyl)thiophene analogue, **2**, were used to demonstrate that inhibition of the deacetylase activity of HDAC4 by small molecules does not cause inhibition of cell growth in HCT116 cells. These results strongly suggest that inhibition of the catalytic activity of HDAC4 by small molecules is not a suitable strategy for cancer therapy.

Experimental Section

Solvents and reagents were obtained from commercial suppliers and were used without further purification. Organics extracts were dried over anhydrous sodium sulfate (Merck). Flash chromatography purifications were performed on Merck silica gel (200−400 mesh) as the stationary phase or were conducted using prepacked cartridges on a Biotage system, eluting with petroleum ether/ethyl acetate. Purity of the compounds was determined by analytical RP-HPLC, data was obtained by two methods on an Acquity Waters UPLC using a flow rate of 0.5 mL/min, an Acquity UPLC BEH C_{18}, 1.7 μm, 2.1 mm × 50 mm column as the stationary phase, and mobile phase comprised of MeCN + 0.1% HCO_2H (solvent A) and H_2O + 0.1% HCO_2H (solvent B). Method 1: 10% solvent A (0.2 min) to 100% solvent A (2.0 min) then 100% solvent A (0.8 min). Method 2: 10% solvent A (0.2 min) to 100% solvent A (2.5 min) then 100% solvent A (0.2 min). All the compounds described in this article showed purities higher than 95% in both analytical methods. Nuclear magnetic resonance spectra (^{1}H NMR) were recorded at 300 K in the indicated solvent on Bruker AM series spectrometers operating at (reported) frequencies between 300 and 400 MHz. Chemical shifts (δ) for signals corresponding to nonexchangeable protons (and exchangeable protons where visible) were recorded in parts per million (ppm) relative to tetramethylsilane and were measured using the residual solvent peak as reference. Signals are tabulated in the order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad, and combinations thereof); coupling constant(s) in Hz; number of protons. HPLC-MS were performed on a Waters Alliance 2795 apparatus, equipped with a diode array and a ZQ mass spectrometer using an X-Terra C_{18} column (5 μm, 4.6 mm × 50 mm). Mobile phase comprised a linear gradient of binary mixtures of H_2O containing 0.1% formic acid (solvent A) and MeCN containing 0.1% formic acid (solvent B). Compounds were purified by RP-HPLC (Waters Xterra C_{18}, 7 μm, 19 mm × 100 mm; flow: 20 mL/min; gradient: A: H_2O (+ 0.1% TFA); B: MeCN (+ 0.1% TFA); 20% A linear to 80% A in 12 min). High resolving power accurate mass measurement electrospray (ES) mass spectral data were acquired by use of a Bruker Daltonics apex-Qe or Waters Synapt Q-TOF Fourier transform ion cyclotron resonance mass spetrometer (FT-ICR MS). External calibration was accomplished with oligomers of polypropylene.

1-(5-Bromo-2-thienyl)-2,2,2-trifluoroethanol (4). A solution of 5-bromothiophene-2-carboxaldehyde **3** (1.0 g, 5.3 mmol) and CsF (80 mg, 0.53 mmol) in DME (20 mL) was treated with $TMSCF_3$ (0.92 mL, 6.3 mmol) and then stirred for 2 h at RT. The reaction mixture was quenched by adding 3N HCl and stirred for 30 min. The mixture was diluted with CH_2Cl_2, and the organic phase was separated, washed with brine, and dried. Evaporation of the solvent under reduced pressure gave a crude, which was purified by silica gel chromatography (1−10% EtOAc/petroleum ether gradient) to give **4** (1.3 g, 95%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00 (d, J = 3.7 Hz, 1H), 6.95 (d, J = 3.7 Hz, 1H), 5.20 (q, J = 6.2 Hz, 1H), 3.62 (br s, 1H). MS (ES) $C_6H_4F_3BrOS$ requires 260, 262; found 243, 245 $(M-OH)^+$.

1-(5-Bromo-2-thienyl)-2,2,2-trifluoroethanone (5). To a solution of the alcohol **4** (0.5 g, 1.92 mmol) in CH_2Cl_2 (10 mL) at RT

was added Dess−Martin periodinane (0.8 g, 1.92 mmol) and the reaction mixture was stirred for 3 h and then quenched by pouring into aqueous $NaHCO_3$ (saturated solution) containing 7-fold excess of $Na_2S_2O_3$. The mixture is stirred for 30 min, and then the layers were separated and the organic layer was washed with water and brine and dried. Evaporation of the solvent gave the crude product, which was purified by silica gel chromatography (1−10% EtOAc/petroleum ether gradient) to give **5** (0.35 g, 70%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74−7.68 (m, 1H), 7.23 (d, J = 4.2 Hz, 1H).

General Procedure for the Synthesis of 6a−l. A mixture of **5** (25 mg, 0.1 mmol) and the appropriate boronic acid (1.3 equiv) in DMF (2 mL), together with 2N Na_2CO_3 aqueous solution (0.1 mL, 0.2 mmol) was degassed with a stream of Ar for 10 min. $Pd(PPh_3)_4$ (6 mg, 0.005 mmol) was added and the reaction heated overnight at 90 °C. The reaction mixture was concentrated under reduced pressure and CH_2Cl_2 was added. The organic phase was washed with 1N NaOH, brine, dried, and concentrated under reduced pressure. The crude material was purified by preparative RP-HPLC, and the desired fractions were freeze-dried to give **6a−l**.

2,2,2-Trifluoro-1-(5-phenylthiophen-2-yl)ethanone (6a). Yield: 17%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.20−8.10 (m, 1H), 7.92−7.82 (m, 3H), 7.57−7.47 (m, 3H). HRMS (ESI) m/z calcd for $C_{12}H_8F_3OS$ 257.0242, found 257.0247. RP-HPLC method 1, t_R = 2.04 min; method 2, t_R = 2.35 min.

2,2,2-Trifluoro-1-[5-(4-methoxyphenyl)thiophen-2-yl]ethanone (6b). Yield: 14%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12−8.08 (m, 1H), 7.84 (d, J = 8.8 Hz, 2H), 7.73 (d, J = 4.4 Hz, 1H), 7.08 (d, J = 8.8 Hz, 2H), 3.84 (s, 3H). HRMS (ESI) m/z calcd for $C_{13}H_{10}F_3O_2S$ 287.0348, found 287.0354. RP-HPLC method 1, t_R = 2.02 min; method 2, t_R = 2.33 min.

2,2,2-Trifluoro-1-[5-(3-methoxyphenyl)thiophen-2-yl]ethanone (6c). Yield: 17%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.16−8.12 (m, 1H), 7.87 (d, J = 4.2 Hz, 1H), 7.46−7.42 (m, 2H), 7.40−7.38 (m, 1H), 7.10−7.06 (m, 1H), 3.84 (s, 3H). HRMS (ESI) m/z calcd for $C_{13}H_{10}F_3O_2S$ 287.0348, found 287.0354. RP-HPLC method 1, t_R = 2.04 min; method 2, t_R = 2.34 min.

2,2,2-Trifluoro-1-[5-(2-methoxyphenyl)thiophen-2-yl]ethanone (6d). Yield: 39%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12−8.08 (m, 1H), 8.04−8.00 (d, J = 7.8 Hz, 1H), 7.96−7.92 (d, J = 4.4 Hz, 1H), 7.49 (t, J = 8.4 Hz, 1H), 7.26 (d, J = 8.4 Hz, 1H), 7.12 (d, J = 7.6 Hz, 1H), 4.01 (s, 3H). HRMS (ESI) m/z calcd for $C_{13}H_{10}F_3O_2S$ 287.0348, found 287.0353. RP-HPLC method 1, t_R = 2.03 min; method 2, t_R = 2.33 min.

4-[5-(Trifluoroacetyl)thiophen-2-yl]benzonitrile (6e). Yield: 26%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22−8.18 (m, 1H), 8.12−8.04 (m, 3H), 8.02−7.96 (m, 2H). HRMS (ESI) m/z calcd for $C_{13}H_7F_3NOS$ 282.0195, found 282.0200. RP-HPLC method 1, t_R = 1.88 min; method 2, t_R = 2.15 min.

1-[5-(4-Acetylphenyl)thiophen-2-yl]-2,2,2-trifluoroethanone (6f). Yield: 7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22−8.18 (m, 1H), 8.10−8.02 (m, 4H), 8.02−7.98 (m, 1H), 2.63 (s, 3H). HRMS (ESI) m/z calcd for $C_{14}H_{10}F_3O_2S$ 299.0348, found 299.0354. RP-HPLC method 1, t_R = 1.89 min; method 2, t_R = 2.16 min.

4-[5-(Trifluoroacetyl)thiophen-2-yl]benzoic Acid (6g). Yield: 12%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.14 (br s, 1H), 8.20−8.16 (m, 1H), 8.05 (d, J = 8.4 Hz, 2H), 8.01 (d, J = 8.4 Hz, 2H), 7.95 (d, J = 4.0 Hz, 1H). HRMS (ESI) m/z calcd for $C_{13}H_8F_3O_3S$ 301.0141, found 301.0147. RP-HPLC method 1, t_R = 1.72 min; method 2, t_R = 1.96 min.

3-[5-(Trifluoroacetyl)thiophen-2-yl]benzoic Acid (6h). Yield: 12%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.31 (br s, 1H), 8.18−8.15 (m, 1H), 8.13 (d, J = 7.4 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 4.2 Hz, 1H), 7.66 (t, J = 7.4 Hz, 1H). HRMS (ESI) m/z calcd for $C_{13}H_8F_3O_3S$ 301.0141, found 301.0146. RP-HPLC method 1, t_R = 1.70 min; method 2, t_R = 1.93 min.

2,2,2-Trifluoro-1-[5-(pyridin-2-yl)thiophen-2-yl]ethanone (6i). Yield: 4%. ^{1}H NMR (400 MHz, CD_3CN) δ 8.63 (d, J = 4.6 Hz, 1H), 8.09−8.05 (m, 1H), 7.98 (d, J = 7.7 Hz, 1H), 7.90 (t, J = 7.7 Hz, 1H), 7.84 (d, J = 4.2 Hz, 1H), 7.42 (dd, J = 7.2, 5.5 Hz, 1H). HRMS (ESI) m/z calcd for $C_{11}H_7F_3NOS$ 258.0200, found 258.0205. RP-HPLC method 1, t_R = 1.23 min; method 2, t_R = 1.34 min.

2,2,2-Trifluoro-1-[5-(quinoxalin-6-yl)thiophen-2-yl]ethanone (6j). Yield: 4%. ^{1}H NMR (400 MHz, CD_3CN) δ 8.95−8.90 (m, 2H), 8.53 (d, J = 1.7 Hz, 1H), 8.25−8.19 (m, 2H), 8.15−8.12 (m, 1H), 7.88 (d, J = 4.3 Hz, 1H). HRMS (ESI) m/z calcd for $C_{14}H_8F_3N_2OS$ 309.0309, found 309.0308. RP-HPLC method 1, t_R = 1.78 min; method 2, t_R = 2.02 min.

2,2,2-Trifluoro-1-[5-(1*H*-indol-5-yl)thiophen-2-yl]ethanone (6k). Yield: 25%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.42 (br s, 1H), 8.12 (s, 1H), 8.12−8.09 (m, 1H), 7.75 (d, J = 4.2 Hz, 1H), 7.59 (d, J = 8.6 Hz, 1H), 7.52 (d, J = 8.6 Hz, 1H), 7.47−7.44 (m, 1H), 6.57−6.54 (m, 1H). HRMS (ESI) m/z calcd for $C_{14}H_9F_3NOS$ 296.0351, found 296.0359. RP-HPLC method 1, t_R = 1.91 min; method 2, t_R = 2.19 min.

1-[5-(2,3-Dihydro-1,4-benzodioxin-6-yl)thiophen-2-yl]-2,2,2-trifluoroethanone (6l). Yield: 69%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10−8.07 (m, 1H), 7.72 (d, J = 4.2 Hz, 1H), 7.40 (d, J = 2.2 Hz, 1H), 7.35 (dd, J = 8.4, 2.2 Hz, 1H), 6.98 (d, J = 8.4 Hz, 1H), 4.35−4.28 (m, 4H). HRMS (ESI) m/z calcd for $C_{14}H_{10}F_3O_3S$ 315.0297, found 315.0303. RP-HPLC method 1, t_R = 1.98 min; method 2, t_R = 2.28 min.

2,2,2-Trifluoro-1-(4-phenylthiophen-2-yl)ethanone (8). A solution of 4-phenylthiophene-2-carboxaldehyde **7** (150 mg, 0.8 mmol) and CsF (12 mg, 0.08 mmol) in DME (10 mL) was treated with $TMSCF_3$ (153 μL, 0.96 mmol) and then stirred for 2 h at RT. The reaction mixture was quenched by adding 3N HCl and stirred for 30 min. The mixture was diluted with CH_2Cl_2, and the organic phase was separated and washed with brine and dried. Evaporation of the solvent under reduced pressure gave a crude that was purified by silica gel chromatography (10−90% EtOAc/petroleum ether gradient) to give an oil that was diluted in CH_2Cl_2 (10 mL). The resulting solution was treated with Dess−Martin periodinane (340 mg, 0.8 mmol) and stirred for 3 h at RT. The reaction mixture was quenched by addition of aqueous sodium thiosulfate (saturated solution) and extracted with CH_2Cl_2. The combined organic extracts were dried. Evaporation of the solvent under reduced pressure gave a crude that was purified by silica gel chromatography (50% EtOAc/petroleum ether) to give **8** (35 mg, 17%) as a solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54−8.49 (m, 1H), 8.23−8.20 (m, 1H), 7.70 (d, J = 7.2 Hz, 2H), 7.53 (t, J = 7.2 Hz, 2H), 7.43 (t, J = 7.2 Hz, 1H). HRMS (ESI) m/z calcd for $C_{12}H_8F_3OS$ 257.0242, found 257.0246. RP-HPLC method 1, t_R = 1.99 min; method 2, t_R = 2.29 min.

2,2,2-Trifluoro-1-(2-phenyl-1,3-thiazol-5-yl)ethanone (10). A solution of 2-phenyl-1,3-thiazole-5-carbaldehyde **9** (50 mg, 0.26 mmol) and CsF (8 mg, 0.052 mmol) in DME (3 mL) was treated with $TMSCF_3$ (0.26 mL, 0.52 mmol, 2 N solution in THF) and then stirred for 3 h at RT. The reaction mixture was quenched by adding 1N HCl and stirred for 30 min. The mixture was diluted with EtOAc, and the organic phase was separated, washed with aqueous $NaHCO_3$ (saturated solution), and dried. Evaporation of the solvent under reduced pressure gave a residue, which was diluted in CH_2Cl_2 (5 mL). The resulting solution was treated with Dess−Martin periodinane (165 mg, 0.39 mmol) and stirred for 2 h at RT. The reaction mixture was quenched by addition of aqueous sodium thiosulfate (saturated solution) and extracted with CH_2Cl_2. The combined organic extracts were dried. Evaporation of the solvent under reduced pressure gave a crude that was purified by RP-HPLC (Waters SYMMETRY C_{18}, 7 μm, 19 mm × 300 mm; flow: 20 mL/min; gradient: A: H_2O (+ 0.1% TFA); B: MeCN (+ 0.1% TFA); 60% A linear to 10% A in 14 min) to give **10** (7.5 mg, 10%) as a solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.60 (s, 1H), 8.07−8.03 (m, 2H), 7.59−7.47 (m, 3H). HRMS (ESI) m/z calcd for $C_{11}H_7F_3NOS$ 258.0200, found

258.0200. RP-HPLC method 1, t_R = 1.50 min; method 2, t_R = 1.69 min.

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Supporting Information Available: Enzyme and cell-based assays conditions, stability experiments conditions. Experimental procedures and analytical data for compounds **2**, **11**, **12**, and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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